An understanding of the prostate cancer pathophysiology for the identification of biomarkers that support an early diagnosis

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Introduction

Prostate Cancer (CaP) is a condition whose etiology is multifactorial, it goes through genetic, infectious, as well as environmental, among others. Currently, PCa represents the most frequent cancer among men and the second in mortality, however, there are still multiple gaps in the knowledge of this condition. One of them is related to a molecular biomarker that allows early and accurate diagnosis of PCa. We have the Prostate Specific Antigen (PSA), however, its diagnostic performance alone is very low, and it must always be associated with digital rectal examination to obtain the best of both diagnostic methods.

Problem statement

Over the years, the focus of prostate cancer has been on the treatment. A patient with the condition is identified and they are offered invasive and non-invasive procedures that can generate adverse deleterious effects for the quality of life. Some efforts have been made for prevention without obtaining clear results according to the available evidence, and related to food and medication. Other efforts have focused on early diagnosis, with emphasis on the measurement of prostate-specific antigen (PSA) and digital rectal examination, however, there are already systematic reviews and meta-analyzes that show how population screening is not suggested of opportunity, given the large population that would have to sift to prevent a 10-year death (1). On the other hand but complementary, PSA and digital rectal examination, although they are fundamental elements, have an operative performance (Sensitivity, specificity, predictive values and probability reasons) that is not high and therefore, has room for false positives and negatives that would limit the diagnostic suspicion of prostate cancer.

There are some biomolecules that have been studied both in Prostatic Hyperplasia and in Prostate Cancer, however its validity in the general population is still limited. There is only one identified genomic variation (AR-V7) that shows the probability of resistance to hormonal management with enzalutamide and abiraterone in patients with castration-resistant prostate cancer and therefore would require the use of chemotherapy(2–4). However, there is no biomolecule or genomic variant that can be distributed at the time as an early detection test or risk for the development of prostate cancer, which supports the realization of this work.

Justification

Prostate cancer (PC) is the second most common type of cancer in the world's male population (5). It is estimated that one in seven men will be diagnosed at the end of their life with CP, and one in 38 men will die as a result of this in the long term. Prostate Cancer is diagnosed more frequently as a consequence of the introduction in 1980 of the Prostate Specific Antigen (PSA) test as a diagnostic tool (3).
During the last decade, there has been a notable decrease in mortality from prostate cancer in developed countries, but it has not been evidenced in developing countries. GLOBCAN in 2008 reported that in northern European countries (Denmark, Norway and Sweden) the diagnosis has

increased by 8.2% per year, with a mortality rate falling since 2000 of 3.1% per year (6). Similar data were evidenced in the United States and Canada, in which the incidence in the diagnosis of PC remains stable, but with a decrease in mortality of 4.3% and 3.1% respectively. On the other hand, in developing countries, mortality has increased (although there are trends towards an increase in diagnosis, mortality has increased in countries such as Colombia, 3.4% per year, Costa Rica, 3.4% year, Chile 2.8% per year and Cuba 5.5% per year) (6,7). With respect to the global context, Colombia has one of the lowest incidences in Latin America (6). Additionally, the mortality rate from prostate cancer has decreased in the last four years (7) and the largest number of cases reported originate in the cities of Bogotá, Valle and Antioquia (The most populated regions with the largest number of Urologists) (7).

On the other hand, when considering an appetite for the treatment of localized prostate cancer, which is the focus of the present work, there are currently studies that show that there are no differences in 10-year mortality when comparing monitoring, radical surgery of prostate and radiotherapy (8). Even when classifying the patient according to the risk of disease progression (D'amico) (9), it is evidenced that when comparing radical prostatectomy with clinical observation, there are no differences at 10 years of follow-up for the specific cancer mortality outcome (10) in localized low risk PC.

This is important to note because we still have gaps in knowledge. For several decades, urologists have performed radical surgeries that generate adverse events such as erectile dysfunction, urinary incontinence and urethral stricture. Given that we have elements of uncertainty such as those already demonstrated, we could consider the search for markers that identify which patients do not require a surgical procedure or those that have the risk, could be evaluated the possibility of genetic manipulation for prevention of prostate cancer.

Recent and interesting investigations have been carried out that, although they are not yet conclusive, do encourage us to continue searching for risk markers and genomic alterations that allow us to classify and treat our patients even before developing the disease. Inhibitory markers such as mitochondrial autophagy have been identified, related to the poor prognosis in prostate cancer. Epigenetics have identified alterations in DNA methylation and microRNA expression that occur in the transition from the normal cell, precancerous, primary and metastatic tumors. The circulating DNA fragments are another important strategy to generate the genetic profile and sequencing of the genes of patients with prostate cancer(11). In such a way that we still have gaps in the knowledge of localized prostate cancer that deserve to be evaluated in works of this type.

Objectives:

- To describe the frequency of Inherited DNA-repair genes and their variants associated to Prostate Cancer in a Cancer-Free Southwest Colombian population

- To identify the association between the TMPRSS2:ERG fusion gene, their variants and the onset of localized prostate cancer.

- To describe the frequency of allelic variants of TMPRSS2 gene in this population

- To analyze the metabolomic profile in patients with malignant disturbances of the prostate compared with non-cancer patients.

Chapter 1: An updated and global review on prostate cancer

Type of article: Narrative Review

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Introduction

Prostate cancer given its frequency in the populations is a pathology of importance in public health not only national, but of high global impact (12). Given the difficulties of the Health System, the limited availability of specialists and the high prevalence of this condition, the knowledge of this condition should be the domain of any general practitioner, and should not remain in specialized medicine areas such as urology and oncology. However, the treatment in an integral way, must be given by centers of excellence in cancer (13).

Objective
To obtain an updated view through a detailed and up-to-date review of the epidemiology, risk factors, classification, diagnosis and treatment of prostate cancer.

Methods
A search was performed in Embase and Medline (through OVID) from January 2000 to March 2017. The keywords used were: "prostate" OR "prostate, neoplasm" AND "diagnosis" OR "treatment". Additionally, an exhaustive manual evaluation of the bibliography provided in the articles found was also made.

Results
Epidemiology
Prostate cancer (PCA) is the most frequent neoplasm in men in the world, and represents the second cause of cancer death in this population in the United States (14). It has an incidence of 131.5 / 100,000 inhabitants (14) with a race distribution of 123 / 100,000 inhabitants in the white and 208 / 100,000 inhabitants in the African American population (15). It is estimated that 1 in 7 men will be diagnosed throughout their life with PCA, and 1 in 38 men will die as a consequence of it (16).
The GLOBCAN study reported that in the Northern European countries (Denmark, Norway and Sweden) the diagnosis of PCA has increased 8.2% per year, accompanied by a declining mortality of 3,1% per year since 2000 (6). In the United States and Canada, similar data have been found, with a stable incidence but with a decrease in mortality of 4.3% and 3.1% respectively; however,

in developing countries, mortality has increased (6,7). Regarding the national epidemiology, Colombia has one of the lowest incidences of PCA in Latin America and a proportion of 28% between incidence and mortality for it, very close to the world average of 28.6% and lower than countries like Ecuador (40.41%), Cuba (46.65%) and Peru (37.74%) (6). Mortality has decreased in the last four years (7) and the regions with the highest number of CAP patients reported are Bogotá, Valle and Antioquia (The most populated regions and with the largest number of Urologists) (7,16).

Risk factors

Race

Black patients have a higher prevalence of PCA (17), furthermore, they present at younger ages with greater tumor volume, a greater prostatic antigen level and worse prognosis (18,19). Some authors associate these results with social inequities and difficulties in accessing health services, to which this population is exposed (20), however there is much more evidence that supports this factor as a risk factor for PCA. On the other hand, lower rates have been found in Asians, which has been additionally related to diet, lifestyles and environmental factors (21).

Family History

Approximately 10-15% of men with PCA have at least one relative with history of this condition (17,22). It has been estimated that having a relative of first degree of consanguinity with PCA increases the RR 2 to 4 times and is 5 times higher if there are two relatives with that diagnosis.

Inflammation

Chronic inflammation is considered a risk factor because it leads to cellular hyperproliferation, which generates an alteration in antioxidant levels, in DNA repair and apoptosis. It has also been found that the history of a sexually transmitted infection has an OR 1.5 (23) and history or current prostatitis an OR of 1.57 (24). Despite being one of the strongest hypotheses, it is still unclear the mechanism that would lead to producing the PCA or if it is a sufficient cause for its development.

Oxidative Stress

Some studies have suggested that reactive oxygen species (such as superoxide or peroxide) create an environment of mutagenesis conducive to the initiation of PCA (25,26). This element could be related to the hypothesis of chronic inflammation.

Androgens

There is evidence that an increase in testosterone levels increases the incidence of PCA, although a dose-response relationship or a concentration of which the risk is increased has not been found; additionally, a higher risk of PCA has not been found in patients with hypogonadism treated with testosterone replacement therapy (27).

Estrogens

It has been shown that estrogens that can predispose and even cause PCA. It is necessary to emphasize that 17β-estradiol has already been classified as a carcinogen, especially in breast and endometrial cancer. It is believed that the effect of estrogen in PCA is caused by direct mutations, through regulation by epigenetic effects or by endocrine disruption (28).

Diet

Several studies have suggested that a diet low in fat, calcium, with an increase in the consumption of vitamin E and lycopene, as well as regular exercise, could behave as protective factors for the development of PCA, on the other hand, the high intake of saturated fats of animal origin and red meat have been described as risk factors; however, the findings are not consistent between the different studies. For example, in the SELECT study, the protective factor for the use of vitamin E and selenium was not demonstrated (29).

Increase in levels of insulin-like growth factor (IGF-1)

Insulin-like growth factor is a mitogenic and antiapoptotic factor. High levels imply more PCA risk (30). However, other studies do not find it as a risk factor.

Genetic

Alterations have been found in suppressor genes such as p53, PTEN, these are related to increased incidence, progression and aggressiveness of the PCA. Among other altered genes have been found: the RAS oncogene, EIF3S3, BCL2 (anti-apoptosis), EGFR, FGFR2c, ERBB2, BRCA 2, MET among many others under study. Some mutations in Chromosome 1 (family PCA risk) and 8 (sporadic cancer), among others. Genetic polymorphisms have been demonstrated in some enzymes such as:

• 5 alpha reductase (higher in black race)

• Vitamin D receptor (VDR) which has been recognized as a protective factor, however it is decreased in black patients, thus increasing the risk of PCA.

• Androgen receptor (AR): Increases the risk of family PCA.

• Telomerase: It is a risk factor for sporadic cancer.

Much attention has been focused on the BRCA2 gene (Breast Cancer susceptibility protein type 2), which has an autosomal dominant inheritance pattern with incomplete dominance. Codifies for a protein of the same name, whose function is to act as a center by recruiting regulatory proteins, in order to repair double-strand breaks by homologous recombination, in addition it facilitates the repair of simple chains promoting the formation of the RAD51-ssDNA complex (Simple chain of DNA) (31).

Historically, the BRCA2 gene has been linked to breast cancer, however recent findings indicate that it may play an important role in PCA. It has not been possible to identify with certainty the mechanism by which the mutations in this gene predispose to the development of the PCA, nevertheless by its function it is deduced that alterations of this predispose to a minor repair of the damages of the genome, which could result in alterations of the cell cycle and consequently a greater cellular proliferation.

It has been found that patients with mutations of the BRCA2 gene generally present a higher incidence of PCA (32), more advanced stages (T3-T4), more aggressive phenotypes and lower survival despite receiving a local treatment with similar curative intent (33).

Obesity

Some authors suggest that obesity plays a role in the development of PCA. It is believed that the resistance to insulin produced by obesity leads to an elevation of this hormone, which due to its anabolic capacity could generate cancer development or its progression (34). It is believed that the obese are less likely to have high Prostate-Specific Antigen (PSA) and therefore less likely to perform biopsy and thus, less likely to diagnose PCA; This, together with the associations with the circulating levels of metabolic and sexual hormones, leads to the suggestion of obesity as a risk factor for aggressive PCA (35).

Alcohol

The relationship of alcohol intake with PCA is controversial. Rota et al in a meta-analysis (50 case-control studies and 22 cohorts) with a total of 52,899 cancer cases, found no evidence between alcohol intake and PCA, there were even no statistical differences in the high-risk group intake (> = 4 alcoholic drinks per day) (36).

Smoke

The carcinogenic capacity of tobacco is known as well as the mechanism by which genetic damage is generated. In PCA, an increase in incidence has not been described, however it has been found

that it can generate higher rates of death due to PCA, although it is modest, it could have an impact at public health level because it is a modifiable risk factor (37).

Natural History

In autopsies, a 30-40% prevalence of PCA has been found in men older than 50 years, on the other hand, <5% in those under 30 years and approximately 60-70% in older people 79 years (38). It has been calculated that 1.5% of these are detectable by clinic every year. The PCA is progressive and its biological aggressiveness is directly related to the degree of cellular differentiation (Gleason Scale), the TNM, the PSA value, among other factors.

Histopathologic Classification

For classification of histopathology, the Gleason classification system is used, which exposes the degree of cell differentiation found in the prostatic stroma. It consists of two values, the first is the degree found more frequently and the second the next, thus a final value is obtained (For example: 4 + 5 = 9), the score goes from 2 to 10. In the case where the two values are in the same proportions, the most undifferentiated is placed first.

The new classification of Gleason performed by the American College of Pathology relates the score with the prognosis of each group (see Table 1) (39).

Table 1. Gleason classification of the American College of Pathology. Adapted from Epstein et al.(39).

Classification	Score	Characteristics
1	<= 6	Only well differentiated glands.
2	3 + 4 = 7	Predominantly well differentiated glands with less component of poorly differentiated, fused or cribiform glands.
3	4 + 3 = 7	Predominantly poorly differentiated, fused or cribriform glands with less component of differentiated glands
4	8 (4 + 4 ; 3 + 5; 5 + 3)	The different ways are: Only poorly differentiated, fused or cribiform glands; predominantly well differentiated glands and minor component that lacks glands; Predominant lack of glands and less component of well-differentiated glands.
5	9 o 10	It lacks gland formation (or with necrosis) with or without glands, poorly differentiated, fused or cribriform glands.

Pathologic and clinical classification

It is based in TNM classification 2016 (Table 2) (40).

Table 2. TNM classification for Prostate Cancer. Adapted from American Joint Committee on Cancer (40).

TNM Classification for Prostate Cancer	
Primary tumor, (T) clinical	
TX	The primary tumor cannot be evaluated
T0	There is no evidence of primary tumor
T1	Primary tumor is not clinically apparent (not visible, not palpable)
T1a	Incidental tumor in 5% or less of resected prostate tissue
T1b	Incidental tumor in more than 5% of resected prostate tissue
T1c	Tumor identified by needle biopsy (due to elevation of the PSA).
T2	Primary tumor confined to the prostate
T2a	Tumor compromises <50% of a lobe
T2b	Tumor that compromises> 50% of a lobe
T2c	Tumor compromises both lobes
T3	The tumor extends beyond the prostatic capsule (Invasion to the prostatic apex or to the prostatic capsule is classified as T2).
T3a	Unilateral or bilateral extracapsular extension.
T3b	The tumor involves seminal vesicles
T4	Fixed tumor or tumor that invades adjacent structures different from the seminal vesicles: bladder neck, external sphincter, rectum, levator ani and / or pelvic wall.
* The tumor detected by biopsy in one or both prostatic lobes, which is not palpable or visible by imaging, is classified as T1c. * Positive margins should be indicated as R1 (residual microscopic disease).	
Regional lymph nodes (N)	
Nx	Regional metastasis not evaluable.
N0	There are no regional metastases.
N1	Metastasis in one or several regional nodes.
Distant metastasis (M)	
Mx	Distant metastasis not evaluable.
M0	There is no distant metastasis.
M1	Remote metastasis
M1a	To non-regional lymph nodes
M1b	To bone
M1c	To another site
When there is more than one site of metastasis it is classified as M1c.	

Staging

The staging of the PCA is shown in table 3. For this, it must be taken into account that if the PSA or the Gleason value are not available, the classification must be determined by the T and/or the PSA value or the Gleason score available.

Table 3. Prostate cancer staging.

Status	T	N	M	PSA	Gleason
I	T1a-c	N0	M0	< 10	<=6
	T2a	N0	M0	< 10	<=6
	T1-T2a	N0	M0	X	X
IIa	T1a-c	N0	M0	< 20	7
	T1a-c	N0	M0	>=10 - <20	<=6
	T2a	N0	M0	< 20	<=7
	T2b	N0	M0	< 20	<=7
	T2b	N0	M0	X	X
IIb	T2c	N0	M0	Any	Any
	T1-2	N0	M0	>=20	Any
	T1-2	N0	M0	Any	>=8
III	T3a-b	N0	M0	Any	Any
IV	T4	N0	M0	Any	Any
	Any	N1	M0	Any	Any
	Any	Any	M1	Any	Any

Risk classification for localized carcinoma:
The risk classification for localized carcinoma used historically for PCA is the D'amico Classification (see Table 4) (41). However, during the last years different changes have been generated according to the heterogeneous prognosis that can be presented with different factors, for which modifications and a new classification suggested by the National Comprehensive Cancer Network (NCCN) have been made (see Table 5) (42).

Table 4. D'Amico Classification for localized carcinoma. Adapted from D'Amico et al (41).

Risk	PSA	Gleason	TNM
Low	<= 10	<= 6	<= T2a
Intermediate	10 a 20	7	T2b
High	>20	>= 8	>= T2c

Table 5. New classification for localized carcinoma suggested for National Comprehensive Cancer Network (42).

Risk	PSA	Gleason	TNM	Others
Very low	<10	<= 6	T1c	Less than 3 cores of the positive biopsy, all with <50% of the core compromised; PSA density <15 ng / ml / gr
Low	<10	<= 6	T1-T2a	

Intermediate	10 a 20	7	T2b-T2c	
High	>20	8 to 10	T3a	

Diagnosis

Currently, prostate-specific antigen (PSA) in conjunction with digital rectal examination are mainly diagnostic methods used in the clinic to detect prostate cancer, however, these have low diagnostic performance, both individually and together (16).

Prostate-Specific Antigen (PSA)

PSA, also called kallikrein III is a 34kDA glycoprotein which is almost exclusive of prostatic epithelial cells. It circulates bound to alpha-1-antichymotrypsin and alpha-2-macroglobulin and its duty is to divide semenogelin I and II into smaller polypeptides, thus preventing the formation of the seminal clot (43–47). It is found in prostatic fluid at concentrations of 1,000,000 ng / mL, under normal conditions a small amount, less than 4ng / mL is released into the bloodstream, but in a neoplastic process these levels rise (47). For this reason, it is considered to perform a prostate biopsy on men with a serum PSA level greater than 4ng / mL (47). However, it has also been found elevated in other pathologies such as breast cancer, renal cell carcinoma, ovarian cancer and adrenal neoplasia (48). Similarly, other urological diseases such as benign prostatic hyperplasia (BPH), prostatitis, cystitis, instrumentation and surgery of the recent urinary tract may be elevated (1,47). It should be noted that digital rectal examination does not increase PSA values.

According to the American Cancer Society (ACS), the PSA sensitivity for reference values of 4ng/ml and 3ng/ml for cancer diagnosis is 21 and 32% respectively. A specificity of 91% for cut-off values of 4ng / ml and 85% for PSA values of 3ng/ml (49).

In the PLCO study, regarding PCA, men between 55-74 years were evaluated, who underwent annual screening with PSA for 13 years, as a result it was obtained that screening with PSA does not lead to a decrease in the incidence of PCA (RR 1.09, 95% CI 0.87-1.36) (50). Another large study was the ERSPC where PSA screening was carried out for 11 years to men from certain European countries, assessing mortality by PCA, the results indicated a relative reduction in mortality rates of 21% (RR 0.79, 95% CI 0.68 to 0.91) (51).

A Cochrane meta-analysis conducted in 2011 summarized the results of 5 population experiments with a total of 341,351 participants and showed that screening with PSA is effective for the detection of PCA (RR 1.35, 95% CI 1.06-1.72) However, this test did not decrease mortality (RR 0.95, 95% CI 0.85-1.07) (52), so that population screening for PCA is currently not recommended. However, there is not enough evidence to determine the best measure for screening in public health, for now it is suggested opportunity screening, men between 50 and 70 years (according to the life expectancy of the population) who enter the urologist's office and patients with risk factors (black race and family members with prostate cancer).

It is of vital importance to clarify that in deciding the start of the search for the diagnosis of PCA, rectal examination should be performed in conjunction with PSA.

Other biomarkers in the PCA diagnosis

The failure of the PSA has led to the need to identify new biomarkers, with greater sensitivity and specificity, which allow an early diagnosis of PCA to be achieved (16). PSA, due to being elevated

in both benign conditions (benign prostatic hyperplasia) and in conditions of malignancy (53), has led to expensive and unnecessary biopsies being requested from patients who did not require it from the beginning (53). As a consequence, other techniques and molecules have been explored to make a more specific diagnosis, such as PCA3, microglobulin, mucins, among others (16). Some of these were included in a detailed review previously published by Esquivel Parra et al (16) and it is suggested to review to deepen the topic.

Other tools that try to decrease the number of unnecessary biopsies that have not yet been successful and are used for patients with PSA values between 4 and 10 ng/ml are:
The Free PSA/Total PSA Ratio: Raises the specificity for the diagnosis of PCA, in those cases with the mentioned levels in the presence of doubt as to the indication for biopsy.
PSA can transit in serum freely (fPSA) or accompanied by protease inhibitors (cPSA) in order to avoid proteolysis. When adding the fPSA and cPSA results in the total serum PSA (tPSA), a large part of this, around 70-90% can be linked to alpha-1-antichymotrypsin, in smaller proportion to alpha-2-macroglobulin, alpha-1 antitrypsin or a protein C inhibitor (46). Consequently, about 10-30% of the total PSA (tPSA) circulates freely (fPSA) (16).
With indexes <0.07, the probability of PCA is almost 90%. A limit value is not yet defined, however the use of 0.20 is recommended to decide between biopsy or follow-up, the values above this suggest a diagnosis and adequate treatment for benign prostatic hyperplasia, on the other hand, the realization is suggested of biopsy in values lower than this due to the high probability of PCA.
The PSA density (total PSA/volume of the prostate in cubic centimeters (cc) determined by ultrasound) before values > 0.15 ng /ml/cc should be biopsied since they suggest adenocarcinoma.
The PSA velocity > 0.75 ng/ml/year (used in follow-up after prostatectomy or initial PSA > 4ng/ml) suggests the presence of cancer; at present it has been lowered even to values like 0.35 ng/ml/year (for PSA <4 ng / ml).
Although all the tools are available to raise the specificity of PSA, prostate biopsy is currently considered in all patients with PSA> 4 ng / ml, however some authors suggest that taking a single PSA value of <2.5 ng/ml (It can be applied for patients under 50 years of age, since studies are needed to confirm its value).

Transrectal prostate ultrasound guided biopsy:
It is the gold standard for the diagnosis of PCA. The samples are taken in the prostatic periphery, which is the site with the highest frequency of carcinoma; usually for the first biopsy, at least 6 cylinders should be taken for each lobe, in case of having a negative biopsy with persistence of elevated PSA, a saturation biopsy will be performed (> 10 - 12 samples/lobe). There are studies that show that prostate biopsy requires adequate analgesia/anesthesia and the use of antibiotic prophylaxis.
Transrectal ultrasound of the prostate is only indicated, if it is accompanied by a biopsy, it should not be done in another condition. The indications for prostate biopsy are PSA >4ng/ml and the presence of alterations in the prostatic surface (nodule or stone prostate) predominantly, although there are variants that are not the subject of this review.
Nuclear medicine
Bone scintigraphy should be performed initially, in patients with PSA> 10 ng/ml or in those classified as intermediate and high risk patients. The probability that patients with PSA levels <10 have a positive scintigraphy is below 1%. On the other hand, a PSA> 49 has a LR + (positive likelihood ratio) >6, which means that there is a 6 times higher probability of finding a positive scintigraphy in patients with PCA than in patients without PCA (54).

Computed tomography (CT)

Abdominal CT (like magnetic resonance imaging) indirectly evaluates nodal invasion by measuring the diameter of the lymph nodes. However, its sensitivity is low and microscopic invasion can not be detected. Using a threshold of 10 mm, the sensitivity is <40%. The median sensitivity, specificity, negative predictive value and estimated positive predictive value are 7%, 100%, 85% and 100%, respectively.

Although fine needle aspiration biopsy (BACAF in Spanish) can be a good complement in cases with positive images, the difficulty of reaching the ganglia due to its position, makes it not very sensitive for staging and has a false negative rate of 40%. %. For CT (like MRI), the detection of microscopic lymph node invasion is <1% in patients with a Gleason score <8, PSA <20ng / ml, or localized disease. Its use should be reserved for high risk patients.

Although bone CT has low specificity, its use over other techniques to evaluate bone metastases is preferred. It is recommended to be performed in symptomatic patients regardless of PSA levels, Gleason score or clinical stage. (55).

Magnetic resonance (MRI) of prostate/pelvic

The T2-weighted image is the most useful for local staging in MRI. At 1.5 T (Tesla), MRI has low sensitivity to detect extraprostatic extension of carcinoma (22-82%) or invasion of seminal vesicles (0-71%), but greater specificity (61-100% and 62-100). %, respectively). The overall accuracy of MRI to distinguish T1 / T2 stages from T3 is 50-85%. These results are due to the fact that MRI cannot detect the microscopic extra-prostatic extension, in such a way that its sensitivity increases with the radius of extension within the periprostatic fat (55).

The use of the endorectal probe improves the accuracy of the stage at 1.5T, and a better precision has been demonstrated in the combined use of endorectal and external probes compared to the use only of external one. The high field strength allows a high resolution T2-WI and the results at 3T seem better than at 1.5T, although the experience of the reader is still of utmost importance, the precision of the RM to 3T varies between 67% and 93% depending on of the reader's experience. Even the prediction of the pathological stage by MRI can be improved when combined with clinical data. Given its low sensitivity for focal extra-prostatic (microscopic) extension, multiparametric prostate MRI is not recommended for local staging in low-risk patients. However, it may be useful in planning treatment in selected low-risk patients (56).

PET/CT

Positron emission tomography of 11C- or 18F-choline (PET) / CT has a good specificity for lymph node metastases, but the sensitivity is 10-73%. In a meta-analysis of 609 patients, the sensitivity and specificity of PET/CT for pelvic node metastases were 62% (95% CI, 51-66%) and 92% (95% CI: 89 -94%) (57). Because of its low sensitivity, PET/CT is not recommended for initial staging in lymph node metastases. Currently, studies are underway with psmaPET-CT (prostate-specific membrane antigen-PET CT).

The PET / CT of 18F-choline shows a superior sensitivity to conventional bone CT when evaluating bone metastasis. It is not clear whether 11C-choline PET / CT is more sensitive than conventional bone scan, but has greater specificity, with fewer indeterminate lesions. However, the cost-effectiveness of these interventions has not yet been evaluated. Therefore, bone CT is preferred based on availability and cost.

Treatment
The treatment of choice depends on the stage of the tumor at the time of diagnosis. Six treatment modalities could be used:

- Surgical
- External conformational radiation therapy
- Brachytherapy
- Hormonotherapy
- Active surveillance.
- Observation

Localized prostate cancer < cT2c, Nx, M0
The approach to localized prostate carcinoma depends on the level of risk previously called by D'Amico and the NCCN guidelines, as follows:

- Very low risk: Any treatment modality can be performed. Good results have been observed with observation and active surveillance to avoid overtreatment. However, in patients with life expectancy greater than 10 years, surgical treatment could be considered.
- Low risk: As in the previous group, any treatment modality can be performed. Life expectancy also plays an important role, since surgical treatment is probably not justified in patients with <10 years of age. In those who have a life expectancy> 10 years, who are considered candidates for surgical treatment, radical prostatectomy is the treatment of choice, without the need to perform lymphadenectomy that has been shown to be more effective when compared to radiotherapy (58).
- Intermediate risk: Retropubic radical prostatectomy with bilateral pelvic lymphadenectomy is the management of choice in these patients. Usually in patients with life expectancy greater than 10 years. As any surgical procedure can have complications such as bleeding, infection, however, the most important for the patient are urinary incontinence and erectile dysfunction, clarifying that the frequency of these has decreased along with the improvement of the surgical technique and the preservation of the neurovascular bundles together with the external sphincter. Another modality of treatment is external radiotherapy which can be performed in these patients, it can be 3D conformational, with modulated intensity (74-80 Gy), or low rate brachytherapy (preferable for low risk patients). All have related complications, similar to surgery such as incontinence, erectile dysfunction, proctitis and/or cystitis.
- High Risk: In this group of patients the treatment is performed in the same way as in the intermediate risk patient

Locally advanced prostate cancer cT3-4, Nx, M0
One of the therapeutic options is radical retropubic prostatectomy with bilateral pelvic lymphadenectomy in selected young patients, with clinical stage T3, gleason < 8 and PSA less than 20 ng/ml, this given that up to 25% may be overstaged.
Another option that could be performed is external radiotherapy combined with hormonal therapy (neoadjuvant, concurrent and adjuvant LHRH analogues (for 1-3 years)).

Advanced prostate cancer; Any T, N1, M1
Hormone therapy (surgical or medical) is the treatment of choice in patients with advanced prostate cancer. The drugs currently used for medical orchiectomy are the LHRH analogues (leuprolide

acetate, goserelin acetate, and triptorelin acetate) and the gonadotropin-releasing hormone (GnRH) receptor antagonists (Degarelix), whose use it must be by specialized personnel. These can be added to emerging medications such as Abiraterone (inhibitor of testosterone synthesis), Enzalutamide (Androgen receptor inhibitor) and Radio-223, when the patient is called castration-resistant (CRPC) (9).

The criteria to be defined as resistant to castration are (9): Testosterone level below 50ng/dl or 1.7nmol/l plus

- Biochemical progression: Three consecutive elevations of the PSA, at least one week apart and resulting in two increments of 50% above the nadir and a PSA> 2, or;
- Radiological progression: Appearance of new lesions: two or more lesions in a bone scan or a soft tissue lesion using the RECIST criteria (Response Evaluation Criteria in Solid Tumors).

Antiandrogens are used as adjuvants in the treatment, there are two types: steroids and non-steroids. Among the non-steroids are flutamide, bicalutamide, among others, however its indications, dosage and monitoring should be performed by the specialist (9).
In those patients with metastatic and castration-resistant PCA, different classifications can be made: 1. According to the functional status and 2. According to the presence of symptoms or visceral metastasis. For those with impaired functional status, even the treatment is under investigation given the poor prognosis of the patients. For those with good functional status, we have different treatments among which are the new generation antiandrogens and chemotherapy. Asymptomatic or mildly symptomatic patients may receive: Abiraterone, Enzalutamide and Radio 223, on the other hand, those who are symptomatic and/or have visceral metastases, are candidates for chemotherapy (Docetaxel or Cabazitaxel (As a second line)), whose management is given by the specialist in oncology and/or in interdisciplinary meetings (9).

Discussion

The present review on PCA presents an updated view on different aspects of prostate cancer.
It is a very frequent pathology, with an incidence that varies between being stable or increasing and a prevalence that is clearly increasing, probably due to the improvement for diagnosis and treatment. With regard to risk factors, we have classically recognized as race, age and genetic factors clearly recognized as such, those that have not yet been able to find the role that plays as food and some that were formerly believed were factors protective and now aim to play a carcinogenic role as estrogens.
It is important to perform an adequate classification and clinical, anatomical and pathological staging, since the process depends on the approximation and use of imaging and radiology techniques, as well as timely, individualized and indicated treatment based on evidence-based medicine.
The genetic characterization and the microbiology of the PCA is what most efforts and researches aim for and point to, since tests with greater sensitivity and specificity are required that lead to an earlier, more accurate diagnosis and, if possible, to reach therapies more specific and less morbid

Conclusion

We have made a journey through the conditions of risk, screening, diagnosis, new biomarkers and treatment of prostate cancer. Several elements have changed in recent years, mainly about the

understanding of the physiology of cancer, the associated factors, the search for new biomarkers in each of the stages of cancer and various elements related to the treatment, however, much research there is ongoing on the prevention, diagnosis and treatment of this condition so important, relevant and relevant to men around the world.

Chapter 2: Molecular alterations associated with prostate cancer

Type of article: Narrative Review

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Introduction

Recently, the quantity and quality of tools available for the genetic study of cancer and the whole genome have increased, with even greater detail available for the exome alone. Many molecular signaling pathways provide negative or positive regulatory signals that control cell proliferation in a way that attempts to preserve cell number and homeostasis, but this process is completely altered in cancer cells (59,60).

Normal cells must acquire at least eight attributes to transition from a normal cell to a cancer cell. These attributes include the following: 1. Genetic instability and mutation, 2. Autonomous growth, 3. Insensitivity to internal and external anti-proliferative signals, 4. Resistance to apoptosis and other forms of induced cell death, 5. Unlimited cell division potential, 6. Ability to form new blood vessels (Angiogenesis), 7. Local invasive behavior that enables the distinction of benign and malignant neoplasms, and 8. Evasion of the immune system.

Additionally, cancer cells require energy for autonomous growth and unlimited replication. Tumor-associated inflammatory mediators also cause preneoplastic cells to progress to invasive cancer cells; finally, cancer cells gain the ability to metastasize, that is, to migrate and colonize organs or tissues (59,61,62). The purpose of this article was to review the main biomolecular mechanisms associated with prostate cancer.

Therefore, the somatic genetic alterations that are involved in the pathogenesis of prostate carcinoma progression are shown in Figure 1 (63,64). The objective of this review was to describe some of the different biological mechanisms associated with prostate cancer

Methods

We performed a systematic literature search in Medline via Ovid, Scopus (Includes Embase) and Lilacs from the inception to nowadays with the following keywords: Prostate neoplasms; Prostate cancer; Molecular Medicine; Genomics; pathways; cell cycle. We included reviews, systematic reviews, basic science studies and analytical studies, which tried to explain the molecular disturbances associated with prostate cancer. According to the heterogeneity expected, we synthesized information based on the molecular mechanism. Information about most promising biomarkers associated with prostate cancer can be found elsewhere (16).

Tumor suppressor genes and oncogenes

Suppressor genes negatively regulate cell growth, and therefore, they play an important role in the normal cell cycle, DNA repair and cell signaling. The loss of the function of both alleles of a suppressor gene leads to carcinogenesis; thus, different pathways can result in cancer, such as 1. Homozygous gene deletion, 2. Loss of one allele and mutational inactivation of the second, 3. Mutations in both alleles, or 4. Loss of one allele and epigenetic inactivation of the second allele (e.g., DNA Methylation)(59). The two best characterized suppressor genes thus far are the retinoblastoma gene (RB1) and the TP53 gene, which are described later.

Oncogenes are positively associated with cell proliferation and are the mutated form of normal genes (proto-oncogenes). Two such oncogenes are MYC and MET. MYC is responsible for the regulation of cell proliferation. This amplified gene is frequently present in prostate cancer (PCa), and its expression in prostatic cells has been associated with immortalization(65). In contrast, MET has been reported in renal cell carcinoma (RCC), primarily in the hereditary type(66).

The mechanisms by which a proto-oncogene can become an activated oncogene are as follows: 1. Proto-oncogene mutation, 2. Gene amplification and 3. Chromosomal rearrangement. An example of the latter mechanism is the translocation that leads to the fusion of the TMPRSS2 gene with the ERG oncogene in a large proportion of PCa cases(67). Figure 2 schematically represents the cell cycle and describes how a cell in G0 is allowed to proliferate based on a signal, is duplicated in S phase, the phase in which DNA is synthesized, and subsequently segregates its genomic complement, which results in two daughter cells in a process called M phase (Mitosis). These two processes are separated by two critical gaps termed Gap 1 and Gap 2. The entire cycle lasts approximately 24 hours, and each phase depends on the previous one. In addition, some mechanisms function to verify the integrity of the DNA. If any alterations are found, the cell attempts to repair the damage, but if repair is not possible, the cell enters an active process termed apoptosis, which will be described later. The loss of the ability to respond to DNA damage leads to genetic instability, increases the mutation rate and mutates genes associated with cancer, thereby contributing to carcinogenesis and progression of the disease(68,69).

Retinoblastoma protein (RB1)

RB1 is important for controlling the R-point, which is a decisive point in late G1 phase during which the cell is committed to undergo division. Thus, if this control is lost, the cell continues to proliferate. All of the above events are due to inactivation of the RB1 pathway, which is mutated in at least 30% of bladder and prostate tumors, although RB1 mutations have not been strongly associated with these cancer types. It has also rarely been associated with renal carcinoma(70,71).

Cyclin-dependent kinase inhibitors

The temporal sequence of events during the cell cycle is dependent on cyclins and cyclin-dependent kinases (CDKs). CDKs phosphorylate protein substrates that are involved in the execution of specific activities in each phase. In contrast, cyclin-dependent kinase inhibitors (CDKIs) bind directly to CDKs and suspend their activity and their ability to phosphorylate other proteins(72). CDKIs belong to one of two classes: the Cip / Kip Family, which includes the CDKN1A (p21), CDKN1B (p27) and CDKN1C (p57) proteins, and the INK4 (inhibits CDK4) family, which includes the INK4B (p15), INK4A (p16), INK4C (p18) and INK4D (p19) proteins. The p16 protein binds to CDKs 4 and 6 and inhibits their interaction with cyclin D1; normally,

active CDK4 and 6 mediate the passage of the cell through G1 phase via the phosphorylation of RB1(59,73). The latter has also been associated with bladder cancer (by deletion of INK4A) and with renal cancer, and p16 inactivation has been shown to occur by hypermethylation of the DNA (epigenetic mechanism)(74). In prostate cancer, hypermethylation of INK4A is typically seen in 60% of cases, although INK4B is rarely inactivated (75).
Decreased CDKN1B has been correlated with decreased overall survival and disease-free survival after radical prostatectomy. In addition to positive CDKN1B in prostate biopsies, it has been associated with increased biochemical recurrence, and in mice, absence is associated with prostatic hyperplasia (76,77).

Tumor suppressor TP53
TP53 is a suppressor protein that plays an important role in response to cellular damage. It signals a halt to the cycle or leads to damage repair pathways (Figure 3), but if repair is not possible, the cell will undergo apoptosis. This suppressor is often mutated in genitourinary cancers. Additionally, Figure 4 shows the possible causes of alterations in TP53 and its responses in the cell cycle.

TP53-induced apoptosis is mediated by Bcl-2 through an intrinsic pathway, and alterations in the regulation of this pathway have direct relevance in the etiology of cancer. This pathway is associated with the activation of transcriptional genes and the inhibition of other genes that block the cascade. On the one hand, TP-53 is dependent on the activation of the Apaf-1/caspase-9 pathway, but on the other hand, Bax (Bcl-2 family) is not essential for TP-53-dependent apoptosis. In addition, different tumor suppressive pathways are associated with TP-53, and some examples are the response to DNA damage, cell senescence and apoptosis, and thus, it is logical to consider that TP-53 is frequently mutated in cancer (59,78).

Methylation of DNA
The covalent modification of the C5 position of the cytosine by a methyl group is mediated by DNA methyltransferase and results in the formation of 5-methylcytosine, which is an epigenetic modification that occurs in vertebrates and is part of normal and essential embryological development. As vertebrates have evolved, CpG dinucleotides have become depleted within the genome, but in some areas, this depletion is not seen; these areas are termed CpG islands and represent approximately 1% of the genome. The epigenetic properties of methylation can affect the genetic activity without alterations in the DNA sequence and represent an alternative means to inactivate the gene apart from a mutation or deletion. The three major pathways that DNA methylation uses to result in genetic alterations include the following: 1. Inherent mutational effects of 5-methylcytosine, 2. Epigenetic effects of the promoter on transcription and 3. Activation and potential induction of a gene due to instability of the chromosome by DNA hypomethylation(59).

DNA methylation and prostate cancer
Glutathione S transferases are a family of enzymes that are responsible for the detoxification of a large group of xenobiotics that catalyze the nucleophilic attack of reduced glutathione in potentially harmful electrophilic compounds. The aberrant methylation of the CpG island at the glutathione S transferase pi (GSTP1) locus is the most frequent somatic alteration reported in PCa (79). This methylation has been detected in up to 90% of PCa and in 70% of Prostatic

Intraepithelial Neoplasia (PIN), however it might be present in normal or hyperplastic tissue (79). Due to oxidative stress, this aberrant methylation leads to the overexpression of GSTP1 in prostatic epithelial columnar cells. These findings are also associated with worse clinical outcomes(80).

The gene of the ras association domain of the familial protein 1 isoform A (RASSF1A) is located on chromosome 3p21. This is a suppressor gene that is methylated in 60-70% of prostate carcinomas, and it has been observed that this alteration is more frequent in high-grade tumors than in less aggressive tumors(81,82). The methylation patterns are not consistent since they can consist of either hypo or hypermethylation, and in addition, the methylation is conserved in all metastases, suggesting an alteration that follows clonal selection(83,84).

DNA damage and repair

Cancer is fundamentally a genetic disease. Alterations in different genes will lead to the activation of different pathways associated with cancer, leading to changes in tumor suppressor genes and oncogenes through epigenetic, mutational and copy number distortions.

To counteract these elements, the cell employs different defense mechanisms including the use of free radicals such as alpha tocopherol, vitamin C, carotenoids, bilirubin and urate and protective enzymes such as superoxide dismutase, glutathione peroxidase and glutathione transferase. In addition to the previously described methylation of GSTP1, associations have been found between the polymorphisms of this gene and the risk of biochemical recurrence in patients with PCa (85).

The cell additionally employs a series of mechanisms termed the DNA damage response (DDR), which involves a number of genes. DDR relies on the replication machinery, as well as on specific mechanisms such as repairs of base cleavage, nucleotide cleavage, double helix rupture and imbalance (59).

Chromosomal abnormalities

Deletions of chromosomal segments are frequently found, although gains and amplifications are seen more frequently in cases of advanced disease(69). These changes have been demonstrated through cytogenetic techniques such as genomic hybridization, fluorescence in situ hybridization or detection of microsatellites. Cytogenetic methods detect numerical changes, while molecular analytic methods identify recombinations that do not lead to changes in copy number(69).

The most frequently altered autosomes are 8, 13, 7, 10, 16, 6 and 17, likely in this order. In addition, gains or amplification of parts of the X chromosome and losses of the Y are also observed. A decreased copy number and loss of heterozygosity of chromosome 8p are also consistent in previous studies (observed in approximately 50% of cases). Specific alterations are observed in each of the chromosomes, but special attention must be paid to the functional impact each alteration may have on the tumor phenotype and the indication or expression of the tumor suppressor genes or oncogenes in the affected regions (69).

Recurrent genetic rearrangements in PCa

Recurrent gene fusions have been identified, primarily between the androgen-regulated gene TMPRSS2 and ERG, which is a member of the ETS (E26 Transforming sequence) family. This fusion occurs in 90% of all fusions that involve ETS genes in prostate cancer(86). The other fusions occur as a result of more complex types of translocations(67,87). In 60% of cases, the fusion occurs due to a deletion of the sequence that separates the two genes (3 Mb).

These rearrangements can be readily identified through reverse transcription polymerase chain reaction (RT-PCR) or by multicolor fluorescence in situ hybridization (FISH).
At present, several clinical studies have evaluated these markers in urine and blood, while other studies have evaluated the expression of the ERG protein using a simpler method (immunostaining)(88–90). The TMPRSS2-ERG fusion status is considered a possible diagnostic marker, although its prognostic significance is still unclear, which is a fundamental part of patient follow-up(91,92).
The TMPRSS2 gene can be merged with other members of the ETS family including ETV1, ETV4 and ETV5(93,94).
Studies have been performed with different technologies including next generation RNA sequencing; in these studies, it was found that some fusions are single events or events that occur in only one patient, which might imply that we actually know very little about PCa (95).

PTEN and PI3K/mTOR
The PI3K/mTOR (mammalian target of rapamycin) pathway plays an important role in cell growth, proliferation and oncogenesis in PCa (96–98). PTEN is a negative regulator of this pathway. In retrospective studies, it has been shown how the loss of PTEN and consequently, the activation of the mTOR pathway lead to a poor prognosis in PCa (63). PTEN deletions have been found in up to 20% of patients with PCa and have been associated with earlier biochemical relapse, metastasis, resistance to castration, presence of ERG gene fusions and the accumulation of nuclear TP53(63).

Association with telomeres
A potential association between telomere length and prostate cancer has been found. Initially, this association was found only in studies with small sample sizes, but subsequently, some studies with larger sample sizes were performed in which associations were observed between a short telomere length and decreased overall survival and increased biochemical recurrence. These findings have been consistent even when adjusted for age, Gleason score and lymph node involvement. It has been proposed that cancer that develops from these areas can lead to greater genotype and phenotype heterogeneity, as well as to an increase in aggressiveness(99). Some studies have even suggested that the risk of death is increased up to 14 times in patients with short telomeres compared with patients with long telomeres(100).

Apoptosis
Apoptosis is an orderly, energy-requiring process in which the cellular content is degraded and condensed into an apoptotic body that is finally digested by neighboring cells or macrophages(101). The positive or negative signals of the apoptotic process finally converge in a family of proteases termed cysteine proteases with aspartic acid specificity. Caspases total at least 13, and some of them are initiators (caspase-8, caspase-9, caspase-10), whereas others are executioners (caspase-3, caspase-6 and caspase-7). Caspases are derived from procaspases, which are larger inactive forms that require proteolytic cleavage in order to become active. They are frequently activated by other caspases (initiator) to generate an activating cascade of executioner caspases. The latter type attacks different anti-apoptotic intracellular proteins such as Bcl-2 and Bcl-XL. They not only destroy their anti-apoptotic functions but also release carboxyl-terminal fragments to remove the cell(102). They also degrade DNA repair and replication proteins such as DNA-PKcs and replication factor C, leading to nuclear dysregulation.

Nuclear proteins such as laminin, NuMa and SAF-A are fragmented and undergo nuclear dissolution and nuclear condensation (milestones in the cell that lead to apoptosis). Proteolysis of cytoskeletal proteins such as keratin and actin also occurs, which leads to the destruction of the integrity of the internal structure. A final step is the breakdown of cell-to-cell interaction proteins such as beta-catenin and focal adhesion kinase, which precipitates the phenotypic and irreversible changes associated with apoptosis(102).

Global defects in apoptosis

In both PCa and PIN, a high level of apoptosis is seen, although compared with other malignancies, PCa has low apoptotic activity along with increased replication. As PCa progresses, it is unclear whether androgen-resistant cells have an increased or decreased apoptosis rate because both have been found in patients with castration-resistant prostate cancer(103). In contrast, an advanced infiltrative tumor whose DNA is mutated and that is fast-growing may have a high rate of apoptosis despite the protective mechanisms that the cell has acquired.

Apoptosis can be initiated by two pathways: the intrinsic and the extrinsic pathways (Figure 5). The intrinsic pathway monitors conditions within the cell and responds to various forms of stress. Pro-apoptotic signals can originate from damaged and unrepaired DNA or from the lack of signals from the cell surface (cell-cell or cell-matrix contacts, including hormones or diminished growth factors).

Mitochondria and the Bcl-2 family are major components of the intrinsic pathway. The Bcl-2 family contains 12 pro-apoptotic proteins including Bax, Bak, Bok, Bik, Bas, Bid and Bim. It also contains six pro-survival proteins including Bcl-2, Bcl-XL, Bcl-W and Mcl1(104).
Each protein in the family responds to different stimuli; however, their primary function is to increase the permeability of the mitochondrial membrane(105). Subsequently, cytochrome c is released from the intermembrane space into the cytoplasm, where it binds to Apaf-1 proteins and forms the apoptosome complex. Caspase-9 is activated, which then activates the entire cascade described above.
In addition, other activations occur such as ones that involve Bid, which is regulated by initiating caspases in the cytosol. Caspase-8 allows the dimerization of Bid with Bax or Bcl-2. This active form of Bax inhibits Bcl-2, which leads to apoptosis. This process can be blocked by anti-apoptotic proteins and by the IAP proteins that inhibit specific caspases(59).

The extrinsic pathway mediates apoptosis after receiving external signals from surface receptors called "death receptors", such as TNFR1 (tumor necrosis factor receptor 1) and Fas receptor. The death receptor domain is located in its intracellular region and allows binding to adapter proteins that also contain a death domain (RIP, TRADD, FADD). Additionally, these proteins have an effector domain that binds to the caspase recruitment domain (CARD) of the initiating caspase(106). Subsequently, the initiator caspase is cleaved and is able to activate the cascade.

The most well-known death receptor is CD95 or Fas, but this receptor does not appear to have a direct effect on the etiology of cancer. In contrast, IGF-1 can activate the PI3K / AKT anti-apoptotic pathway and stimulate the expression of Bcl-like proteins along with Bax suppression. In addition, the expression of IGF binding proteins may also be altered in PCa (69,107,108).

Androgens and prostate cancer

Most treatments for PCa are based on androgenic suppression, but they are rarely curative. To cure the disease, different mechanisms should be considered and are described as follows (Figure 6): 1. Some carcinomas do not express androgen receptor (AR) in some cases because the gene is silenced by a hypermethylated promoter; 2. Several peptide growth factors and cytokines such as fibroblast growth factor 7 (FGF7), epidermal growth factor (EGF) and interleukin-6 (IL-6) can activate the AR synergistically with or independently of a steroid ligand; 3. In some carcinomas, somatic mutations in the AR alter its receptor specificity and cause it to respond to estrogen, progesterone, dehydroepiandrosterone or synthetic anti-androgens; 4. Amplification of the AR gene can occur in up to 30% of cases, even in the presence of depletion, which leads to increased sensitivity to a minimal androgen level; 5. Different coactivating proteins have been identified as mediators of the effects of AR on chromatin structure, as well as transcriptional initiators and their interactions with other signaling pathways. All of the circumstances described above could lead to androgen-independent tumor growth(69).

Expression profiles

With the advent of the analysis of gene expression patterns by cDNA or oligonucleotide microarrays, research related to the diagnosis, prognosis and new therapeutic markers has become increasingly important. For example, it has been found that hepsin is not a good marker since its down-regulation increases tumor heterogeneity in prostate carcinomas(109). Another marker is the P504S protein, which is identical to Alpha-Methyl-Acyl-CoA Racemase (AMACR); the latter is a peroxisomal enzyme that is involved in the metabolism of branched-chain amino acids, which might be useful for the differentiation of hyperplasia and atrophy from cancer(110,111).

Epigenetics / Environmental factors

Lifestyle and dietary habits have been found to be triggers of the oncogenic cascade in PCa. For example, dietary carcinogens, estrogens and oxidants act as a trigger for chronic inflammatory changes within the prostatic tissue and thus act as a promoter of PCa (63,112,113). It has been suggested that the intake of red meat (formation of heterocyclic aromatic amines and polycyclic aromatic hydrocarbon, which have carcinogenic properties) or animal fat is a risk factor for PCa. However, when prevention studies on both of these micronutrients and other elements of the diet were performed, the suppression of those elements was not found to prevent prostate cancer(29). Additionally, sexually transmitted diseases as part of a system that triggers chronic inflammation in epithelial cells have been associated with the development of PCa (Figure 7)(114–116).

Given the change from persistent oxidative stress, a survival response is generated by glutathione S transferase, cyclooxygenase-2 and other mediators. In general, the epigenetic silencing of multiple genes occurs, including the silencing of a fundamental gene, GSTP1, which is found throughout all stages of prostate cancer progression(63).

Conclusions

The natural history of prostate cancer involves numerous genetic and molecular alterations that cause the normal prostatic epithelial cell to become cancerous and resistant to castration. Different biological mechanisms have been associated with the development of prostate cancer, such as alterations in tumor suppressor genes, oncogenes (TP53, RB1, among others) and CDKIs; DNA methylation; chromosomal alterations and rearrangements; changes in PTEN and PI3K / mTOR; global defects in apoptosis; alterations in the AR; and epigenetic mechanisms. These are not the

only mechanisms, but they have been found to be associated with prostate cancer at a higher frequency than others. Similarly, the development of prostate cancer does not have a unique etiology, but rather, it is predominantly multifactorial and can be explained by the different mechanisms described here.

Legends

Figure 1. Natural history of prostate cancer and the molecular alterations involved

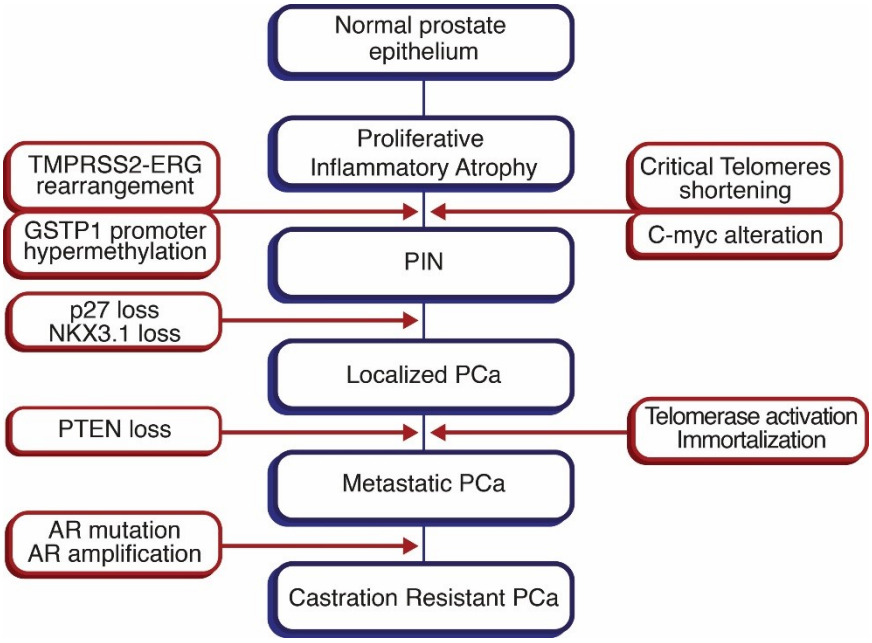


Figure 2. Cell cycle scheme

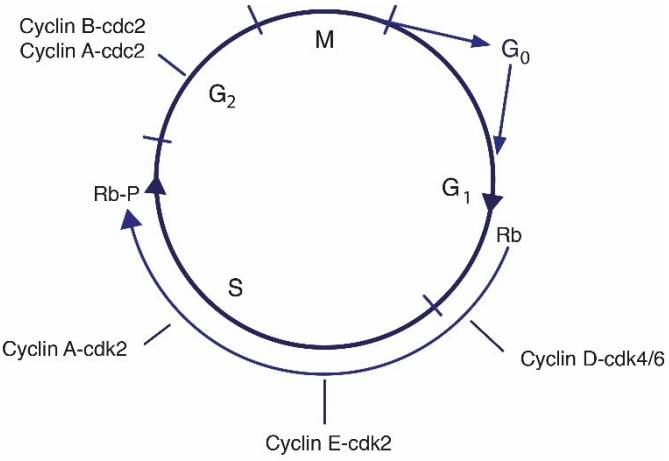


Figure 3. TP53-dependent repair mechanisms

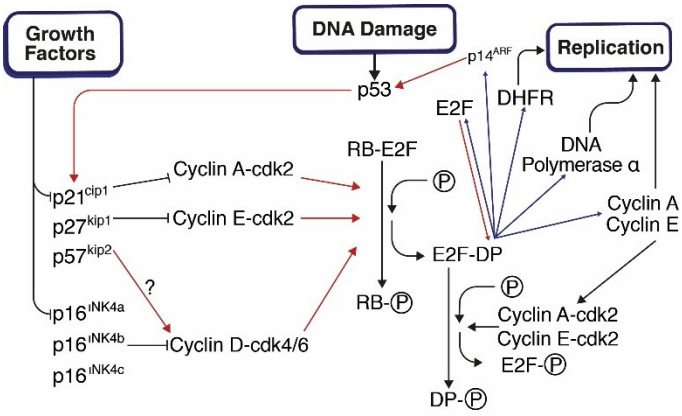


Figure 4. Causes of alterations in TP53 and its response in the cell cycle

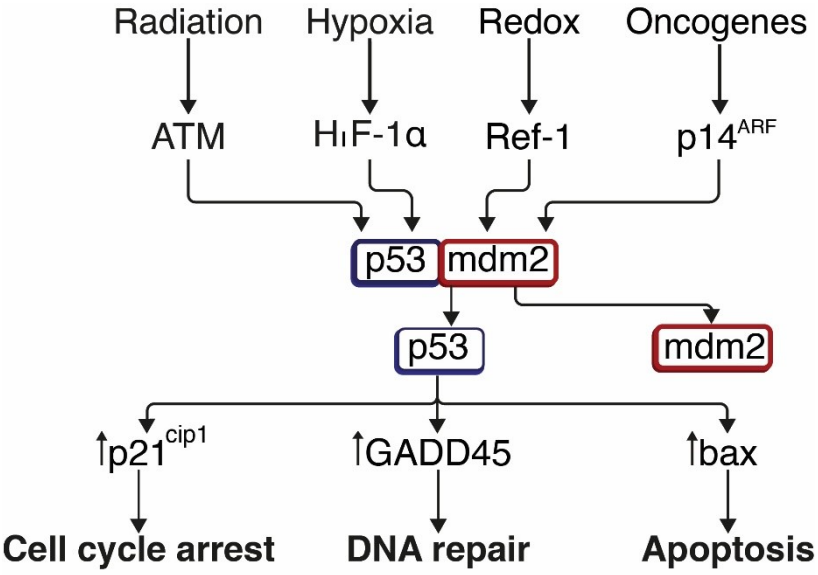


Figure 5. Intrinsic and extrinsic pathways of apoptosis

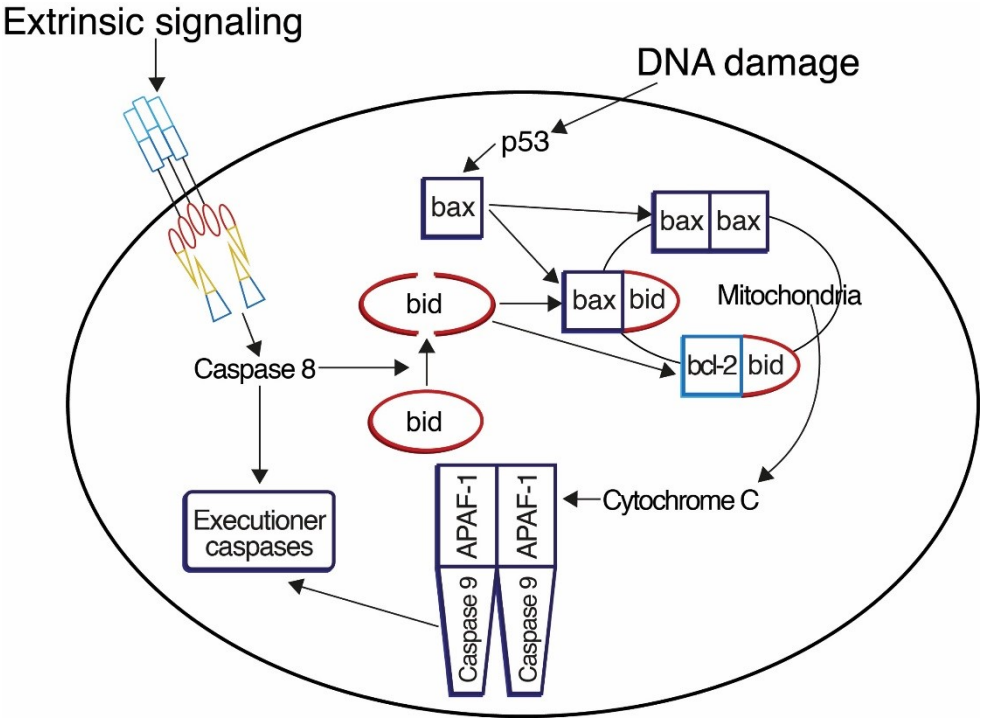


Figure 6. Mechanisms of altered androgen signaling in prostate carcinoma*

*1: The androgen receptor (AR) is absent in some carcinomas. 2: Growth factors activate the AR or a coactivator in a ligand-independent fashion. 3: AR mutations alter ligand specificity and affinity. 4: AR expression is increased by gene amplification. 5: Coactivators are differentially expressed or mutated.

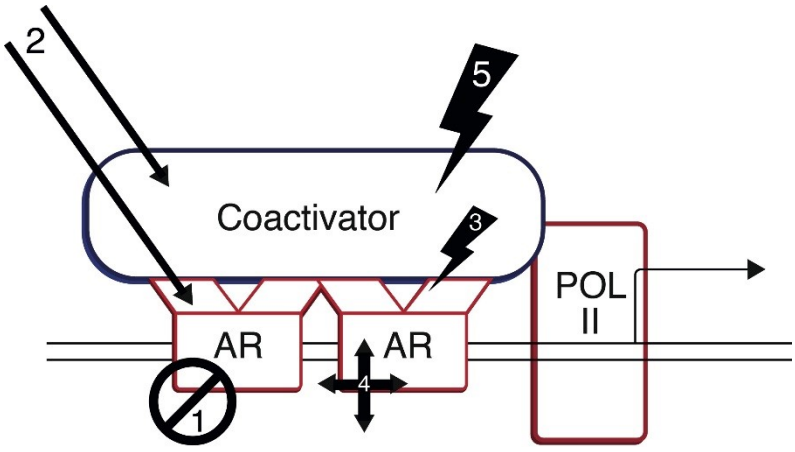
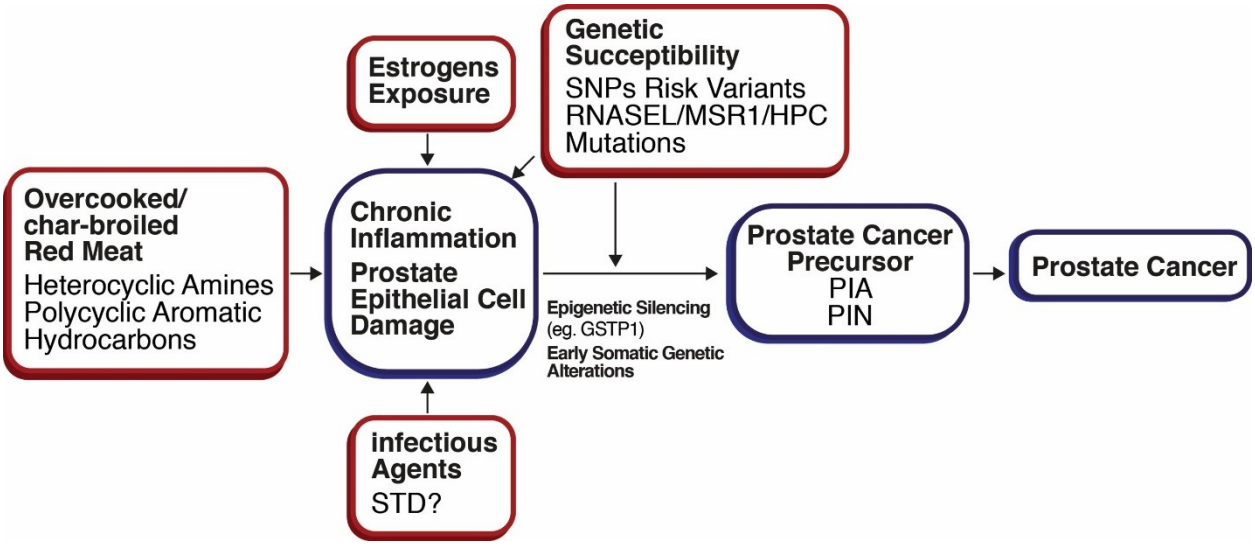


Figure 7. Epigenetic factors associated with prostate cancer



Chapter 3. A general overview of biomarkers for the screening and early diagnosis of prostate cancer

Type of article: Narrative Review

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Introduction

Currently, prostate-specific antigen combined with digital rectal examination are the screening methods mainly used in the clinic to detect prostate cancer, however, these have low diagnostic performance, both individually and as a whole.

The importance of finding new biomarkers, which are more sensitive and specific for screening and early diagnosis of prostate cancer, has been especially reinforced by the PSA failure. The fact that the latter is elevated in a benign condition such as prostatic hyperplasia and also in conditions of malignancy (53), has led to the requesting costly and unnecessary biopsies to patients who did not require it from the beginning (53). As a consequence of this, other techniques and molecules have been explored to make a more specific diagnosis, such as PCA3, microglobulin and mucins, among others.

The aim of the present work was to describe some of the new biomarkers involved in the screening and early diagnosis of prostate cancer.

Epidemiology

Prostate cancer (PCA) is the second most common type of cancer in the world's male population (5). It is estimated that 1 in 7 men will be diagnosed throughout their life with PCA, and 1 in 38 men will die as a result of it. Similarly, in the United States 6 out of 10 men diagnosed with prostate cancer are older than 65 (117). It is the type of cancer that is most often diagnosed as a result of the introduction in 1980 of the Prostate-Specific Antigen (PSA) test as a diagnostic tool (3). It is estimated that by the year 2030 there will be 1.7 million new cases of PC in the world, with an expected mortality of 499,000 cases (29.3%) (7).

During the last decade, there has been a notable decrease in mortality from prostate cancer in developed countries. The GLOBOCAN study reports that the incidence of PC is variable in the global context: Rates are higher in countries such as Australia/New Zealand, North America (ASR) 111.6 and 97.2 per 100,000 respectively) and in Western and Northern Europe, because screening with PSA and subsequent biopsy are performed routinely (118). In countries of North America (United States and Canada) the mortality by PCA has decreased to 4.3% and 3.1% respectively, and in countries such as Denmark, Norway and Sweden (Northern Europe) the mortality rates have declined from the year 2000 to 3.1% per year (6); however, in developing countries, mortality has increased (although there are trends towards increased diagnosis, mortality has increased in countries such as Colombia, 3.4% per year, Costa Rica, 3.4% per year, Chile 2.8% per year and Cuba 5.5% per year) (6,7). With regard to the global context, Colombia has one of the lowest PCA incidences in Latin America and a 28% share of incidence and mortality for it, very close to the

world average of 28.6% and lower than countries like Ecuador (40.41%), Peru (37.74%) and Cuba (46.65%) (6). Finally, in Colombia the mortality rate from prostate cancer has decreased in the last four years (7) and the highest number of reported cases originate in cities such as Bogotá, Valle and Antioquia (The most populated regions with the largest number of Urologists) (7).

Biomarkers in PCA diagnosis

What we had?

Prostatic-Specific Antigen

The history of use of the Prostate-Specific Antigen (PSA) dates from the 1980s, when it was used in the follow-up of patients diagnosed with prostate cancer PCA (119), subsequently, for the year 1994 FDA (US - Food and Drug Administration) approved the use of PSA along with digital rectal examination as screening methods for PCA (47).

The PSA, also called kallikrein III is a glycoprotein of 34kDa produced almost exclusively by the epithelial cells of the prostate gland, which circulates bound to alpha 1-antichymotrypsin and alpha 2-macroglobulin and its duty is to split semenogelin I and II in smaller polypeptides, thus avoiding formation of the seminal clot (43–47). Under normal conditions a small amount, less than 4ng/mL, is released into the bloodstream, but in a neoplastic process these levels rise (47). For this reason, it is considered to perform a prostate biopsy on men with a serum PSA level greater than 4ng / mL (47). However, PSA has also been found elevated in other pathologies such as breast cancer, renal cell carcinoma, ovarian cancer and adrenal neoplasia (48). Similarly, benign prostatic hyperplasia (BPH), prostatitis, cystitis, perineal trauma and recent urinary tract surgery may elevate it (1,47). PSA can be in serum freely (fPSA) or accompanied by protease inhibitors (cPSA) in order to avoid proteolysis. When adding the fPSA and cPSA results in the total serum PSA (tPSA), a large part of this, around 70-90% can be linked to alpha-1-antichymotrypsin, in smaller proportion to alpha-2-macroglobulin, alpha-1 antitrypsin or a protein C inhibitor (46). Consequently, about 10-30% of total PSA (tPSA) circulates freely (fPSA), this free form of PSA is characterized by assuming three specific molecular forms (120). One of them is predominantly in the transition zone of the prostate in patients with BPH and is called BPSA (121), the second representation is called intact PSA (iPSA) and finally there is the proPSA (pPSA), found in its most in the peripheral zone of the prostate gland, which is associated with prostate cancer (122).

According to the American Cancer Society (ACS), the PSA sensitivity for reference values of 4ng / ml and 3ng / ml for cancer diagnosis is 21 and 32% respectively. A specificity of 91% for cut-off values of 4ng / ml and 85% for PSA values of 3ng / ml (49).

In the USA, the study PLCO was performed to evaluate the incidence of ovarian, colorectal, pulmonary and prostate cancer. In the case of PCA, men between 55-74 years were evaluated, who underwent annual screening with PSA for 13 years, as a result it was obtained that screening with PSA does not lead to a decrease in the incidence of PC (RR 1.09 , 95% CI 0.87-1.36) (50). Another large study was the ERSPC (The European Randomized Study of Screening for Prostate Cancer), where PSA was screened for 11 years to men from certain European countries, evaluating the mortality by PC, the results indicated a relative reduction in the rates of Mortality of 21% (RR 0.79, 95% CI 0.68 to 0.91) (51).

A Cochrane meta-analysis conducted in 2011 summarized the results of 5 population experiments with a total of 341,351 participants and showed that screening with PSA is effective for the detection of prostate cancer (RR 1.35, 95% CI 1.06-1.72) , however, this test did not decrease mortality (RR 0.95, 95% CI 0.85-1.07) (52). Another meta-analysis of the year 2010 obtained similar results, aimed to show evidence of the benefits of screening with the prostate-specific antigen, for which results were taken from 6 experiments with a total of 387,286 participants. The results showed that screening with PSA is related to an increased probability of diagnosing prostate cancer (RR 1.46, 95%, CI 1.21-1.77), but as in the previous study, no decrease in PCA mortality was observed. (RR 0.88, 95% CI 0.71- 1.09) (123), so that population screening for prostate cancer is not recommended at present.
This suggests the importance of determining other more specific biomarkers, which lead to a decrease in unnecessary biopsies, as well as an early detection of prostate cancer and thus improve patient survival.

What is more recent?

Prostate Cancer Gen 3 (PCA3)

The PCA3 (Long non-coding RNA prostate cancer associated 3 gene test) has been implicated in a significant number of investigations that reflect the rationale for the study of prostate cancer (124). This is involved in the survival of the cancer cell, this effect is achieved in part by modulating the signaling to the androgen receptor (AR) and exerts its main function in the nucleus and in the microsomal fraction of the cell (125).
Taking this test requires that the process be divided into two large parts; First a prostate massage must be performed and then the urine sample is obtained, this will facilitate the appearance of the biomarker in the urine (125).
The studies have shown promising results, but controversial at the same time for different reasons. They are promising because it has been found that the measurement of this biomarker can differentiate between cancer and chronic prostatitis/benign prostatic hyperplasia (53). In part, the reason why this is possible is related to the high expression of the molecule in cancer tissue 60 to 100 times more than in inflamed tissue but without neoplasia and it should also be noted that this was observed in 95% of patients (124). Christos K et al., (53) mention that this biomarker is useful to differentiate between benign prostatic hyperplasia (BPH) from localized cancer, however, they suggest that the use of the molecule for screening should not be unique, but rather, should be a panel of new biomarkers that provide greater diagnostic precision (124). The use of Beta 2 microglobulin (B2M) and intestinal mucin (MUC3) associated with PCA3 (53) is also mentioned. It was known in this study that the values taken into account to say if the increase of PCA3 is relevant are: 195 DU (with this reference value the diagnostic accuracy is improved up to 77%) (53).
Although the advantages of the use of PCA3 for the diagnosis of prostate cancer have been elucidated and the idea of using it in therapeutic programs as a support has been supported (124); there is a counterpart that discusses the clinical validity of the findings found in the different studies. This controversy is based on the fact that there are few studies that provide a greater knowledge of the biomarker and those that currently exist are criticized that have used small population samples that make the final result less reliable. More studies are needed that include a larger proportion of patients and are followed up over a longer period of time (124). As a result of the above, it has been found that there is a failure to demonstrate that the associations between PCA3 and prostate cancer have any relevance in the patient's prognosis (125). According to this

study, using biomarkers in this way, the predictive value of the test is increased and it is possible to differentiate between benign inflammatory conditions and those that require special follow-up, such as neoplasia (125).

β2 - microglobulin

The b2-microglobulin (B2M) is a low molecular weight polypeptide (11,815 D), synthesized by nucleated cells and complexed with the Major Histocompatibility Complex I (MHC I) (126) (whereby it is assumed that it plays a role in the immune response). Initially identified in the urine of patients with renal tubular disease (127) and later in patients with breast cancer (128) and gastric cancer (129).

It has been reported that B2M stimulates growth and increases the expression of genes coding for osteocalcin (OC) and bone sialoprotein (BSP) in cells of PCA by activating the protein kinase-A enzyme cascade, Cyclic AMP (cAMP) dependent. Thus, when there is an overexpression of B2M in prostate cancer cells, an increase in the coding of genes that will ultimately be responsible for generating metastases is generated, such as OC, SBP, cyclin A, cyclin D1 and vascular endothelial growth (VEGF). These gene pathways suggest that signaling mediated by B2M could be a therapeutic target for the treatment of PCA with bone metastasis (Figure 8) (130–132). Similarly, another molecular pathway in which B2M has been involved is in the interaction with iron homeostasis. When B2M interacts with the protein hemochromatosis (HFE) (which acts as its receptor), modulation occurs in iron homeostasis and subsequent epithelial-mesenchymal transdifferentiation (EMT), which ends up promoting growth, invasion and metastasis to bones and soft tissues (Figure 9) (133).

In studies carried out by Zhang et al, it was demonstrated that the serum B2M marker could be used as a diagnostic marker for PCA and useful in the differentiation between malignant processes and BPH when finding that serum levels of B2M were significantly elevated in patients with PCA, unlike levels of the same marker in patients with BPH or in the control group. However, no marked difference was found between B2M levels between patients with BPH and those in the control group.

Likewise, patients with higher PSA levels (≥ 20 mg / mL) have higher B2M levels and vice versa (134). Therefore, B2M levels are associated with greater clinical aggressiveness (134).

B2M can, therefore, be an efficient biomarker for the clinical diagnosis, follow-up and prognosis of PC and, in turn, a potentially effective therapeutic target to reverse EMT and thus prevent metastatic progression (135).

4Kscore (4 kallicrein assay)

The 4Kscore test, as the name implies, combines the plasma values of four kallikreins used as prostate markers: total PSA (tPSA), free PSA (fPSA), intact PSA (iPSA) and kallikrein -2 (hK2) (136). Most plasma PSA is bound to protease inhibitors and a small amount is found freely (fPSA), this free PSA assumes three molecular forms: iPSA, pro-PSA and BPSA. A lower value of fPSA in proportion to tPSA is more related to PCA, whereas a higher value is associated with benign disease. Kallicrein-2 has been found to be increased in patients with high-grade prostate cancer. Additionally, in the 4K test, data such as the age of the person, digital rectal examination findings (nodules) and previous biopsy history are taken (136).

In different studies: Gothenburg ERSPC (137), ProtecT (138), Rotterdam ERSPC (139), Braun K, et al. (140), Parekh Dj, et al. (141) among others it has been concluded that the use of the 4K test

practically halves the practice of biopsies in patients with PSA in the gray area (4-10ng / ml), since it allows identifying those individuals susceptible to developing cancer clinically relevant to those with non-malignant disease.
In conclusion, the 4K panel allows the prediction of prostate cancer to be individualized even if the patient has not previously been screened or biopsied. This panel decreases around 41-71% of biopsies performed unnecessarily (136). However, there are limitations regarding the availability of the panel in the daily clinical scenario. Currently it is only available in the United States and the cost for patients is around $ 1185 USD (136).

Mucins
Mucins are glycosylated proteins of high molecular weight, present in most epithelial tissues, including the urinary tract and the reproductive system (142).
Currently a total of 21 mucin-encoding genes are known, these have been divided into two groups: MUC1, MUC3 and MUC4 coding for membrane mucins, these being necessary in the cell-cell interaction, while the genes MUC2, MUC5AC, MUC5B and MUC6 code for mucus-secreting mucins (143).
The function of mucins in general is to protect and hydrate the epithelium. However, in neoplastic processes its function has been affected (144).
MUC1 has been the most studied in the development of different cancers, among those prostate cancer, where it has been implicated in angiogenesis, proliferation and transduction of tumor signals, as well as in tumor invasion and metastasis (144), and that diminishes the immune response and promotes tumor propagation to other tissues, as cell-cell and cell-extracellular matrix interactions are lost (142).
In malignant prostatic tissue, through tissue microarrays, an overproduction of MUC1 has been reported, together with an aberrant distribution and alterations in the glycosylation of this mucin (142,144). In addition, in studies conducted by Cozzi et al., Overexpression of MUC1 was demonstrated in 58% of neoplastic prostate tissue samples and in 90% of metastatic lymph nodes, whereas in cancer free tissue it was not evidenced (143).

Other Biomarkers:
Recently there has been an interest in proteins involved with mitochondrial autophagy, as these may be associated with carcinogenesis (11). These proteins can be seen as new diagnostic and prognostic biomarkers in prostate cancer. However, they are not the only new biomarkers on which attention has been focused; it has been seen that by making a genetic profile of the cancer and also by observing the presence of stem cells in the neoplastic tissue, new markers of the disease can be obtained. Here are four of them:

Leucine-rich pentatricopetide repeat motif-containing protein (LRPPRC)
The LRPPRC protein stabilizes the BCL-2 oncogene and blocks autophagy, thus altering the regulatory mechanisms of cell death (11). In the comparison of the immunohistochemistry of LRPPRC expression by Mancini et al, between neoplastic tissue and BPH; it is found that this protein was expressed more in cancer tissue (11). This finding confirms that the LRPPRC can be seen as a new diagnostic biomarker and the mitochondria as a therapeutic target (11).

Genetic profiling of prostate cancer: Circulating tumor DNA (ctDNA)

The analysis of circulating DNA fragments of the tumor supposes the possibility of being able to find these pieces of free DNA in bodily fluids and in the plasma of patients with solid tumors (11). The advantage of these sequences is that they contain the complete genetic information of the tumor; which would allow analyzing it to determine the prognosis and response to treatment (11). In addition to the benefits mentioned above, ctDNA allows non-invasive analysis, since it would not be necessary to obtain a biopsy as such (11). Finally, the ctDNA allows the identification of Novo mutations, which ultimately impacts the course of treatment.

Stem cells

Recent studies have shown the presence of stem cells in the prostate and its possible role in the progression to the disease and the response to treatment (11). What has been observed is that from these cells some neoplasms originate and their presence could constitute a prognostic predictor factor (11). Some biomarkers associated with stem cells could in the future be used as an aid in the approach to personalized therapy of prostate cancer (11).

Alpha methylacyl CoA Racemase

Alpha methylacyl CoA Racemase (AMACR) or also called P504S is a peroxisomal and mitochondrial enzyme necessary for the production of bile acids and the beta-oxidation of branched-chain fatty acids (145). In prostate cancer it is used as a molecular marker, since through DNA microarrays, a high expression of AMACR has been detected in the affected prostate tissue, compared with benign prostatic tissue (146,147).

There are different biomarkers that are currently under investigation to determine their diagnostic performance in patients with prostate cancer. It should be noted that those described are not all biomarkers in development and it is still too early to take these tools because studies with larger sample size, validity and reliability are lacking, which allow us to work with certainty on our male population, however it is clear that the present and future should be focused on the investigation of this type of biomarkers in prostate cancer, as well as in other types of tumors.

Legends:

Figure 8. MHC I (Major Histocompatibility Complex1), B2M (Beta 2 microglobulin), GTP (Guanosine triphosphate), ATP (Adenosine triphosphate), AMPc (Adenosine monophosphate cyclic), PKA (Protein kinase A), DNA (Deoxyribonucleic acid), CA (Cyclin A), C-D1 (Cyclin D1), SBP (Bony Sialoprotein), OC (Osteocalcin), VEGF (Vascular Endothelial Growth Factor).

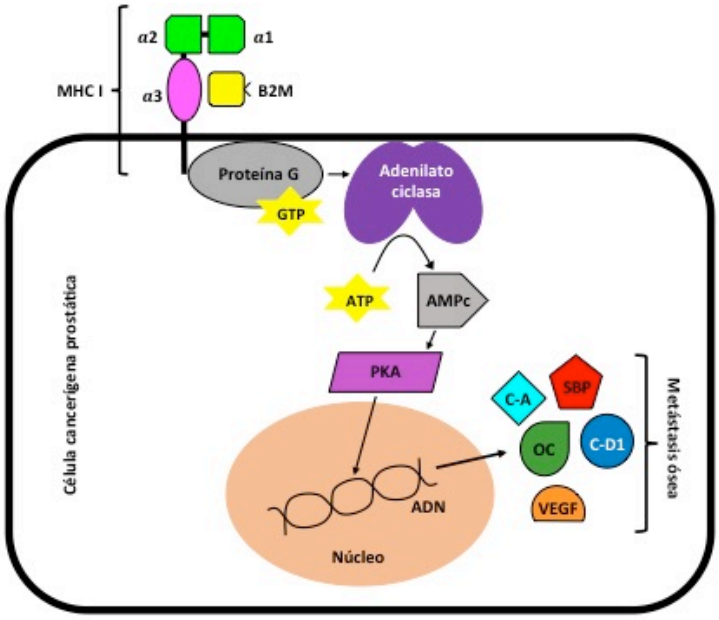
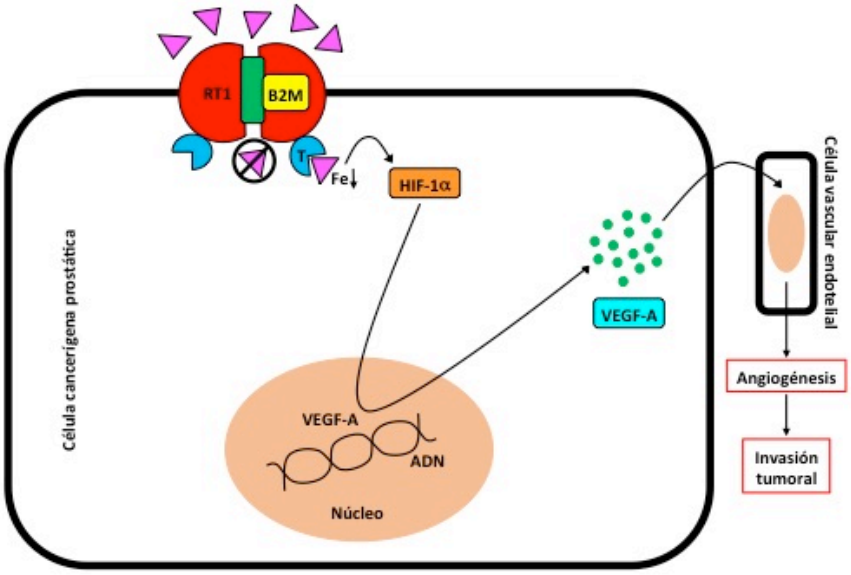


Figure 9. Beta 2 microglobulin (B2M) directly regulates the levels of cellular iron (Fe) by forming complexes with the protein hemochromatosis (HFE), which block the Transferrin receptor 1 (RT1) and prevent the entry of iron into the cell and as a consequence a decrease in its intracellular concentration. This phenomenon promotes the expression of the Hypoxia Inductor Factor 1a (HIF-1a), which will act on the cell nucleus to generate the synthesis of the Vascular Endothelial Growth Factor A (VEGF-A), which will induce the process of angiogenesis in vascular and endothelial cells and, as a consequence, tumor growth, invasion and metastasis. T (Transferrin), DNA (deoxyribonucleic acid).


RT1
B2M
Fe
HIF-1α
Célula cancerígena prostática
VEGF-A
VEGF-A
ADN
Núcleo
Célula vascular endotelial
Angiogénesis
Invasión tumoral

Chapter 4: Metabolic profiling based on Nuclear Magnetic Resonance Spectroscopy and Mass Spectrometry as a tool for clinical application

Type of article: Narrative review

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Introduction

The metabolic state of an organism depends on its genome, transcriptome, proteome, epigenome, microbiome and exposome (environment) among other elements, including some that are still undiscovered. Metabolomics as a tool of study provides an abundance of information with the potential to accurately describe the physiological state of an organism (148). One of the objectives of metabolomics is to identify and characterize small molecules under physiological conditions and identify those that may be important biomarkers for the identification and treatment of health problems (149).
In this review, we will describe a few aspects of metabolomics, the methods used for quantification and some clinical applications, mainly in cancer.

General concepts of metabolomics

The term metabolomics is a rapidly growing field that was defined as the quantitative study of metabolites (molecules smaller than 1,500 kDa) in a biological system and alterations to their concentrations due to environmental or genetic effects. Research has focused on areas such as toxicology, biomedical sciences, nutrition, genetics, innate errors of metabolism, diabetes, cancer, diagnostic tests, neuronal diseases and others. These applications are based on the theory that metabolites are the functional outputs of an organism, and measuring metabolites is a means to identify an alteration in the system's homeostasis that could occur before the appearance of symptoms of a particular disease, as a single metabolite may be the substrate for a number of different enzymes involved in complex metabolic pathways (150,151).
One of the main advantages of applying metabolomics is the ability to detect hundreds of metabolites in parallel (152), thereby efficiently monitoring biochemical alterations.
The metabolic profiles of biological specimens can be affected by factors such as diet, age, ethnicity, lifestyle, medications or microbiota, and these factors should be controlled for to obtain disease-specific information (153).

Types of fluids used in metabolite analysis

Analyses of metabolites can use various fluids including urine, blood plasma, serum, cerebrospinal fluid, saliva and tissues. The former three are the most commonly used bio-fluids in metabolomics studies because they contain thousands of detectable metabolites and are obtained noninvasively

or minimally invasively (154). The use of each fluid type presents processing and analysis challenges and different possible associations with diseases and the effects of drugs, among others.

Urine

The analysis of urine samples offers a number of advantages over the use of other fluids such as blood (serum), plasma, saliva, cerebrospinal fluid and homogenized tissues. Among these advantages are the following: 1. Urine can be collected in large quantities. 2. The sample collection process is not invasive, and samples can be taken repeatedly, sometimes without inconveniencing the organism from which the sample is collected. 3. Urine requires less complex pretreatment steps for analysis because it has low levels of proteins and high concentrations of low molecular weight compounds (152). Thus, urine is a sample with low complexity of analysis and few molecular interactions. For these reasons, urine is the most commonly used fluid in the analysis of metabolites for the early detection of diseases (155,156). However, given its high salt content, MS measurements of urine are slightly more challenging than measurements of other fluids and require pretreatment of the sample (148).

Blood plasma and serum

The use of these fluids for metabolite analysis allows the determination of the presence and stage of several diseases, but these fluids contain a wide range of macromolecules that, when analyzed, can overlap with the results from small molecule metabolites (156).

Relationships can be found between serum and plasma metabolic profiles that can provide an overview of metabolic status at a point in time. In NMR data, this relationship includes narrow signals from small molecule metabolites and broad signals from proteins and lipids (152).

Homogenized tissues

The metabolic profiling of intact tissue has gained interest in recent years; its purpose is to obtain an approximate understanding of the molecular basis of disease and pathway analysis (157). The global determination of metabolite concentrations in anatomical tissues might provide information on unique aspects of the tissue during pathological development, which cannot be derived from measurements taken in other fluids, such as the relationship between metabolism and changes in tissues. Nonetheless, the information derived from analyzing tissue samples is inconvenient to obtain, requiring the use of invasive sampling and sample treatment before analysis and storage and involving potential interactions between compounds (156).

Metabolite analysis techniques

Two of the most important techniques currently used in metabolomics are Nuclear Magnetic Resonance Spectroscopy (NMR) and Mass Spectrometry (MS). NMR requires little to no preparation, is rapid and non-invasive, does not destroy tissue and has highly reproducible results (coefficient of variation 1-2%). Combining NMR with MS might increase the diagnostic yield, but the data obtained from NMR/MS experiments are quite complex, as they provide qualitative and quantitative information on several metabolites, and distinguishing statistically between disease and control markers can be difficult (Figure 10) (152).

We would like to go deeper describing the techniques used for metabolite analysis in the following paragraphs:

Nuclear Magnetic Resonance Spectroscopy (NMR)

At present, single-pulse (1D) and one-dimensional nuclear Overhauser enhancement spectroscopy (NOESY) with water suppression is one of the most widely used methods in metabolomics. This technique is robust, provides a flat baseline under similar conditions and allows pulses to suppress elevated signals from water, leaving metabolite signals intact (158,159). Two additional important methods used to eliminate large signals from large molecules (e.g., tissue and serum) are the Carr-Purcell-Mieboom-Gill (CPMG) sequence and the "edited diffusion" method that allows the observation of large molecules such as lipids (148).
Other methods, such as 2D NMR, have been used in the detection of metabolites to reduce spectrum complexity, although these methods are expensive, time consuming and complex to analyze. These methods include 2D-J Spectroscopy, Correlation Spectroscopy (COSY), Total Correlation Spectroscopy (TOCSY), Heteronuclear Single Quantum Coherence Spectroscopy (HSQC) and Heteronuclear Multiple Bond Correlation (HMBC) (160,161).
HR-MAS-NMR (High-Resolution Magic Angle Spinning NMR) is another technique used with tissues. This technique obtains high-resolution spectra from heterogeneous samples that are neither solids nor pure liquids, such as solvated resins, allowing the *in situ* characterization of organic molecules and the quantification of compounds in the solid support phase (Table 6)(162).

Mass spectrometry
Mass spectrometry (MS) is used with various separation methods, such as gas chromatography (GC), liquid chromatography (LC) and capillary electrophoresis (CE), to provide chemical information for metabolomic studies (Table 6) (163,164).

Liquid chromatography coupled to mass spectrometry (LC-MS or HPLC-MS):
LC-MS is one of the most important techniques used for metabolic analysis, with high sensitivity and the ability to provide a wide range of information on metabolite content (152). This technique separates a sample by liquid chromatography for subsequent analysis by mass spectrometry. LC-MS is widely used to analyze complexed substances, up to non-volatiles and can be used to separate macromolecules such as proteins (165). LC-MS is considered a moderate- to high-performance method, and ultra-high pressure LC (UPLC) increases the chromatographic resolution of this technique 3- to 5-fold (166). Urine can be input directly into an LC system, but serum and other liquids require preparation for protein precipitation (152).

Gas chromatography coupled to mass spectrometry (GC-MS)
GC-MS is usually used to analyze samples of fluids such as urine and for the stable analysis of volatile metabolites including fatty acids, steroids and flavonoids. GC-MS has lower costs of analysis than HPLC-MS, but its use is limited for nonpolar thermally stable metabolites. Thus, it is commonly used to derivatize compounds that produce other volatile compounds. The advantages of GC-MS include its rapid ability to identify metabolites, supported by the commercial availability of extensive libraries (167,168).
The use of different agents in the derivatization of compounds has been studied in human urine samples treated with N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA), N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and Methyl bis-trifluoroacetamide (MBTFA) and analyzed in GC-quadruple MS. BSTFA and MSTFA exhibit similar efficiency with respect to the number of peaks detected, peak intensity and reproducibility. MBTFA combined with BSTFA exhibits better secondary and tertiary amine derivation. Some amino acids were detected when

derivation with BSTFA was followed by the use of MBTFA; however, these results were not reproducible, and BSTFA is recommended as an optimal derivation agent for GC-MS (168).

Combining NMR and MS

NMR has low sensitivity for the identification of metabolites, but compared to MS, it offers the possibility for broad observation and quantification of the most abundant compounds in biological fluids and tissues without the need to prepare or fractionate samples. Additionally, NMR allows the identification of compounds with identical masses and the identification of dynamics that can reveal metabolic pathways and their compartmentalization (148). Combining NMR and MS yields increased identification and quantification of compounds, especially in complexed ones. Additionally, data from both techniques can be cross-analyzed to increase the number of compounds identified by using the principle of a relatively constant abundance/intensity ratio for the same metabolites across different samples (169,170). The combination of NMR and MS also allows the performance of isotope screening experiments and metabolic flow analysis (NMR provides position, and MS provides quantification) (171).

Platforms to establish standards in NMR-based metabolomics

Information on biological molecules associated with NMR spectra is available in databases including HMDB, BMRB, TOCCATA and COLMAR, but information on many metabolites is still needed (169,170,172,173). Repositories of results from metabolomics studies have been generated by the NIH Common Fund Centers (174) and by initiatives to coordinate metabolomics standards, e.g., COSMOS, which currently develops a robust infrastructure that allows the exchange of data or metadata between researchers and the development of applications (http://nmrml.org and http://metabolomexchange.org) (175). Many other important databases (pathway analysis and viewers) are now available for metabolomics analysis, these include: KEGG (http:// www.genome.ad.jp/kegg/), MetaCyc (http://meta cyc.org/), AraCyc (http://www.Arabidopsis.org/ tools/aracyc/), MapMan (http://gabi.rzpd.de/ projects/MapMan/), KaPPA-View (http:// kpv.kazusa.or.jp/kappa-view/), the data model for plant metabolomics experiments ArMet (http:// www.armet.org/), functional genomics databases MetNet (http://metnet.vrac.iastate.edu/) and DOME (http://medicago.vbi.vt.edu). In addition, standards and best practices have been published for the metabolic phenotyping of biological fluids (176,177).

Analysis of metabolites in clinical conditions

The development of a pathology can be identified through different metabolic pathways, but we are only describing three of them: **glycolysis**, in which ATP production is increased, mediated by the increased activity of enzymes such as hexokinase and lactate dehydrogenase and the increased production of lactate induced by the overexpression of hypoxia-inducible factors (HIF-1). Under these conditions, the action of *pyruvate dehydrogenase kinase* 1 is induced, which in turn inhibits *pyruvate dehydrogenase*, thus reducing pyruvate input to the tricarboxylic acid (TCA) cycle and reducing levels of associated metabolites. The **consumption of glutamine** also increases via the action of the glutaminase enzyme. This effect presents a possible pathway for supplementing the induced deficit in TCA under anaerobic conditions. Some studies suggest that this pathway provides the main energy source used under aerobic conditions by proliferating cells such as lymphocytes, fibroblasts and some cancer cells. This pathway is also a precursor of oxaloacetate, which is involved in the synthesis of fatty acids and cholesterol. The alteration of these pathways in turn alters the **metabolism of fatty acids**, which are produced in greater quantities in cancer

cells due to the hyperactivity of enzymes such as ATP citrate lyase (ACL), acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS). The latter is expressed at low levels in normal cells and tissues but is highly expressed in cancerous tissues. Cancerous tissues also exhibit an increase in messenger lipids such as phosphatidylinositol-3,4,5-triphosphate [PI (3,4,5) P3], which is formed by the action of phosphatidylinositol-3-kinase (PI3K) and active protein kinase B/Akt(178). Alterations in these pathways can be used in the detection of metabolites and the preventive diagnosis, treatment, and identification of biomarkers for diseases. Therefore, the study of alterations to the levels of these compounds in tissue, plasma or urine has been used to study various pathologies.
Urological cancer is a condition in which early detection based on biochemical methods is required to offer rapid treatment based on personalized medicine. Metabolomic tools based on NMR and MS have the potential to assist the provision of an early diagnosis and targeted therapy. Various biomarkers for urological cancer based on different pathways of disease in human and animal models are currently being studied.

Bladder cancer:
Metabolomics has been extensively used in the study of cancer, with studies on bladder cancer using LC/MS to compare the urine metabolites of healthy controls and those of patients with bladder cancer and identify differences (179).
Recently Cheng et al (180) pooled 11 studies which described metabolites to detect bladder cancer in a systematic review, with different techniques and high heterogeneity: Gas chromatography-time-of-flight mass spectrometry (Two studies); Liquid chromatography-mass spectrometry / Capillary electrophoresis-mass spectrometry (Three studies); High resolution-magic angle spinning-nuclear magnetic resonance spectroscopy (One study); Proton nuclear magnetic resonance (Three studies); Reversed phase liquid chromatography-mass spectrometry / hydrophilic interaction chromatography-mass spectrometry (One study); High-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (One study). Regarding the altered glucose metabolites expression, they found no conclusion about glucose, fructose and lactic acid since they were found up in some studies and down in others, nevertheless pyruvic acid was found down in only one study (anaerobic oxidation). Similarly, citric acid and fumaric acid were found also down in different studies (aerobic oxidation).
Regarding the altered amino acid metabolites expression, they found that threonine, phenylalanine, valine, isoleucine, lysine, methionine, leucine (Essential amino acids), glutamate, histidine, arginine, aspartic acid, tyrosine, glutamine and serine (non-essential amino acids) were found up. They found some studies regarding the lipid and nucleotide metabolites expression, however they were not enough to pool information and recommend for the diagnosis of bladder cancer.
Another study found that metabolite profiles in urine can discriminate between bladder cancers with and without muscular invasion and between healthy patients and those with bladder cancer and can trace relationships between different metabolic pathways and identify where pathology-associated alterations appear (181).
There are few studies using different samples and analytical platforms with similar results for some metabolites nonetheless, it is important to standardize these two fundamental variables to establish a way to diagnose Bladder Cancer nowadays.

Prostate cancer:

Plasma and tissue samples from prostate cancer (PCa) patients have been evaluated using different methods such as HRMAS and LC/MS, therefore alterations in amino acid levels have been found, such as increased levels of lactate, phospholipids and choline; low levels of citrate and polyamines; and regulation of spermine (182–186). One of the advantages of this marker is that non-invasive methods can be used for the detection of PCa, as it can be measured in urine (187).
Regarding the complete spectrum of metabolites, so far, the most promising biomarkers for PCa diagnosis are: Sarcosine (AUC 0.67)(187), choline, phosphocholines (AUC 0.982) (186), phosphorylcholines, carnitines (AUC 0.97)(188), citrate (AUC 0.89) (189), amino acids (lysine, glutamine and ornithine) (190–193), arachidonoyl amine (AUC 0.86) (188) and lysophospholipids (Steroid hormone biosynthesis pathway and bile acids – Sensitivity and Specificity 92- 94%) (192).
The following five constituents are also important when discriminating between prostate cancer and hyperplasia: dihyroxybutanoic acid, xylonic acid, pyrimdine, xylopyranose and ribofuranoside, with an AUC 0.825 (194). Additionally, citrate, glutamate and taurine have important discriminatory roles, with sensitivity of 100% and specificity of 96% (195).
Regarding the metabolic pathways, many of them have been associated with PCa. The following are the most described in literature:
1) Energy metabolism, including TCA cycle intermediates (195–197), lactate (196,197), citrate (198), phosphoenolypyruvate, and adenosine diphosphate (197);
2) Cell growth and proliferation pathways including: common amino acids (199,200), bile acids (201), polyamines (202), glycerol-3-phosphate (200), long chain fatty acids (198,203,204), phospholipids (200), phosphocholines (205), and choline (202,206);
3) Growth and function pathways and osmoregulation including: Steroid hormones (198,207) and inositol and its isomers (202) respectively;
4) Chronic stress is another important pathway involved in developing cancer, and cortisol (208) is thought to be related by this way (209);
5) Cell proliferation pathway through de novo lipid biosynthesis has participated by different metabolites such as: citrate, inositol, lactate (210), and cortisol (208,209), along with phosphoethanolamine, glycerophosphoethanolamine (211) and acetate (210).
As readers can see, there are many metabolites that might be associated or altered in PCa, therefore, there is still place to study through this molecular method how to diagnose and establish a prognosis in patients with this condition.

Renal cancer:
Kidney Cancer is one of the most important urological cancers since it has high mortality. Research has been focused on the clear cell type since it is the most frequent and different studies have shown results from human and animal samples. Here we present some of the most promising metabolite biomarkers for this kind of tumor according to critical revisions from Rodrigues et al and Ari Hakimi et al (212,213): Regarding the amino acid metabolism, they found that Creatine, glutamate, glutamine and quinolinate were found upregulated mostly in renal tissue, however, 4-Hydroxybenzoate, Gentisate and hippuric acid were found downregulated mainly in urine. Regarding the fatty acid metabolism, they showed that carnitine, acylcarnitines and acetylcarnitines were found upregulated in renal tissue, but Choline/choline-Containing Compounds were found downregulated in the same kind of sample. The glutathione (reduced form) was found upregulated in renal tissue and regarding the glycolysis, glucose was found to be inconclusive in different kind of samples, on the other side, lactate and pyruvate were found

upregulated in different samples. Citrate, fumarate, malate and succinate had inconclusive findings, regarding the tricarboxylic acid metabolism. Additionally, alfa-tocopherol (vitamin E metabolism) was found upregulated in renal tissue. Most of the inconsistencies are because of differences in type, collection, handling and manipulation of the samples; experimental designs and the kind of analytical platforms (212).
The identification of biomarkers has been used in renal cancer patients, allowing differentiation between healthy controls and cancer patients and suggesting the involvement of alterations in the quinolinate pathways (pathways of nicotinate and nicotinamide metabolism), gentisate (benzoate degradation pathway) and alpha-ketoglutarate (alanine, aspartate and glutamine metabolism pathway) in the disease. However, these results have not been validated for use in clinical practice (214,215).

Conclusions

Metabolomic profiling using NMR and/or MS provides an important diagnostic tool for identifying metabolites under different conditions. These tools have been tried for conditions that greatly affect quality of life, such as cancer; in such cases, the fast, valid and reliable identification of biomarkers is required. Different methods involving NMR and/or MS can result in variations in their results; however, the appropriate control of confounding variables and the use of statistical and bioinformatic analyses can offer a wide range of information, useful for clinical applications.

Legends:

Figure 10. Process for obtaining a metabolic profile

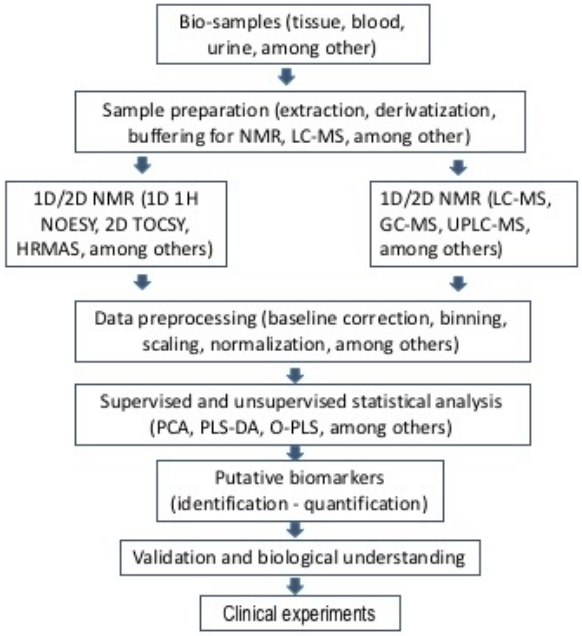

Table 6. NMR and MS Techniques

Technique	Principle	Advantage	Disadvantages
NMR methods			
1D sequences	Detects signals from heavy metabolites (small and large).	High performance screening. Quantitative.	Spectrum complex to analyze. Molecular signals detectable at high and low concentrations.
CPMG (Carr-Purcell-Mieboom-Gill)	Suppresses signals of large molecules and generates those of small molecules.	Easy to interpret. High-performance technique that allows for screening and can be used in HRMAS-NMR studies.	Poor results with urine.
Edited diffusion sequence	Generates information on large molecules (e.g., lipids).	High-performance technique with good results in the screening of large molecules in plasma and serum.	Not used in urine, as there are no appreciable differences in diffusion coefficients.
2D sequences	Simple spectrum. Multiplicity from J-coupling is eliminated.	Easy to interpret and resolve overlapping signals.	Time-intensive and less quantitative than other techniques.
MS Methods			
LC-MS (HPLC/UPLC-MS)	Metabolites are separated by liquid chromatography and detected with Mass Spectrometry.	Simple preparation. The most commonly used technique and high performance.	Suppression of ions in samples with multiple anions and cations may affect results.
GC-MS	Separation is based on gas chromatography.	More sensitive and reproducible than LC-MS. High separation capacity and more quantitative than other techniques.	Requires more preparation than other techniques and cannot detect all compounds. May produce a more complex analysis than other techniques.
2D GC-MS	Separation occurs in two dimensions.	Resolution allows better analysis of complex biological samples.	More sample preparation required. Cannot detect all compounds.
Electrospray Extractive Ionization (EESI-MS)	Direct analysis method using two spray sources: one is nebulized and the other provides drops of charged solvent.	No sample preparation required.	Less quantitative, and the results can be affected by ion suppression.

Desorption Electrospray Ionization (DESI-MS)	Ambient ionization method that directly analyzes a sample.	Sensitive, does not require sample preparation and has a high tolerance for salt.	Less quantitative, and the results can be affected by ion suppression.
Direct Analysis in Real-Time (DART-MS)	Uses hot helium and nitrogen. Generates real-time information and direct analysis.	High-performance, sensitive and does not require sample preparation.	Less quantitative, and the results can be affected by ion suppression.

Chapter 5. Frequency of inherited DNA-repair genes mutations and their variants associated to Prostate Cancer in a Cancer-Free Southwest Colombian population

Type of article: Original article

Authors: Herney Andrés García-Perdomo; Mailyn Bedoya; Adalberto Sanchez

INTRODUCTION

Prostate cancer (PCa) is the most frequent malignant neoplasia diagnosed in men, considering developing countries and the developed ones (excluding skin cancer); besides it is one of the main causes of death (216–218). This condition has a high incidence and prevalence, however it is possible to find substantial variation according to geographic location and some ethnic groups (219), for example: In Asian countries like China, PCa has the lower incidence, while in NorthAmerica, especially for afroamerican people, incidence is too high (220–222). Additionally, having a first-grade relative with PCa, the risk is increased by two and with two relatives, it increases five times the risk (OR 2 y 5 respectivamente)(223).

Another important factor associated is the general classification as sporadic versus familiar PCa (221), being the last one, the most frequently associated with early ages (~ 45 years), in members from the same family. This one has been reported as frequent as 15%, while in the esporadic, the genetic material is damaged by enviromental exposition to different factors, during the lifetime (Epigenetics) and its prevalence could be as high as 80 - 90% (221,224). PCa is known as one the most inherited cancers around the world (Almost 50%), accordingly, it increases the autosomic dominant cancer predisposition (225,226).

Mutations in DNA-repair genes like BRCA2, BRCA1, CHEK2, HOXB13, NBN and a series of single nucleotide polimorfism (SNPs) (223) have been linked to PCa and have been used as biomarkers for PCa in order to predict clinical outcomes in a population (223,227–229). According to this intention, researchers need to continue studying genomic variants in different populations because the clinico-pathological and genomic diversity.

Up to date, there are no studies about genomic diversity regarding the risk for this inherited condition in a southwest population. For consolidating the knowledge about this clinical condition, we need to translate information about mutations from similar predisposing populations to germline cancers to serve as centinels for identifying high-risk families to develop this condition. The previous could serve to diagnose earlier and to perform an intervention according to the individual genotype, which focuses on personalized medicine as an standard way to diagnose and treat nowadays.

The objective was to describe the frequency of Inherited DNA-repair genes and their variants associated to Prostate Cancer in a Cancer-Free Southwest Colombian population.

METHODS

We designed an observational descriptive design which took place from 2014 to 2016 and included people located in Southwest Colombia (Nariño, Cauca, Putumayo y Valle) from all ages.

Sample size
According to the expected frequency for hereditary Prostate Cancer (~ 15%), alfa 15% and an expected error of 5%, the calculated sample size was 162 people and the sampling was by convenience.

DNA extraction
Each patient underwent blood extraction to obtain DNA. All drops were collected in filter paper until dried. These filters were immersed in phosphate buffer along with the DNeasy package from QUIAGEN ® company. Each extraction was quantified and quality verified to continue the sequencing processing.

Sequencing protocol
DNA aliquots from each samples underwent a preparation process with the TruSeq Exome Library Prep®, then the obtained libraries were normalized to be sequenced using the TruSeq Rapid Exome®. These packages were provided by Illumina® from San Diego, California, USA. The normalized fragments with its corresponding adaptors for sequencing were charged in HiSeq2500 machine.
We sequenced the full exome and identified the variants associated, specifically the single nucleotide polymorphisms (SNPs) for twelve genes which have been associated with Prostate Cancer (PCa) (ATM, BRCA1, BRCA2, CHEK2, EPCAM, HOXB13, MLH1, MSH2, MSH6, NBN, PMS2 y TP53)(230).

This project accomplished all ethical international standards. Descriptive statistics were performed in R (231) and the results are shown in frequency tables for each gene and its variants associated. We finally searched the variants in the following public databases: Exome Aggregation Consortium (ExAC) (232), PharmGKB (233), Clinvar (234) and Ensemble (235), in order to look for some pattern to use the variants we found as markers.

RESULTS

Frequency of genes

We obtained samples from 162 people from Southwest Colombia. 9203 genes associated with PCa along with 7.315.466 data were sequenced in this population. The most frequent genes found were ATM 1221 data (13.2%), BRCA1 1178 data (12.8%), BRCA2 1484 data (16.12%) and NBN 965 (10.42%) (Figure 11).

Associated variants

The missense and stop variants associated with these 12 genes were present in 22% (2043 data) and 0.13% (12 data) (Table 7). On the other side, ATM, BRCA1, BRCA2 and PSM2 were the most frequently associated with missense variants (247, 445, 490 and 289 data respectively). Additionally, BRCA2 and MLH1 had the highest frequency of stop variants (4 and 3 data respectively) (Table 8).

SNPs associated with Prostate Cancer

The most common SNPs in these 12 genes were the following:
BRCA2 (rs169547 (158 data; 97%), rs206075 (158 data; 97%), rs206076 (158 data; 97%)); ATM (rs659243 (158 data; 97%), rs228589 (134 data; 82%)); TP53 (rs1625895 (157 data; 96%), rs1042522 (141 data; 87%), rs1642785 (145 data; 89%)); PMS2 (rs2228006 (144 data; 88%), rs1805319 (142 data; 87%)); NBN (rs709816 (137 data; 84%)) and MSH6 (rs3136367 (136 data; 83%)) (Table 9).

When comparing with bioinformatics databases, we found that rs169547 (BRCA2), rs 9534262 (BRCA2), rs659243 (ATM), rs228589 (ATM), rs1042522 (TP53) and rs2228006 (PMS2) had the higher Latino Allele Frequency found in ExAC. Rs1042522 (MAF 0.46), rs228589 (MAF 0.46) and rs9534262 (MAF 0.47) coincided with higher Minor Allele Frequency (MAF) found in Ensemble. Additionally, rs799917 (MAF 0.46) and rs799905 (MAF 0.45) had high MAF although they were not found on ExAC (Table 9).

DISCUSSION

Race, diet, family history, environmental factors and hereditary components have been reported as risk factors for PCa (219–222). Regarding the latter, genes such as BRCA1, BRCA 2, MSH2, HOXB13, ATM, CHEK2 and NBN have been proposed as important candidates contributing to PCa (223,226–229). The existence of single nucleotide polymorphisms (SNPs) for these genes, further increase the risk and even could be used as a prognostic biomarker for this condition (236). Additionally, observing a germline mutation in some DNA-repair gene provides information to patients to look for counseling, identify predisposition to cancer and perhaps to initiate interventions to reduce the risk (226).

Regarding the frequency of these genes, Pritchard et al recently published a paper in patients with metastatic PCa from different series (United Kingdom and United States)(226), however it is important to compare some of the results: The most frequent gene was BRCA2 (5.35%) followed by CHEK2 (1.87%) and the third one was ATM (1.59%). BRCA 1 was present in 0.87% of the samples, while genes like MSH2, NBN, MSH2, MSH6 and PMS2 had lower frequency (0.14%, 0.29%, 0.14, 0.14 and 0.29 respectively). HOXB13 was not assessed in these series. These results are lower than ours, and we might expect to have a higher frequency, coming from metastatic patients data. PCa incidence, at least in Cali, Colombia, has been stable during the last few years (2002 to 2007 – Annual Percent Change APC -0.5%), mortality diminished (12), and afro-descendants population increased from 15% in 1964 to 26% in 2005 (237) however, these data do not explain the results since there are not previous studies that described the frequency of these genes and their variants.

Pilié et al (238) published another important study to discuss, this one was not about frequency of genes, but identifying genes associated with PCa and other concomitant tumors (at least one additional tumor). They also found similar germline genes: BRCA2, ataxia telangiectasia mutated (ATM), mutL homolog 1 (MLH1), BRCA1, checkpoint kinase 2 (CHEK2) and homeobox protein

Hox-B13 (HOXB13), although they found 10% of pathogenic or likely pathogenic mutations in cancer-predisposing genes. They found 525 missense, 7 frameshit, 5 in-frame coding insertion or deletions and 2 nonsense variants (rs80359440, rs80359515, rs61757642, rs587781658, rs373226793, rs180177143, rs759113408, rs17879961 and rs138213197). On the contrary, we found a higher frequency of this type of variants and none of the SNPs they reported.

In our study, -identified as the first study in southwestern Colombia-, the most frequently gene found was BRCA2, which has been considered one of the few genes that confers a high risk of suffering from this and other conditions (218,221,239,240).
This gene has an important role in the repair of double-strand DNA breaks that function by regulating the intracellular transport and activity of RAD51, a critical protein in homologous recombination (241–243). The most frequent variants found for this gene, such as: rs169547, rs206075, rs206076 and rs9534262, are similar with other studies (244,245). These have been considered benign mutations (246–248) -the first three, were reported in Colombia by Arias et al (249)-, and rs9534262 was considered a deletion-type mutation with an unknown clinical relevance according to D´Argenio et al (247), although benign according to ClinVar. This one seems to be a QTL of significant importance since it regulates the levels of the alternative transcripts of BRCA2 (250).

The second most frequent gene was ATM, which exerts control in cell division. When detecting a damage in the genetic material, it leads to either cell cycle arrest, DNA repair or apoptosis (251–253). In addition, it can be considered as the main transducer in the double-strand rupture repair process, where it recruits and cooperates with other sensor proteins such as 53BP1 (p53 binding protein) and BRCA1.
BRCA1 is the third most frequently gene found and associated with an increased risk of sporadic PCa (3.5 times), while for germline mutations in this gene only 0.44% of the cases have been observed of PCa (220,254,255). The Breast Cancer Linkage Consortium (BCLC) described that men aged 65 with mutations in BRCA1 report an increase in the risk of PCa with a relative risk (RR) of 1.82, being linked to a series of cellular processes such as response and repair of DNA damage (220,253,255,256), transcriptional regulation and chromatin modeling. This is the key in cellular control systems to be involved in all phases of the cycle, in such a way that it can block cell proliferation and promote apoptosis of cells with a high risk of malignant transformation (220,255). For this gene, the most frequent variant was rs799917, a nonsense mutation-type polymorphism (247) but benign according to Arias et al (249). This one is located in the coding sequence of the gene and affects the interaction of miR-638 with mRNA of BRCA1 (257). It is also associated with the risk of suffering from breast, stomach and esophagus cancer additionally (257–259). These two genes have not been reported in Colombia, according to literature review.

Another important gene found in this study was NBN, which is a component of the protein complex hMRE11 / hRad50 / NBN that participates in the initiation of a response to DNA damage and is linked to the repair of double-strand rupture (260–262). This acts in the non-homologous junction pathway as a DNA damage sensor and in the homologous recombination pathway, participating in DNA repair and in the cell cycle check point in the S phase (262). rs709816 is one of its most frequent variant, which has been associated with bladder cancer (262); another one was rs3736639, which has been found at a slightly higher frequency in patients with leukemia (260).

On the other hand, the rs1805794 presents positive association in different types of cancer such as bladder (263,264), lung (265), breast, nasopharyngeal (266–268), osteosarcoma (269) and prostate cancers. In this last one, GG carriers have almost twice increased risk for developing PCa, suggesting a role for NBN in the diagnosis and progression of prostate cancer (260). rs1061302 has been associated with an increased risk for lung, larynx and liver cancer. SNPs might affect the proper binding of the complex and thus alter its ability to repair or detect DNA breaks (262,265). Although this variant is considered a synonym polymorphism, the altered nucleotide may affect the stability of the mRNA, the splicing or the transduction rate (262).

Additionally, rs169547 (BRCA2), rs9534262 (BRCA2), rs659243 (ATM), rs228589 (ATM), rs1042522 (TP53) and rs2228006 (PMS2) had the higher Latino Allele Frequency and some of them showed coincidence with a higher MAF. This would be the first description of these kind of SNPs for Colombia population which leads to studying the way to work as a network with them as biomarkers for PCa.

Strengths and limitations

This is the first study performed in southwest Colombian population, which is extremely important to characterize our population and beginning to recognize the risk for developing PCa. The quality of the samples, the methods we followed and the quality and analysis performed with the data are another important issues to highlight from our study.

Regarding the limitations, we could declare that most of the variants found in the present study are considered as benign, nonetheless it is important to go deeper to analyze longitudinally these data and obtaining the real risk in this population. Perhaps another important limitation could be that the extrapolation of these data is limited to people with similar characteristics in southwest Colombia.

Conclusions

To conclude, BRCA2, ATM, BRCA1 and NBN DNA-repair genes were the most frequently found in this Southwest Colombian Population. Additionally, there were non-pathological variants like rs169547, rs206075, rs206076 and rs9534262 and some other variants like rs799917, rs3736639, rs1061302 and rs1805794 associated to NBN gene, which is highly linked to PCa.

Legends

Figure 11. Frequency of genes associated with Prostate Cancer

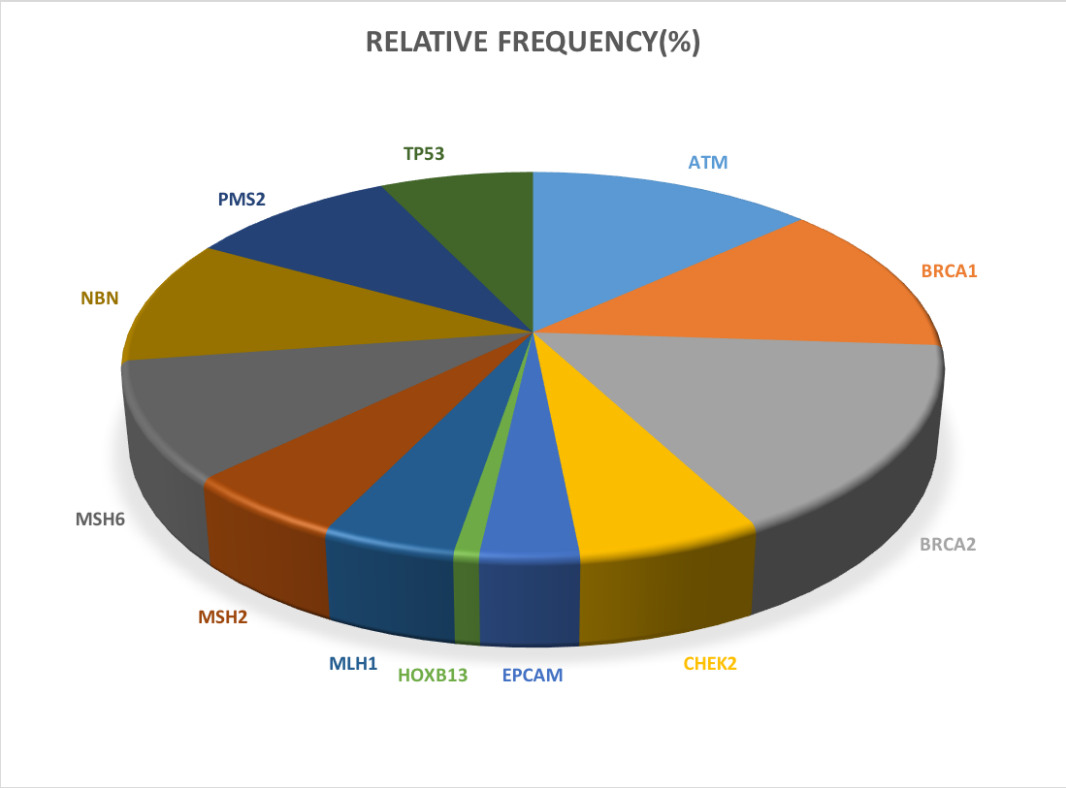

Table 7. Frequency of variants associated with PCa

VARIANT	Absolute Frequency	%Relative Frequency
Downstream	769	8.347
Frameshift	5	0.054
Intron	3906	42.397
Missense	2043	22.175
Nc_transcript	15	0.163
Splice	4	0.043
Stop	12	0.13
Synonymous	1727	18.745
Upstream	479	5.199
UTR	253	2.746

Table 8. Frequency of variants by each gene associated with PCa

GENE	VARIANT	FREQUENCY	% by each gene	% by total variant
ATM	Missense	247	0.202	0.027
	Stop	1	0.001	0
BRCA1	Missense	445	0.378	0.048
	Stop	1	0.001	0
	Frameshift	4	0.003	0
BRCA2	Missense	490	0.33	0.053
	Stop	4	0.003	0
CHEK2	Missense	33	0.057	0.004
EPCAM	Missense	27	0.088	0.003
HOXB13	Missense	2	0.025	0
MLH1	Missense	67	0.16	0.007
	Stop	3	0.007	0
MSH2	Missense	13	0.025	0.001
	Stop	1	0.002	0
MSH6	Missense	70	0.077	0.008
	Stop	1	0.001	0
NBN	Missense	119	0.123	0.013
PMS2	Missense	289	0.323	0.031
	Stop	1	0.001	0
	Frameshift	1	0.001	0
TP53	Missense	240	0.365	0.026

Table 9. SNP by GENE (Only included SNPs with more than 100 data)

GENE	SNP	Frequency	% by population	% by gene	% by snp	Variant	Exome Aggregation Consortium			Clin var	Ensemble		
							Type	Total Allele frequency	Latino Allele Frequency	Type	MAF	Highest MAF (1000 genomes)	Type

BRCA2	rs169547	158	97.53	0.118	0.02	T/C	Missense	0.9937	0.997	Benign	0.02	0.12	Missense
	rs206075	158	97.53	0.118	0.02	A/G	Synonymous	0.9931	NA	NA	0.03	0.13	Synonymous
	rs206076	158	97.53	0.118	0.02	G/C	Missense	0.993	NA	NA	0.03	0.13	Synonymous
	rs9534262	127	78.4	0.095	0.016	T/C	Benign	0.5208	0.51	Benign	0.47	0.5	Splice Region variant
ATM	rs659243	158	97.53	0.167	0.02	A/G	Missense	1	1	NA	0	0	Missense
	rs228589	134	82.72	0.141	0.017	A/T	Intron	0.572	0.6499	NA	0.46	0.49	Intron
	rs148973142,rs3092850,rs3218681,rs4987984	123	75.93	0.13	0.015	NA	NA	NA	NA	NA	NA	NA	NA
	rs2066734	121	74.69	0.128	0.015	TAA/T	Intron	0.4253	0.5695	NA	0.38	0.5	Intron
TP53	rs1625895	157	96.91	0.244	0.02	T/C	Non coding transcript exon	0.8613	NA	NA	0.17	0.35	Non coding transcript exon variant
	rs1642785	145	89.51	0.226	0.018	G/C	Non coding transcript exon	0.6689	NA	NA	0.42	0.5	5 prime UTR varian
	rs1042522	141	87.04	0.219	0.018	G/C	Missense	0.66	0.7115	NA	0.46	0.5	Missense
PMS2	rs2228006	144	88.89	0.172	0.018	T/C	Missense	0.8514	0.6563	Benign	0.12	0.33	Missense
	rs1805319	142	87.65	0.17	0.018	G/C	Synonymous	0.8109	NA	NA	0.17	0.35	Synonymous
	rs1805321	101	62.35	0.121	0.013	G/A	Missense	0.3854	0.3883	Benign	0.36	0.49	Missense
NBN	rs709816	137	84.57	0.147	0.017	A/G	Synonymous	0.4676	0.5891	Benign	0.39	0.49	Synonymous
	rs2308962	108	66.67	0.116	0.013	T/C	Splice region	0.3529	0.3481	Benign	0.38	0.5	Splice Region variant
	rs3736639	108	66.67	0.116	0.013	T/A	Intron	0.3535	0.348	Benign	0.38	0.5	Intron
	rs1805794	106	65.43	0.114	0.013	C/G	Missense	0.3453	0.3451	Benign	0.36	0.5	Missense
	rs1063045	105	64.81	0.113	0.013	C/T	Synonymous	0.3527	0.3477	Benign	0.38	0.5	Synonymous
	rs1061302	104	64.2	0.111	0.013	T/C	Synonymous	0.3451	0.3452	Benign	0.35	0.5	Synonymous
	rs2234744	103	63.58	0.11	0.013	G/A	Intron	0.3446	0.3447	Benign	0.35	0.5	Intron
MSH6	rs3136367	136	83.95	0.186	0.017	C/G	Intron	0.7426	0.6172	Benign	0.19	0.4	Intron
	rs2234731	122	75.31	0.166	0.015	NA	NA	NA	NA	NA	0.24	0.47	Intron
	rs2020911	108	66.67	0.147	0.013	A/T	Intron	0.4043	0.5252	Benign	0.4	0.5	Intron
EPCAM	rs1126497	123	75.93	0.469	0.015	T/C	Missense	0.5198	0.4629	Benign	0.33	0.5	Missense

CHEK2	rs5762757	121	74.69	0.303	0.015	A/C	Intron	0.5939	0.5648	NA	0.42	0.49	Intron
	rs5762756	113	69.75	0.283	0.014	NA	NA	NA	NA	NA	0.43	0.48	Intron
BRCA1	rs799917	113	69.75	0.105	0.014	G/A	Missense	0.41	0.3435	NA	0.46	0.5	Missense
	rs799905	112	69.14	0.104	0.014	G/C	Intron	0.4805	0.4468	Benign	0.45	0.5	5 prime UTR variant
MSH2	rs2303426	102	62.96	0.236	0.013	C/G	Intron	0.5047	NA	NA	0.37	0.5	Intron

Chapter 6: Association between TMPRSS2:ERG fusion gene and the Prostate Cancer: Systematic Review and Meta-analysis

Type of article: Original article – Systematic review and meta-analysis

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Introduction

Prostate Cancer (PCa) is a heterogeneous disease with a variable natural history and slow growth pattern. It may display latency periods of up to 20 years in which it remains organ-confined. Although prostate cancer lesions can remain localized for long periods, more aggressive forms might occur and when metastasis occurs, lymph nodes and bones are affected predominantly with detrimental results(270). PCa is the second most commonly diagnosed worldwide cancer among men (mainly >65 years). It is a public health concern in developed countries, in which elderly men correspond to the greatest proportion of general population(9).

Patients at high risk and susceptibility to develop PCa, require screening over time. Age, race and family history are the most important risk factors(271). A 50% higher risk in monozygotic twins than in dizygotic twins and the higher incidence in African Americans (and lower rate in Americans of Asian ancestry) supports genetic factors as an important determinant of the variation risk at the population level (272). There has been concerns about the diagnosis and early treatment of this disease due to the absence of specific markers(273). Until now, the gold standard for the diagnosis of PCa is an invasive procedure consisting in the histopathological evaluation of the prostate, a procedure with significant morbidity(92).

Currently, the use of prostate-specific antigen (PSA) as a screening and monitoring marker for prostate cancer is widespread and is currently the only widely used serum biomarker for PCa(274), although there is still debate about the screening for PCa among men in the general population. This biomarker is prostate-specific but it has a low specificity and could also increase unnecessary biopsies, without lowering mortality(1).

TMPRSS2:ERG has been assessed as a specific biomarker for PCa, since 2005 (275). This transmembrane protease serine 2 (TMPRSS2) is a promising biomarker located at 21q22.2 and expressed in normal and malignant prostatic epithelium. Additionally, ERG is a member of the E-twenty six family members (ETS), which are key regulators of differentiation, apoptosis, embryonic development, cell proliferation and inflammation. Currently, there are different studies trying to look for the association between this gene, the fusion and the advanced prostate cancer, but less research for this gene as a diagnostic tool(276).

The primary aim of this study was to identify the association between the TMPRSS2:ERG fusion gene, their variants and the onset of localized prostate cancer.

Methods

We performed this review according to the recommendations of the Cochrane Collaboration (1) and following the PRISMA Statement (2). The PROSPERO registration number is CRD42018087071.

Eligibility criteria
We included RCTs, Cohort, case-control and cross sectional studies that involved patients >18 years-old assessing the association between TMPRSS2 fusion gene, its SNPs and PCa. Studies from Molecular biology, translational and clinical research and those that compare men with and those without prostate cancer, were also included. We excluded Observational Descriptive studies, studies with no human subjects and advanced prostate cancer. There was not setting or language restrictions.

TMPRSS2 and associated SNPs
We previously performed a search in Emsemble.org with the Prostate adenocarcinoma as Keyword, and then we Identified associated GENES and chose TMPRSS2 variants in humans. We applied the chosen gene in Genecards.org and identified the associated phenotype, additionally identified superpathways for PCa in KEGG and performed a final search in UCSC Genome Browser on Human Dec. 2013 (GRCh38/hg38) assembly. After all, we found the following SNPs that could be a missense variant when transcript into a protein: rs572530227, rs537370123, rs570454392, rs545726689, rs185312677, rs540987630, rs546335233, rs561063944, rs147233451, rs544474510, rs565237319, rs181414852, rs577684898, rs547544037, rs530689404, rs12329760, rs75603675.

Primary outcome: Prostate Cancer defined by histology of the tumor coming from TRUS guided biopsy, TURP or radical prostatectomy

Information sources
Literature search was conducted in accordance to recommended by Cochrane. We used medical subject headings (MeSh), Emtree language, Decs and text words related.
We searched MEDLINE (OVID), EMBASE, LILACS and the Cochrane Central Register of Controlled Trials (CENTRAL) from inception to February 2018 (Appendix 1). To ensure literature saturation, we scanned references from relevant articles identified through the search, conferences, thesis databases, Open Grey, Google scholar and clinicaltrials.gov, among others. We tried to contact authors by e-mail in case of missing information.
Additionally, we looked information in the following specific databases: dbSNP, GeneSNP, Polyphen, Human Genome Database, Ensemble, among others.

Data collection
Two researchers reviewed each reference by title and abstract. Then scanned full-texts of relevant studies, apply pre-specified inclusion and exclusion criteria and extracted the data. Disagreements were resolved by consensus and where disagreement could not be solved, a third reviewer dissolved conflict.
Two trained reviewers using a standardized form independently extracted the following information from each article: study design, geographic location, authors names, title, objectives,

inclusion and exclusion criteria, number of patients included, losses to follow up, timing, definitions of outcomes, outcomes and association measures and funding source.

Risk of bias
The assessment of the risk of bias for each study was use QUADAS2 tool as recommended by Cochrane

Data analysis / Synthesis of results
The statistical analysis was performed by using Stata 14® and Review Manager 5.3 (RevMan® 5.3). For categorical outcomes we reported information about Odds Ratio (OR), with 95% confidence intervals according to the type of variables and we pooled the information with a random effect meta-analysis according to the heterogeneity expected. The results reported in forest plots of the estimated effects of the included studies with a 95% confidence interval (95% CI). Heterogeneity was evaluated by using the I^2 test. For the interpretation, it was determined that the values of 25%, 50%, and 75% in the I^2 test correspond to low, medium, and high levels of heterogeneity, respectively.

Publication bias
An evaluation was conduct to identify reporting or publication bias using the funnel plot.

Sensitivity analysis
We performed sensitivity analysis extracting weighted studies and running the estimated effect to find differences.

Subgroup analysis
We performed subgroup analysis by: Geographical setting, Sample and Technique.

Results
Study selection.
We found 241 records with the search strategies. After duplicates were removed, there were 223 records. Finally, 18 studies were included in qualitative analysis and 15 studies in Meta-analysis (Albadine 2009; Chan 2013; Dimitriadis 2013; Huang 2011; Laxman 2008; Leyten 2012; Lin 2013; Maekawa 2014; Mosquera 2009; Nguyen 2011; Park 2014; Penney 2016; Robert 2013; Salami 2013; Sanda 2015;Cornu 2013; Tomlins 2011; Tavukcu 2013) (92,273–275,277–290)(Figure 12).
Included studies.
A total of 1057 patients were included, with a median of 25,5 patients per study. All fifteen studies evaluated new biomarkers in different samples for early diagnostic of PCa (Table 10).
Albadine 2009; Dimitriadis 2013; Huang 2011; Laxman 2008; Lin 2013; Mosquera 2009; Park 2014; Penney 2016; Salami 2013; Sanda 2015 performed their analysis based on data from United States (USA) (273–275,279,281,285–287,289,290). On the other side, Chan 2013 and Nguyen 2011 brought data from Canada (278,288). Netherlands was the based for the analysis of Leyten 2012 and Robert 2013(92,280) and Maekawa 2014 was the only one coming from Japan (277).

Regarding the samples: Albadine 2009; Huang 2011; Mosquera 2009; Penney 2016; Robert 2013 and Park 2014 performed their analysis based on tissue samples (275,280,281,285–287). On the

other side, Chan 2013; Dimitriadis 2013; Laxman 2008; Leyten 2012; Lin 2013; Nguyen 2011; Salami 2013; Sanda 2015 were based on urine (92,273,274,278,279,288–290). Only Maekawa 2014 used blood samples for their analysis (277).
Regarding the molecular templates: Huang 2011; Penney 2016; and Park 2014 performed their analysis based on protein expression (275,281,286). On the other side, Chan 2013; Laxman 2008; Leyten 2012; Lin 2013; Nguyen 2011; Salami 2013 and Sanda 2015 were based on techniques related to RNA (92,274,278,279,288–290) and Albadine 2009; Dimitriadis 2013; Maekawa 2014; Mosquera 2009; Robert 2013 based their analysis on techniques related to DNA (273,277,280,285,287)

Regarding Genetic polymorphisms: Cornu 2013 evaluated two single nucleotide polymorphisms (SNPs) at 8q24 locus (rs1447295 and rs6983267) in all patients. These two SNPs correlated to the biopsy outcome in clinical practice (282). Additionally, Penney 2016 evaluated six SNPs, comparing ERG+ to ERG- cancers. They found four significantly associated with ERG+ compared to controls (rs7679673, rs902774, rs11672691, rs1859962). Three were significantly associated with ERG- compared to controls (rs2660753, rs7629490, rs1016343), and the associations trending in opposite directions for ERG+ and ERG- for three of them (rs12653946, rs1512268, rs11704416) (281).
Furthermore, Maekawa et al showed that the rs12329760 polymorphism was significantly associated with the risk of sporadic prostate cancer in Japanese men (277).

Risk of bias within and across the studies

All included studies had no applicability concerns and low risk of bias for flow and timing (92,273–275,277–281,285–290). Regarding Patient selection, index test and reference standard risk of bias, we found that Chan 2013 and Leyten 2012 had low risk, however, Albadine 2009, Huang 2011, Maekawa 2014 and Nguyen 2011 had unclear risk of bias since they used case-controlled studies to perform their analysis and did not describe the blinding assessment for index and reference tests (Figure 13).
Dimitriadis 2013, Laxman 2008, Lin 2013, Mosquera 2009, Park 2014, Penney 2016; Robert 2013, Salami 2013 and Sanda 2015 had unclear risk of bias for index and reference test since they did not describe the blinding assessment appropriately (Figure 13).

TMPRSS2:ERG and Prostate Cancer

15 studies assessed the association between TMPRSS2:ERG and Prostate Cancer (Albadine 2009; Chan 2013; Dimitriadis 2013; Huang 2011; Laxman 2008; Leyten 2012; Lin 2013; Maekawa 2014; Mosquera 2009; Nguyen 2011; Park 2014; Penney 2016; Robert 2013; Salami 2013; Sanda 2015) (92,273–275,277–281,285–290). We found an OR 2.24 95%CI (1.29 to 3.91) I^2=91%, showing a significant association but a high heterogeneity (Figure 14).

Publication bias

We did not identify reporting or publication bias using the Begg´s and Egger´s tests (p=0.631 and p=0.716 respectively).

Sensitivity analysis

We did not find any differences on Effect Estimate (OR) when we performed sensitivity analysis.

Subgroup analysis

Based on sample

When analyzing based on the sample we found: OR 2.98 95%CI (0.71 to 12.46) I2=87% (Six studies), OR 2.79 95%CI (1.12 to 6.98) I^2=94% (Eight studies) and OR 1.33 95%CI (1.02 to 1.72) (one study) for tissue, urine and blood respectively (Figure 15).

Based on the Molecular template

When analyzing based on the Molecular template, we found: OR 1.93 95%CI (0.20 to 18.14) I2=90% (Three studies) , OR 2.43 95%CI (0.93 to 6.36) I^2=95% (Seven studies) and OR 3.55 95%CI (1.08 to 11.65) I^2= 83% (Five studies) for Protein, RNA and DNA respectively (Figure 16).

Based on geographical setting

When analyzing based on the geographical setting, we found: OR 1.73 95%CI (0.77 to 3.82) I2=93% (10 studies), OR 5.83 95%CI (2.86 to 11.86) I^2=16% (Two studies), OR 1.33 95%CI (1.02 to 1.72) (One study) and OR 9.92 95%CI (1.09 to 90.16) I^2= 58% (Two studies) for USA, Netherlands, Japan and Canada respectively (Figure 17).

Discussion

Summary of the main findings

We found that TMPRSS2: ERG fusion was significantly associated with diagnosing PCa, mainly in urine samples and DNA-based molecular templates, however there was an important heterogeneity that could not be explained with the planned subgroups.

Contrast with literature

Penney et al, showed that tumors that develop TMPRSS2-ERG fusion have different genetic etiology, suggesting also that SNPs are differently associated with the risk of developing prostate tumors either with or without fusion (281). Park et al, described that 53% of patients with ERG-positive HGPIN revealed progression to PCa, which needs to be addressed in other studies and systematic reviews. (275). Additionally, Huang et al, determined that the fusion between the androgen regulated TMPRSS2 gene and the members of the ETS transcription factor family (ERG, ETV1, ETV4) has been identified as a genomic aberration in prostate cancer (286).

Tavukcu et al, compared different samples (peripheral blood, pubic hair and urine). They identified higher number of copies of the fusion gene in post-DRE urine and tissue samples compared with blood and pubic hair samples. They concluded that peripheral blood and pubic hair PCR analysis of TMPRSS2:ERG gene fusion seemed to be suboptimal and emphasizing that the samples obtained from urine after prostatic massage seemed to be as effective as direct tissue sampling (284).

High prevalence of TMPRSS2-ERG positive prostate cancer has been found clinically relevant. Studies have shown that TMPRSS2-ERG fusion prostate cancer is associated with higher tumor stage and cancer-specific survival or metastasis (285). Nonetheless, we evaluated the association with TMPRSS2-ERG gene fusion in early detection of PCa. The PSA level of subjects with the TMPRSS2-ERG fusion was also significantly higher than those one who do not have it.

The main mechanisms by which the TMPRSS2-ERG fusion genes are produced are interstitial deletion and balanced translocation (276,291). Because of their specificity, detection of these fusion genes could be a valuable ancillary diagnostic tool in the early detection of PCa. In fact,

these rearranged genes can be detected either by fluorescence in situ hybridization (FISH), reverse transcription polymerase chain reaction (RT-PCR) techniques (292), or branched DNA (bDNA) analysis that is a very sensitive approach (291).
FISH has been considered an standard for the detection of fusion rearrangements; the break-apart strategy is the main approach used for this propose (293). Yoshimoto et al. developed a three-colour assay, which is able to distinguish between the two main mechanisms of gene rearrangement for TMPRSS2-ERG, the interstitial deletion, or the reciprocal translocation(294).
Different questions arise regarding FISH and the discussion of the results, for instance, the presence of multiple signals showing multiple copies of the fusion gene are difficult to interpret (295), the number of nuclei and the score of rearranged nuclei to be assessed as positive (295,296).

On the other side, RT-PCR provides some advantages such as the lower cost and its capacity of discriminating different variants of the TMPRSS2-ERG fusion gene. In this regard, some authors have shown an association between some of these fusion subtypes with good (297) and poor prognoses (298). However, because of its high sensitivity and cross-contamination, RT-PCR may show false positive results. Hence, RT-PCR is an interesting and useful technique in the diagnostic setting and should be considered as potential complement to FISH (291).

Labor-intensive and cost prohibitive methods such as long-range PCR followed by Sanger sequencing or whole genome sequencing have thus far yielded only a handful of TMPRSS2-ERG genomic breakpoints (299).
Assays for detecting *TMPRSS2-ERG* fusion have been limited to those based on RT-PCR or FISH. RT-PCR requires the presence of a stable, full-length transcript that can be difficult to retain in routine clinical processing, whereas FISH requires subspecialty molecular pathology expertise that is not uniformly available (300).
Detection of TMPRSS2-ERG fusions is most commonly carried out using either fluorescence in situ hybridization (FISH) or reverse transcription polymerase chain reaction (RT-PCR), but these methods are costly and require considerable infrastructure and expertise (90).

Strengths and limitations

This is the first systematic review related to the association of this important gene and the prostate cancer in early stages, following the international recommendations for systematic reviews and meta-analysis. There was a very sensitive search strategy, enhanced with specific findings for SNPs searched in Genecards.org, KEGG and UCSC Genome Browser.
The most important limitation of this review is the high heterogeneity, that might be explained by geographical setting, molecular template and type of sample. Even though we analyzed and identified some important findings but we could not explain the heterogeneity, therefore there might be other important variables to have in mind for future studies, making data more homogeneous.

Clinical and population importance

Prostate cancer is characterized by its extensive clinical heterogeneity; early stratification of aggressive disease from a majority of indolent cancers at diagnosis is a critical clinical task in cancer management and treatment.
Based on these findings, we state that TMPRSS2:ERG fusion gene might be the new gold standard biomarker for the diagnosis and stratification of PCa in the very early stages, allowing clinicians to identify and treat locally the cancer with the advent of new technology and to establish which one will become a very aggressive tumor.

As a conclusion, there is an association between TMPRSS2:ERG fusion gene with the diagnosis of Prostate Cancer, mainly based on urine samples and DNA-based molecular templates. TMPRSS2:ERG might be used as the gold standard biomarker for diagnosis and stratification of PCa.

There is still too much work for standardizing the molecular template, the specific technique and the sample to be used. We recommend to increase our efforts to elucidate these issues.

Legends

Appendix 1. Search strategies

Medline through ovid

TMPRSS2.mp or (Transmembrane Protease, Serine 2).mp or (TMPRSS2 protein, human).mp or rs572530227.mp or rs537370123.mp or rs570454392.mp or rs545726689.mp or rs185312677.mp or rs540987630.mp or rs546335233.mp or rs561063944.mp or rs147233451.mp or rs544474510.mp or rs181414852.mp or rs565237319.mp or rs577684898.mp or rs547544037.mp or rs530689404.mp or rs12329760.mp or rs75603675.mp

and/

exp Prostatic Neoplasms/ or exp prostatic intraepithelial neoplasia/ or (prostatic adj2 malignanc$).mp or (prostatic adj2 cancer).mp

and/

exp randomized controlled trial/ or (randomi*ed adj2 controlled adj2 trial).mp. or exp clinical trial/ or (clinical adj2 trial).mp or exp double-blind method/ or Exp cohort studies/ or (cohort adj2 stud$).mp or exp case-control studies or exp Cross-sectional studies

Embase:

'tmprss2 gene'/exp or 'transmembrane protease serine 2'/exp or TMPRSS2:ti,ab or (TMPRSS2 next/3 protein):ti,ab or (Transmembrane next/3 Protease next/3 Serine):ti,ab or (rs572530227 or rs537370123 or rs570454392 or rs545726689 or rs185312677 or rs540987630 or rs546335233 or rs561063944 or rs147233451 or rs544474510 or rs181414852 or rs565237319 or rs577684898 or rs547544037 or rs530689404 or rs12329760 or rs75603675):ti,ab

and/

'prostate cancer'/exp or 'prostatic intraepithelial neoplasia'/exp or (prostatic next/3 malignanc*):ti,ab or (prostatic next/3 cancer):ti,ab

and/

'randomized controlled trial'/exp or (randomi*ed NEXT/2 controlled NEXT/2 trial):ti,ab or 'clinical trial'/exp or (clinical NEXT/2 trial):ti,ab or 'double blind procedure'/exp or 'cross-sectional study'/exp or 'case control study'/exp or 'cohort analysis/exp

Central (Ovid)

TMPRSS2.mp or (Transmembrane Protease, Serine 2).mp or (TMPRSS2 protein, human).mp or rs572530227.mp or rs537370123.mp or rs570454392.mp or rs545726689.mp or rs185312677.mp or rs540987630.mp or rs546335233.mp or rs561063944.mp or rs147233451.mp or rs544474510.mp or rs181414852.mp or rs565237319.mp or rs577684898.mp or rs547544037.mp or rs530689404.mp or rs12329760.mp or rs75603675.mp

and/

exp Prostatic Neoplasms/ or exp prostatic intraepithelial neoplasia/ or (prostatic adj2 malignanc$).mp or (prostatic adj2 cancer).mp

Figure 12. Flowchart

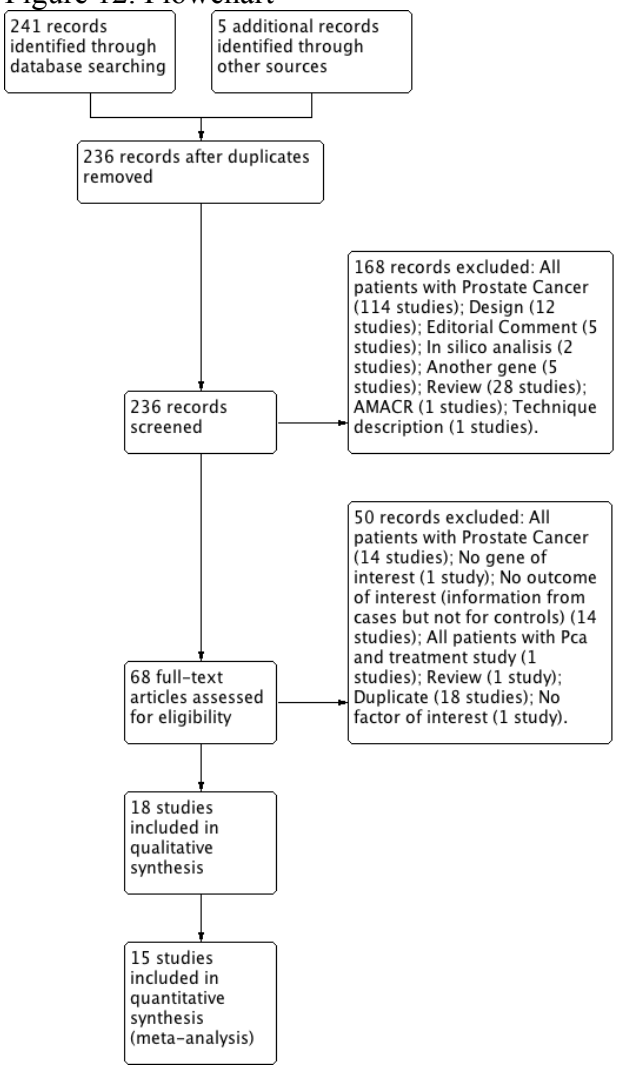
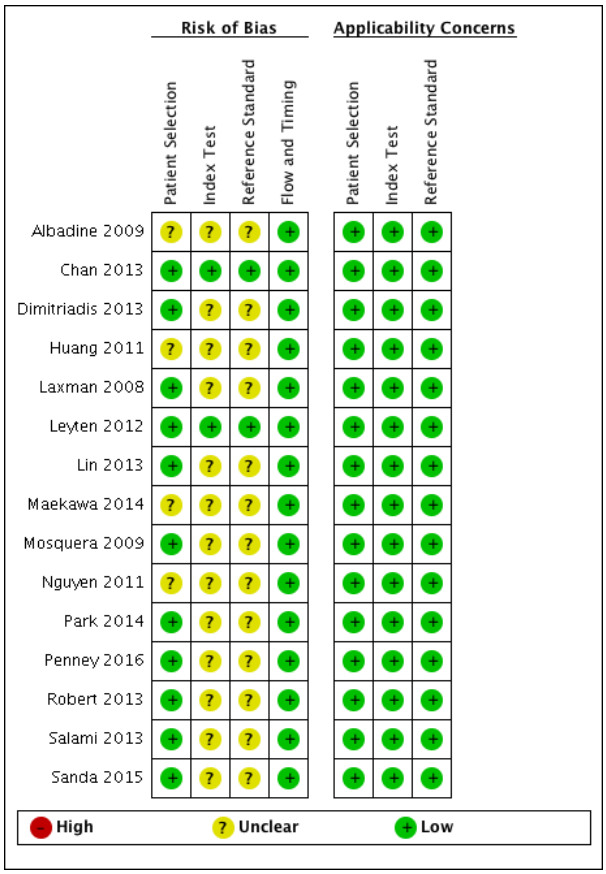


Figure 13. Risk of bias a. Within the studies



b. Across studies

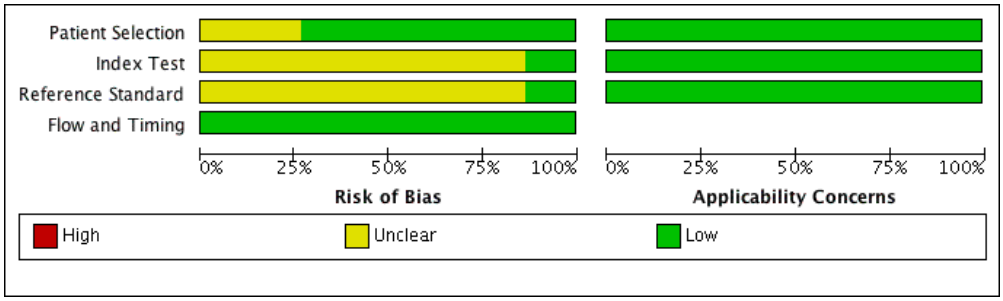


Figure 14. Meta-analysis of the association between TMPRSS2:ERG and PCa

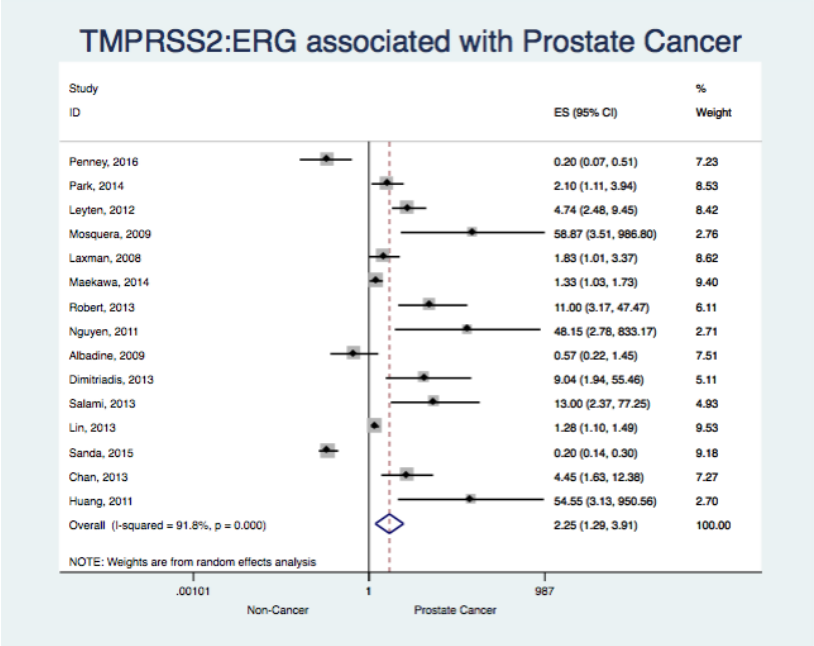


Figure 15. Meta-analysis based on sample

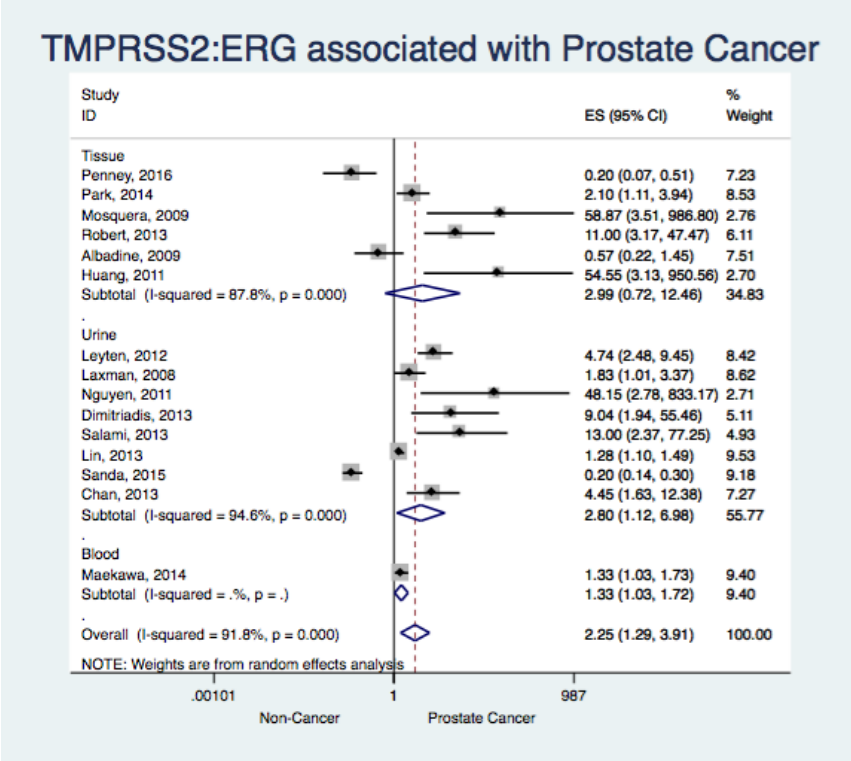


Figure 16. Meta-analysis based on molecular template

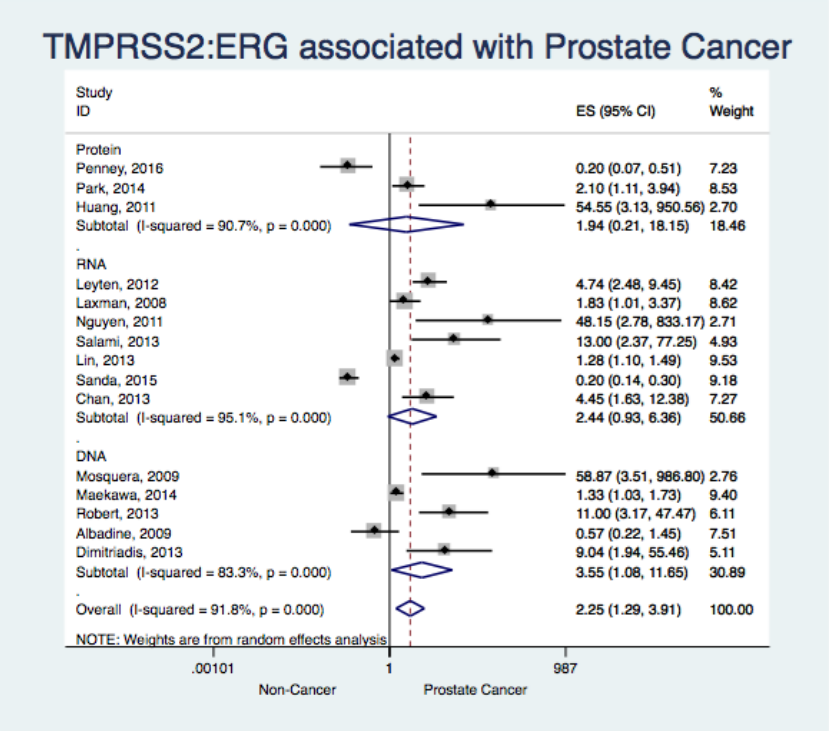


Figure 17. Meta-analysis based on geographical setting

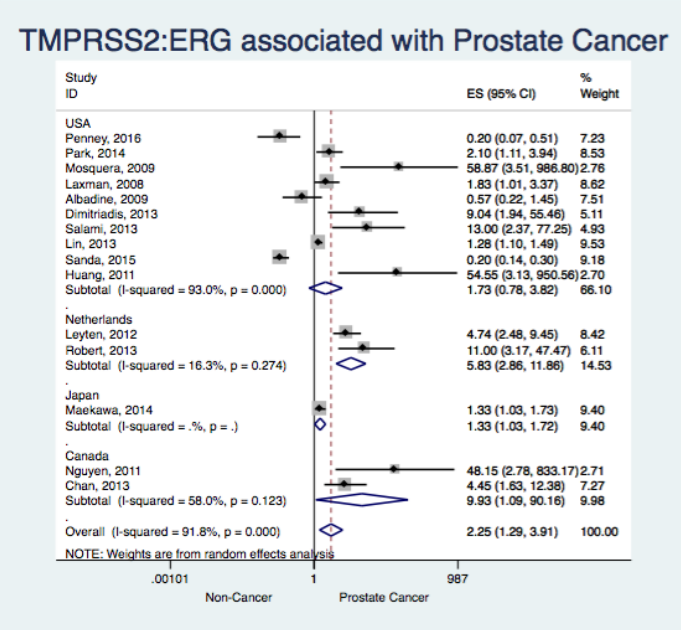


Table 10. Characteristics of included studies

Author	Gene	Technique	Molecular Template	Sample	Setting	Age	Study Design	N
Penney 2016	TMPRSS2:ERG	Immunohistochemical x Microarrays	Protein	Tissue	USA	40 - 84	Cohort	487
Sanda 2015	TMPRSS2:ERG	mRNA	RNA	Urine	USA	55-60	Prospective multi-center study	1077 (516)
Park 2014	TMPRSS2:ERG	Inmunohistochemical x Monoclonal ERG antibody	Protein	Tissue	USA	Median 65	Randomized phase III, double-blinded, placebo-controlled clinical	1590 (461)
Tavukcu 2013	TMPRSS2:ERG	RT-PCR	RNA	Tissue, Peripheral blood, Urine, Pubic hair	Turkey	43-78	Cohort	50
Lin 2013	TMPRSS2:ERG	RT-PCR	RNA	Urine	USA	NA	Prospective, observational, active surveillance study	387
Leyten 2012	TMPRSS2:ERG	mRNA	RNA	Urine	Netherlands	44-86	Prospective multicenter cohort	443
Tomlins 2011	TMPRSS2:ERG	Transcription-mediated amplification (Malign)/ FISH assay (Benign)	RNA/DNA	Urine (Malign) /Tissue (Benign)	USA/Canada	56-65	Cohort	1312
Mosquera 2009	TMPRSS2:ERG	FISH	DNA	Tissue	USA	54-70	Cohort	140 (134)
Laxman 2008	TMPRSS2:ERG	RT-PCR	RNA	Urine	USA	NA	Cohort	234
Maekawa2014	TMPRSS2	Real Time PCR SNP Sonda TagMan	DNA	Blood	Japan	48-100	Cases and controls	518
Chan 2013	TMPRSS2:ERG	RT-PCR	RNA	Urine	Canada	Median 68	Cohort	92?
Cornu 2013	TMPRSS2:ERG	TaqManTM assays	DNA	Urine	France	59-68	Cohort	291
Robert 2013	TMPRSS2:ERG	RT-PCR	DNA	Tissue	Netherlands	NA	Cohort?	128(96)

Huang 2011	TMPRSS2:ERG	Immunohisto chemical microarray	Protein	Tissue	USA	NA	Retrospective cohort	80
Nguyen 2011	TMPRSS2:ERG	RT-PCR	RNA	Urine	Canada	19-88	Cases and controls	101
Albadine 2009	TMPRSS2:ERG	FISH	DNA	Tissue	USA	NA	Cohort	92
Dimitriadis 2013	TMPRSS2:ERG	RT-PCR TagMan	DNA	Urine	Greece	45-83	Cohort	66
Salami 2013	TMPRSS2:ERG	RT-PCR	RNA	Urine	USA	56-71	Cohort	48(45)

Chapter 7. Frequency of allelic variants of TMPRSS2 gene in a Prostate Cancer-Free Southwest Colombian population.

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INTRODUCTION

One of the most prevalent neoplastic pathologies associated with male gender is prostate cancer, which has been estimated for 1.1 million people around the world (301–303). This changes according to ethnicity and geographical location (240). The population mainly affected is Afro-descendant, which had its prevalence increased by 11% in recent years(304). Latin American and Afro population inhabits in southwest Colombia, approximately in same proportion with different rates of disease incidence(6).

Variants in certain genes have been associated with a higher frequency of prostate cancer (BRCA1-2, ATM, NBN, TMPRSS2, among others) (305). Serine proteases like the TMPRSS2, are recognized by their mechanisms of action in inflammatory and immune processes. This gene is located on chromosome 21q22.3, and is expressed at the apex of the secretory epithelium of the glands. The fusion with members of the ETS family is the most frequent chromosomal rearrengement found in 50% of prostate cancers, produced mostly by the microdeletion of a portion of the TMPRSS2 gene (306). The TMPRSS2 gene, and the fusion gene (TMPRSS2:ERG), have been associated with the severity and prognosis of prostate cancer, although the actual pathophysiological process or the variant associated to this condition is not very well known (307). The fusion gene has been widely studied and, at the moment, it has been postulated as one of the most important biomarkers today for diagnostic and prognostic purposes, of prostate cancer population (298). There are some reported SNPs in the literature that have been most frequently related to these clinical scenarios.

The importance of this study is that there are no similar descriptive studies characterizing the presence and its variants for this gene in the southwest Colombia population. This study focuses on describing the frecuency of allelic variants of TMPRSS2 gene in this population.

METHODS

We designed an observational descriptive design which took place from 2014 to 2016 and included people located in Southwest Colombia (Nariño, Cauca, Putumayo y Valle) from all ages.

Sample size

According to the expected frequency for hereditary Prostate Cancer (~ 15%), alfa 15% and an expected error of 5%, the calculated sample size was 162 people and the sampling was by convenience.

We performed a whole exome sequencing which allows to sequence all protein-coding regions (exome) in the genome. This technique permits to identify variants that might alter the sequence of a protein. We performed it as follows:

DNA extraction
Each patient underwent blood extraction to obtain DNA. All drops were collected in filter paper until dried. These filters were immersed in phosphate buffer along with the DNeasy package from QIAGEN ® company (Hilden, Germany – Operational). Each extraction was quantified and quality verified to continue the sequencing processing.

Sequencing protocol
DNA aliquots from each samples underwent a preparation process with the TruSeq Exome Library Prep®, then the obtained libraries were normalized to be sequenced using the TruSeq Rapid Exome®. These packages were provided by Illumina® from San Diego, California, USA. The normalized fragments with its corresponding adaptors for sequencing were charged in HiSeq2500 machine.
We sequenced the full exome and identified the variants associated, specifically the single nucleotide polymorphisms (SNPs) for TMPRSS2 gene, associated with Prostate Cancer (PCa).

This project accomplished all ethical international standards. Descriptive statistics were performed in R and the results are shown in frequency tables for each gene and its variants associated. We finally searched the variants in the following public databases: Exome Aggregation Consortium (ExAC), PharmGKB(308), Clinvar(309), Ensemble, dbSNP(310) in order to look for some pattern to use the variants we found as markers.

RESULTS

We included 162 patients with 7.315.466 data sequenced and TMPRSS2 gene was found in 414 data (4.3%). Missense variants were found in 23% of data, although the most frequent variants were synonymous and introns. Only one stop variant was found in this data (Table 11).

Additionally, the most common variants for the TMPRSS2 gene were: rs140530035 (32.12%), rs17854725 (19.8%) and rs2298659 (13.5%) (Table 12).

DISCUSSION
The transmembrane protease, serine 2 also called TMPRSS2, is a protease of 492 amino acids expressed on the cell surface of multiple organs and it is theorized that they are strategically located to regulate cell-cell interactions. It has been shown to be positively regulated by androgenic hormones in neoplastic tissue and that could modulate the inflammatory response of prostate cells through the activation of PAR-2 (311,312).

The TMPRSS2 gene has historically been associated with the malignant tumor of prostate cancer which is one of the most frequent in the male gender, has been the subject of study by multiple authors during the last decades, in order to link its presence with the frequency of cancer and its

prognosis(276). Although there are studies that have found that this gene does not represent a worse prognosis for prostate cancer (313), an important fusion of this gene with the ERG gene was described, with increasing association with diagnosis and aggressiveness of the prostate neoplasm (Present in 50% of high risk prostate cancer) (97,314).

We found a low frequency of the allelic variant associated with TMPRSS2:ERG fusion gene in this cancer-free population of southwest Colombia. Although this is not the most frequent SNP found in this study, literature reports that the rs12329760 is a variant having a non-negligible allele frequency in populations of East Asia and Northern Europe (0.38 and 0.37 respectively) considering having a ratio of major homocygote (> 7%). Moreover, the Hispanic population has a frequency (0.155) finding low proportion of homozygotes (315). It should be noted that in the southwest Colombia, there is a large volume of Afro-descendant population, in which has been identified that the frequency of this allelic variant is higher (0.29). Therefore this is an important data to keep in mind when identifying new biomarkers for prostate cancer.

Rs140530035 was the most sequenced polymorphism in the present study. It is a very common intron in the world population and the allele frequency of this variant achieves 0.9 (316). Comparing to populations, citizens of northern Europe (Finland) have an AF of 0.99 while in Latino population it is only 0.66, according to Lek et al. 2016 (316).

The rs17854725 and rs2298659 are synonyms variants that, according to the literature is rare in the Latinoamerican population with an AF of 0.15 or less and pathological associations are unknown. Like the previous ones, rs75603675 has no known pathological associations (316).

Strengths and limitations

This is the first study in southwestern Colombia to perform the description of this gene and its allelic variants in relation to the cancer-free population. In addition, the quality of samples, analysis and data was one of the advantages of the project. Finally, a few variants associated with the TMPRSS2 gene were identified, which is a very important information that calls our attention for longitudinal studies in patients without cancer to determine the risk for this condition.

About the limitations of the study, we did not found information regarding whether there is a pathological relationship with prostate cancer.

CONCLUSIONS

The TMPRSS2 gene was not frequent in the cancer-free population of southwestern Colombia. Nonetheless, the most common variants for the TMPRSS2 gene were: rs140530035 (32.12%), rs17854725 (19.8%) and rs2298659 (13.5%).

Legends

Table 11. Associated Variants

Variant	*Absolute Frequency*	*Percentage (%)*
5UTR	3	0.72

Intron	144	34.78
Missense	98	23.67
Stop	1	0.24
Synonymous	168	40.58

Table 12. Variants identified for TMPRSS2 gene

Variants	Absolute Frequency	Percentage (%)
No identifier available	36	8.70
rs12329760	44	10.63
rs140530035	133	32.13
rs143049780	1	0.24
rs148125094	1	0.24
rs149527323	1	0.24
rs17854725	82	19.81
rs181414852	1	0.24
rs2298659	56	13.53
rs3787950	15	3.62
rs61735789	2	0.48
rs61735790	1	0.24
rs61735792	1	0.24
rs61735793	1	0.24
rs61735794	3	0.72
rs61735795	1	0.24
rs75603675	35	8.45

Chapter 8. Metabolomic profile in patients with malignant disturbances of the prostate. An experimental approach

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Introduction

Prostate cancer (PCa) is the most frequent malignant neoplasia diagnosed in men, considering developing countries and the developed ones (excluding skin cancer); besides it is one of the main causes of death (216–218). PCa is the second most commonly diagnosed worldwide cancer among men (mainly >65 years). It is a public health concern in developed countries, in which elderly men correspond to the greatest proportion of general population(9). This relevant condition is a heterogeneous disease with a variable natural history and slow growth pattern. It may display latency periods of up to 20 years in which it remains organ-confined. Although prostate cancer lesions can remain localized for long periods, more aggressive forms might occur and when metastasis occurs, lymph nodes and bones are affected predominantly with detrimental results(270).
Patients at high risk and susceptibility to develop PCa, require screening over time. Age, race and family history are the most important risk factors(271).
There has been concerns about the diagnosis and early treatment of this disease due to the absence of specific markers(273). Until now, the gold standard for the diagnosis of PCa is an invasive procedure consisting in the histopathological evaluation of the prostate, a procedure with significant morbidity(92). Currently, the use of prostate-specific antigen (PSA) as a screening and monitoring marker for prostate cancer is widespread (274), although there is still debate about the screening for PCa among men in the general population. This biomarker is prostate-specific but it has a low specificity and could also increase unnecessary biopsies, without lowering mortality(1).

In the search for new biomarkers, metabolomics is one of the omics sciences that has been studied during the last 5 to 10 years. It mainly indicates and measures the systemic activity and conditions in the human body, suggesting that dysregulation of metabolism has a fundamental role in the development and progression of common conditions and malignancies (317).
Specifically, prostate cells´ metabolism produces the components for prostatic fluid, such as: Prostate Specific Antigen (PSA), spermine, myo-inositol and citrate (Higher levels than in any other organ). In Prostate Cancer, cells loss the ability to accumulate citrate by lowering the zinc

levels (317). Additionally, there is some evidence demonstrating that chronic inflammation and related markers like in metabolic syndrome, increase tumor growth (318)

Metabolomics employ different techniques to detect the components in every tissue with different statistical or bioinformatic methods (319). The major approaches are: 1. Targeted analysis, 2.Metabolite profiling and 3.Metabolic fingerprinting, nevertheless, the three of them have advantages and disadvantages as any other technique. Regarding the metabolites, so far, the most promising biomarkers for PCa diagnosis are: Sarcosine (AUC 0.67)(187), choline, phosphocholines (AUC 0.982) (186) , phosphorylcholines, carnitines (AUC 0.97)(188), citrate (AUC 0.89) (189), amino acids (lysine, glutamine and ornithine) (190–193), arachidonoyl amine (AUC 0.86) (188) and lysophospholipids (Steroid hormone biosynthesis pathway and bile acids – Sensitivity and Specificity 92- 94%) (192).

The purpose of this experiment was to identify the metabolites in patients with malignant disturbances of the prostate compared to non-cancer patients.

Methods
This research was carried out by the UROGIV Research Group along with the research group Labiomol and Development and Applications in Nuclear Magnetic Resonance (DARMN). An exploratory study was carried out based on plasma samples of both benign and malignant pathology.

Sample setting
Samples were taken in the urology section of the Hospital Universitario del Valle and in the Prostate biopsy section of the Rafael Uribe Uribe Clinic.

Population
Two sample groups of patients older than 18 years of male gender who were at risk of Prostate cancer due to elevated PSA or abnormal digital rectal examination were selected and divided as follows: Group 1. Patients with localized prostate cancer (PCa), who were characterized according to the risk classification. Group 2. Patients with negative prostate biopsy for malignancy.

Exclusion criteria
Patients were excluded for the following conditions: Other concomitant cancer types, coagulation disorders, with renal or metabolic disorders such as diabetes mellitus, gout or hyperthyroidism.
Additionally, symptoms of acute diseases two weeks before the sample taken as: Febrile episode, cough, headache, diarrhea, hematuria as well as psychic disorders or episodes of stress trauma. Ingestion of medications up to two weeks before taking samples such as: Antibiotics, hormones, non-steroidal anti-inflammatory drugs and chemotherapy or radiotherapy medication. Those who did not sign the informed consent

Selection of samples and sample size
Sampling was done by convenience and according to the availability of patients upon admission to the urology unit of the HUV and Rafael Uribe Uribe Clinic.

Patients were taken according to convenience sampling at the institutions and had an indication for prostate biopsy, they were instructed and requested informed consent for the handling of samples. He was also informed of the risks and benefits that had both the procedure and the research project. The patients underwent blood sample, as well as urine and the standard procedure of prostate biopsy to confirm the diagnosis.

According to the result of the histology, 12 patients were admitted with confirmation of prostate cancer and 20 patients with negative results.

Handling samples

Blood sampling procedure
The procedure was carried out by trained and trained personnel for sampling in humans. After collecting, the blood was mixed by inverting the tube 8 to 10 times. It was stored in the vacutainer tubes (collection tubes with EDTA) in vertical position at 4ºC until centrifugation. The samples were transported within the next two hours for collection.

Procedure for separating the serum
Prior to the centrifugation of the sample, the centrifuge was switched on and cooled at 4º C. Previously, an aliquot of 500 µL of phosphate buffer pH 7.4 (0.142M Na2HPO4) was taken in 1.5 mL Eppendorf® tubes.
The blood samples were centrifuged in a rotor (oscillating head) for 5 minutes at 4000 rpm at 4 ° C. After centrifugation, 250 µL of the plasma layer was taken with an appropriate micropipette without disturbing the buffy coat layer. It was stored in a -80ºC bio-freezer.

Preparation for resonance
Each sample was thawed at room temperature and centrifuged at 12000g at 4oC for 5 min. 65 µL of Phosphate Buffer pH 7.4 (0.142M Na2HPO4) prepared with D2O and TSP (3-(Trimethylsilyl) propionic-2,2,3,3-d4 acid sodium salt), and 585 µL of sample were added in eppendorf. It was centrifuged again at 12000g at 4oC for 5 min. Finally, 600 µL of supernatant was transferred to a 5mm NMR tube.

1 H-NMR spectra were acquired on Bruker Avance II Ultra Shield 400 MHz spectrometer (Bruker BioSpin, Rheinstetten, Germany) with direct BBO probe equipped with 3 gradients at a temperature of 300K. The spectra were obtained by means of the pulse sequence CPMG at 64 scans and with pre-saturation of the water signal. Through the CPMG sequence, signals belonging to molecules of high molecular weight can be eliminated from the spectrum, improving the resolution of the signals corresponding to the metabolites. The resonance frequency and the homogeneity of the field were adjusted automatically, while the calibration of the solvent suppression was performed manually for each sample. The spectra were referenced with respect to the resonance of the α-glucose doublet at δ 5.233 ppm

Data analysis
The spectral data matrix was pre-processed with scripts in software R (R Development-Core Team) (231) ; firstly there was a base line correction, then the regions of the TSP, water and anticoagulant signals, as well as the spectral regions of the ends, were eliminated. Additionally

binning was performed in the spectral region comprised between δ 0.1 - 9.0 ppm. For the analysis of the spectral data, multivariate methods were used; PCA and OPLS-DA were applied in R, using pareto as a scaling method and combined with the statistical total correlation spectroscopy (STOCSY) analysis method for aiding the identification of potential biomarker molecules.

Ethics

The study accomplishes all international ethical requirements for human research and it was approved by the IRB at Universidad del Valle.

Results

We included 32 patients 12 with prostate cancer diagnosis and 20 with hyperplasia. The median age was 69.5 years (46 to 82). All patients had localized prostate cancer showing no evidence of metastasis in extension studies (Table 13).

Table 13. Characteristics of included patients

Age	Ethnic group	Setting	Past history	Urethral catheter	PSA	Histology	Tumor	Gleason	Risk group
Included patients with Cancer									
61	Multiracial	Cali	No	No	15.3	Adenocarcinoma	T1c	3+3	Intermediate
77	Multiracial	Silvia	No	Yes	75.85	Adenocarcinoma	T2c	4+3	High
63	Multiracial	Cali	No	No	20.9	Adenocarcinoma	T2c	3+4	High
65	Multiracial	Santander de Quilichao	No	No	7.08	Adenocarcinoma	T2c	3+3	High
78	Multiracial	Cali	No	No	20.2	Adenocarcinoma	T2a	3+3	High
82	Black	Zarzal	No	No	16.61	Adenocarcinoma	T2c	3+3	High
57	Black	Cali	No	No	8.01	Adenocarcinoma	T2a	3+3	Low
74	Multiracial	Popayán	No	No	15.99	Adenocarcinoma	T2c	3+3	High
74	Multiracial	Sabaneta	No	No	9.05	Adenocarcinoma	T2a	3+3	Low
82	Multiracial	Cali	No	No	13.74	Adenocarcinoma	T2a	4+4	High
60	Black	Puerto Tejada	No	No	14.82	Adenocarcinoma	T2a	3+3	Intermediate
67	Black	Tumaco	No	No	11.2	Adenocarcinoma	T2a	3+3	Intermediate
Included patients without Cancer									
75	Multiracial	Cali	No	No	4.88	Negative for malignancy			
54	Multiracial	Cali	No	No	10.1	Negative for malignancy			
60	Multiracial	Cali	No	No	8	Negative for malignancy			
65	Multiracial	Cali	No	No	4.37	Atypical Acinar Proliferation / Glandular Atrophy (IHC)			
51	Multiracial	Cali	No	No	4.24	Chronic prostatitis			

77	Multiracial	Cali	No	Yes	10.57	Atypical Acinar Proliferation / Glandular Atrophy (IHC)			
72	Multiracial	Cali	No	No	5.25	Chronic prostatitis			
64	Multiracial	Santander de Quilichao	No	No	3.12	Benign prostatic tissue/Chronic prostatitis (IHC)			
77	Multiracial	Cali	No	No	11.3	Benign Hyperplasia/Chronic Prostatitis/Glandular atrophy/Atypical acini			
73	Multiracial	Cajibio	No	No	8.61	Glanduloestromal Hyperplasia/Chronic Prostatitis/Glandular atrophy			
73	Multiracial	Cali	No	No	8.4	High-grade Prostatic Intraepithelial Neoplasia (IHC)			
70	Multiracial	Pradera	Two relatives with Pca	No	10.8	Glandular Atrophy (IHC)			
54	Black	Cali	One relative with Pca	No	12.16	Chronic prostatitis/Glandular Atrophy (IHC)			
73	Multiracial	Pradera	No	Yes	38	Negative for malignancy			
69	Multiracial	Cisneros, Juntas Dagua	No	No	5.07	Glanduloestromal Hyperplasia/Chronic Prostatitis/Glandular atrophy			
66	Multiracial	Palmira	No	No	6.63	Glandular Atrophy (IHC)			
73	Multiracial	Restrepo	One relative with Pca	No	6.85	Benign Hyperplasia/Chronic Prostatitis/Glandular atrophy/Atypical acini			
46	Multiracial	Dagua	No	No	19.0	Glanduloestromal Hyperplasia/Acute and Chronic Prostatitis/Glandular atrophy			
71	Multiracial	Cali	No	No	11	Glanduloestromal Hyperplasia/Acute and Chronic Prostatitis/Glandular atrophy			
69	Multiracial	Palmira	No	No	9.55	Glanduloestromal Hyperplasia/Acute and Chronic Prostatitis/Glandular atrophy			

Sample metabolomic profile in plasma

We found the following graphical profile for metabolites in a sample of healthy patients (Figure 18). Some of the metabolites represented by their resonances are: the acetone, the acetoacetate, the alanine, the glucose, the valine and some others (Table 14).

Figure 18. Metabolomic profile ^{1}H-RMN of a sample of healthy human plasma

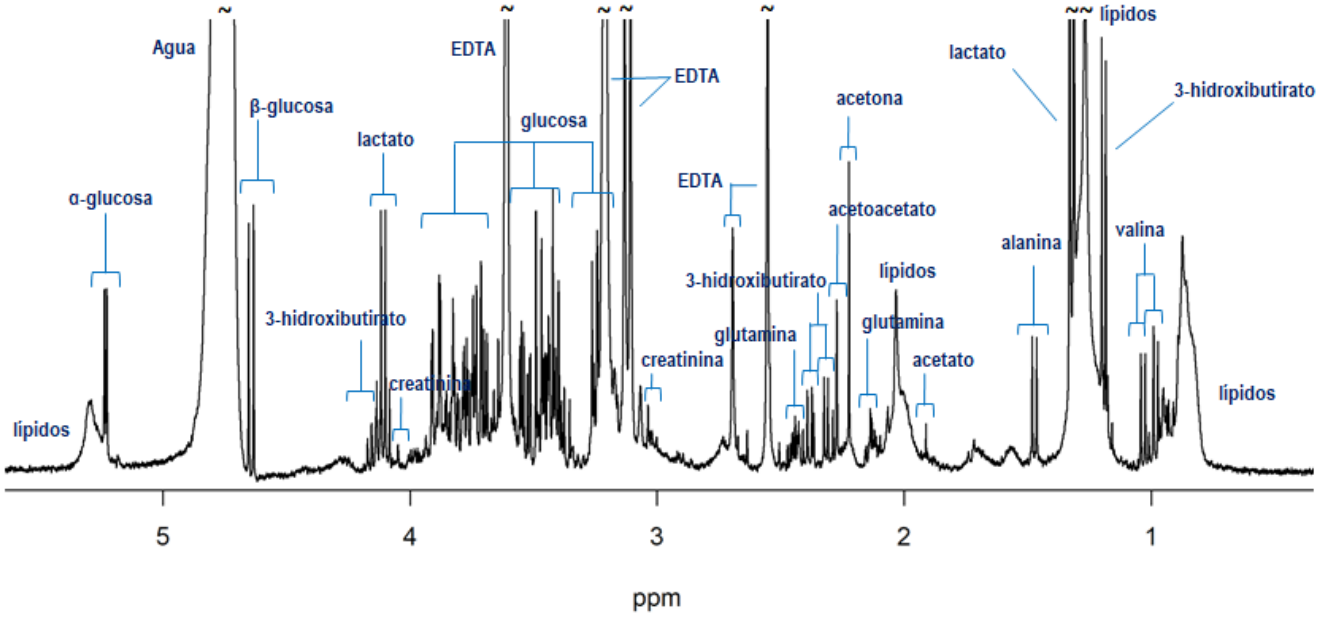


Table 14. Spectrums of resonances ^{1}H-RMN of metabolites of healthy human plasma.

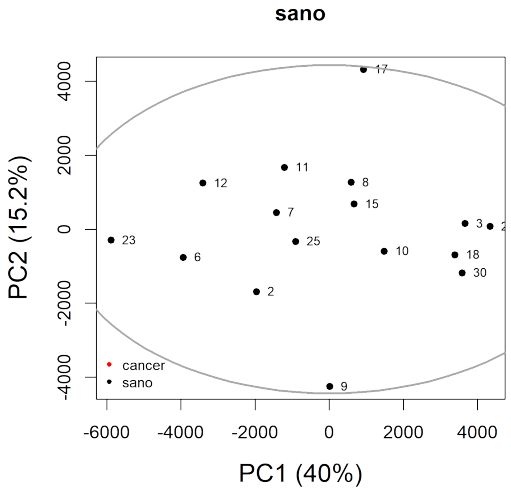
Signal	δ ^{1}H-RMN (ppm)	Signal	δ ^{1}H-RMN (ppm)
Valine	0.98 (d)	Acetate	1.91 (s)
	1.04 (d)	Glutamine	2.14 (m)
	2.28 (m)		2.44 (m)
	3.61 (d)		3.77 (t)
3-hidroxibutirate	1.19 (d)	Acetone	2.23 (s)
	2.30 (dd)	Acetoacetate	2.28 (s)
	2.40 (dd)	Creatinine	3.04 (s)

	4.15 (m)		4.05 (s)
Lactate	1.32 (d)	Glucose	3.22 - 3.92 (m)
	4.11 (c)	β-glucose	4.64 (d)
Alanine	1.48 (d)	α-glucose	5.23 (d)
	3.76 (c)		

Principal component analysis

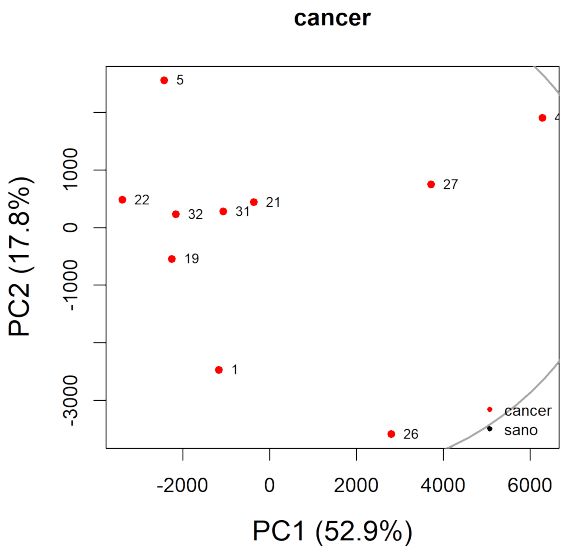
We pretend to obtain with the two first components to explain more than 50% of data. We had to eliminate two data from healthy patients and one for cancer patients due to contamination with ethanol. We obtained 18 non-cancer patients, however we had to exclude two atypical data, therefore we only included 16 non-cancer patients (Figure 19).

Figure 19. PCA for healthy patients (16 samples).



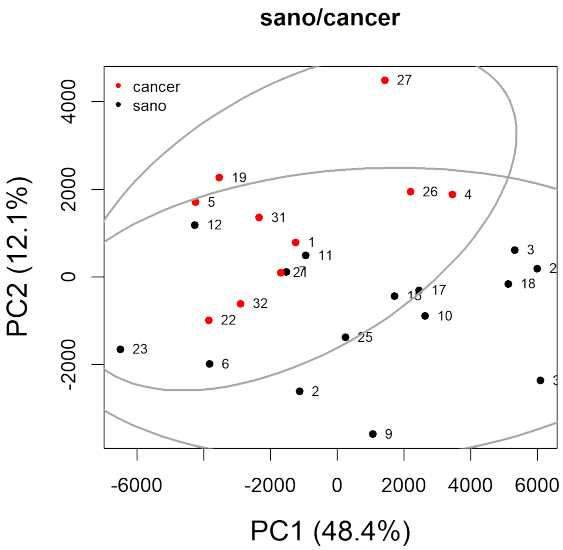
We only had to eliminate one patient (atypical data) after the PCA for cancer patients, therefore the figure is shown with 10 patients (Figure 20)

Figure 20. PCA for cancer patients (10 samples)



After including the 16 samples of non-cancer patients and the 10 samples of cancer patients, we still found one atypical data therefore we excluded and the final analysis included 15 non-cancer patients and 10 cancer patients. In Figure 21, we can show the discrimination of cancer and non-cancer patients with two main components explaining more than 50% of data.

Figure 21. Final PCA Cancer and non-cancer patients included



Subgroup analysis Risk

Although this is a low sample, we can show that the most important discriminative patients with cancer have high risk of progression. The patients with low and intermediate risk are apart from this group (Figure 22 and 23).

Figure 22. Subgroup of cancer patients with healthy patients

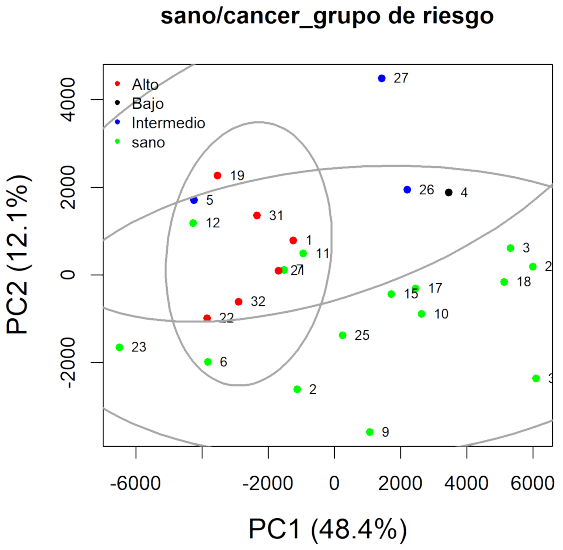
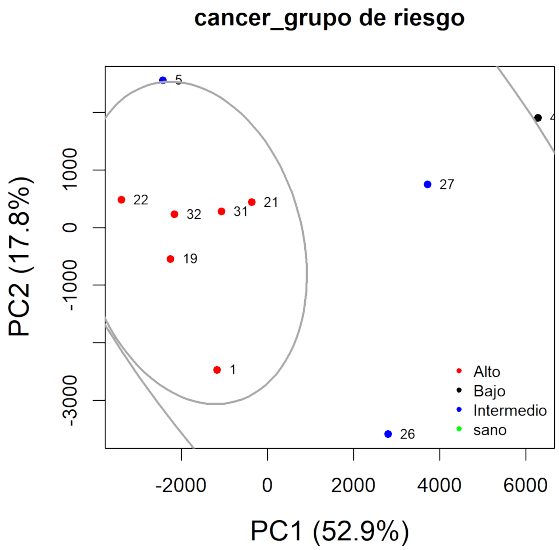


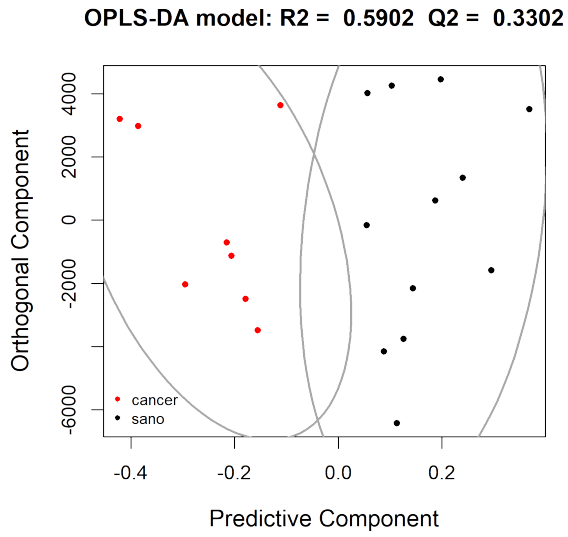
Figure 23. Subgroups of cancer patients.



OPLS-DA model

We included 15 non-cancer patients and 10 patients with cancer, then we applied the 5-fold method. It resulted in a discriminative expression of patients with and without cancer (R2 = 0.5902; Q2 = 0.3302; Figure 24).

Figure 24. OPLS-DA for cancer and non-cancer patients



Additionally, we describe the loadings plot to identify the metabolites associated with cancer (Figure 25 and Figure 26).

Figure 25. Loadings plot

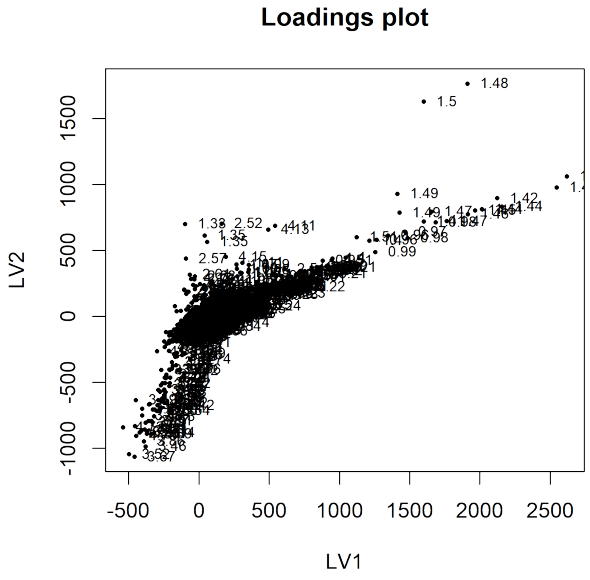
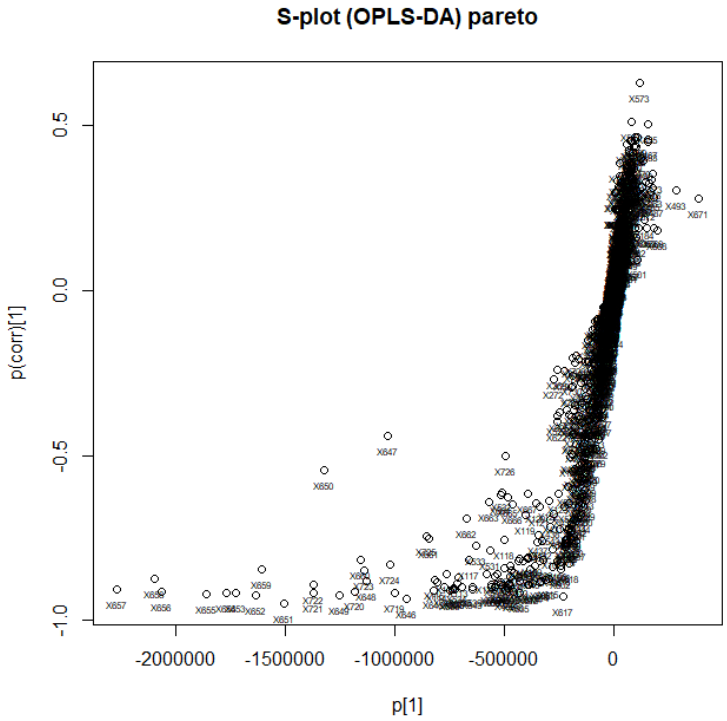


Figure 26. S-plot to discriminate the metabolites



The metabolites associated with prostate cancer were shown in a statistical total correlation spectroscopy (STOCSY) 1D graph to better discriminate metabolites. The figure 27 is for the loading 657 (Lipids) and the Figure 28 is for the loading 647 (The lactate), which were found with positive correlation with prostate cancer.

Figure 27. STOCSY 1D for lipids (A barplot color-coded for positive (red), negative (blue) and null (green) correlations).

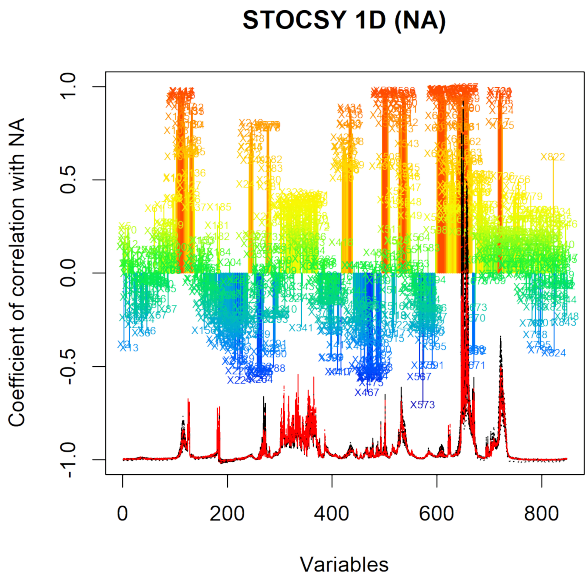
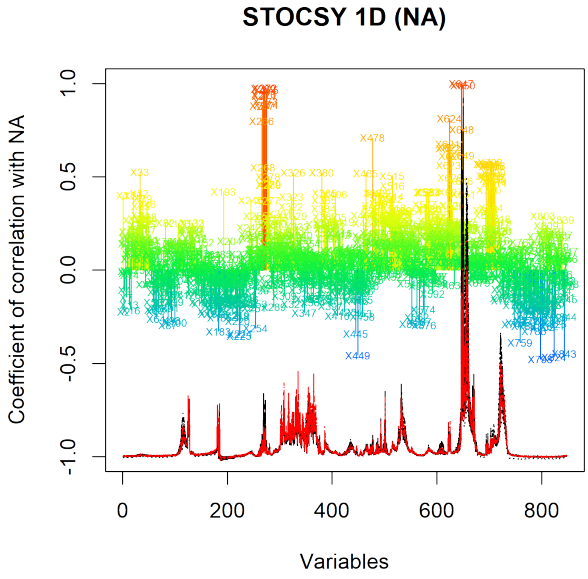


Figure 28. STOCSY 1D for Lactate (A barplot color-coded for positive (red), negative (blue) and null (green) correlations).



Discussion

Summary of the main results

There was a consistent difference between the two groups (cancer and non-cancer patients) according to the PCA, mainly in the high risk of progression PCa patients. The two most important metabolites that discriminate cancer from non-cancer patients were lactate and lipids in plasma samples.

Contrast with literature

In 1920, it was described that cancer cells, even in the presence of oxygen, produce ATP through anaerobic pathway (anaerobic glycolysis) instead of tricarboxylic acid cycle (Krebs cycle). The Warburg effect consists in the ability of sustaining high rates of glycolysis for ATP generation (320). In the prostatic epithelial cells, we find an overexpression of zinc-regulated transporter/iron-regulated transporter-like protein 1(ZIP1) which inhibits the m-aconitase in the tricarboxylic acid cycle. This leads to accumulation of citrate in the healthy prostate (321). In prostate cancer, the ZIP1 lowers, since the malignant tissue cannot accumulate zinc and the anaerobic glycolysis increases, overproducing lactate. This process might be a hallmark in the beginning of prostate cancer, even before of histological changes (321).

According to this, lactate has been associated with tumor progression, as well as pyruvate and alanine. Lactate, for example, has been studied as urine biomarker for non-invasive detection of PCa, nonetheless there are not consistent results (322).

There is an important difference in PCa regarding the glucose metabolism due to the dependence of Androgen receptor (AR) signaling. It has been described that glycolysis differs between cell sensitive to androgen (early stages) and non-sensitive (advanced), according to the dependence to glucose (322,323). AR also regulates the activity of several genes related to glucose consumption such as: Hexokinase-2(HKII), Pentose phosphate pathway (PPP) and Calcium/calmodulin-dependent protein kinase beta (CaMKKB) among others. Overall, AR stimulates glycolysis and anabolic metabolism(322).

Regarding the metabolomics, we performed an experiment based on plasma samples and nuclear magnetic resonance (NMR) which is broadly accepted in literature. These have advantages such as: easy to obtain, less diurnal variation and less invasive than a prostate biopsy. On the other side, the disadvantages are: higher concentration of proteins and more sample preparation than urine(321).

Our results indicate a dysregulation in energetics pathway according to the differences in lactate and lipids between groups. There are only few studies describing the use of 1H-NMR in plasma samples with few patients and their findings were the following: 1.Increased levels of alanine, pyruvate and sarcosine and low levels of Glycine (Disturbances in energetic metabolism and lipogenesis, and alterations in glycine synthesis and degradation)(324). 2. Acylcarnitines, Choline and arginine (Alteration in fatty acids metabolism, alteration in membrane phospholipidic metabolism and alteration in amino acids metabolism)(182).

Some other studies but with different approach (Liquid and gas chromatography/Mass spectrometry) have found similar results with different biomarkers: Alteration in lipid metabolism, disturbances in growth inhibition and induction of apoptosis, alteration in amino acid metabolism and alteration in energetic metabolism (Increased levels of Palmitic acid, linolenic acid, aspartic

acid, choline, alanine, lysine, sarcosine and phosphatidylcholine and low levels of ornithine, stearic acid, glutamine, valine, tryptophan, DHEAS, epiandrosterone sulfate, carnitines, 2-hydroxybutyrate, ketone bodies among others)(191,325–327).

Strengths and limitations

This is the first pilot study in Colombia to identify a biomarker for diagnosis localized prostate cancer in a very early stage through metabolomics. We designed a consistent method to analyze the samples through nuclear magnetic resonance (NMR) and found interesting results to understand the pathophysiology of PCa. The finding of lactate and lipids might be tried in a population study with higher sample size to extrapolate results and to consider using these metabolites and this technique as a way for screening or diagnosing PCa, depend on the context.

Regarding the limitations, we found the following to be considered in next trials: a low sample size and the lack of association with mass spectrometry (MS) to complement and identify low weight molecules (Lipidomics).

Conclusions

The main differences between patients with prostate cancer versus non-cancer regarding their metabolomics profiles confirmed that lactate and lipids are the most reliable biomarkers to trace cancer development in prostate.

We suggest to continue research in this important omics technology to try to elucidate what really happens in the prostate when malignant disturbances are presenting.

Chapter 9. Integrative model

Prostate cancer is one of the most common and studied malignancies around the world, however, the molecular mechanisms underlying its development are poorly understood since most of the research has been focused on progression and advanced stages. Similar to other cancers, PCa has multiple genomic disturbances including microsatellite variations, point mutations and chromosomal alterations such as deletions, insertions, translocations and duplications, nonetheless in this tumor, these alterations result in a high frequency of gene fusions. Additionally, there are important disturbances in physiological and metabolic processes inside the gland which might trigger or complement the tumorigenesis.

The highly heterogeneous nature of PCa provides a real challenge for clinical disease management. In our case, the knowledge of each one of these different elements in conjunction, might increase the better understanding of the complexity of the pathophysiology of the prostate cancer and perhaps in the future we might find different targets to prevent or cure it in early stages.

Inherited Prostate Cancer

Race, diet, family history, environmental factors and hereditary components have been reported as risk factors for PCa (219–222). Regarding the latter, genes such as BRCA1, BRCA 2, MSH2, HOXB13, ATM, CHEK2 and NBN have been proposed as important candidates contributing to PCa (223,226–229). In this compendium, we found that the most frequent genes were ATM 1221 data (13.2%), BRCA1 1178 data (12.8%), BRCA2 1484 data (16.12%) and NBN 965 (10.42%) with missense and stop variants for most of them. On the other side, there were non-pathological variants like rs169547, rs206075, rs206076 and rs9534262 and some other variants like rs799917, rs3736639, rs1061302 and rs1805794 associated to NBN gene, which are highly linked to PCa in the literature.

The existence of single nucleotide polymorphisms (SNPs) for these genes, further increase the risk and even could be used as a prognostic biomarker for this condition (236). Additionally, observing a germline mutation in some DNA-repair gene provides information to patients to look for counseling, identify predisposition to cancer (226).

BRCA2 has an important role in the repair of double-strand DNA breaks that function by regulating the intracellular transport and activity of RAD51, a critical protein in homologous recombination (241–243). The most frequent variants found for this gene, such as: rs169547, rs206075, rs206076 and rs9534262, are similar with other studies (244,245).

The second most frequent gene was ATM, which exerts control in cell division. When detecting a damage in the genetic material, leading to either cell cycle arrest, DNA repair or apoptosis (251–253). In addition, it can be considered as the main transducer in the double-strand rupture repair process, where it recruits and cooperates with other sensor proteins such as 53BP1 (p53 binding protein) and BRCA1.

BRCA1 is the third most frequently gene found and associated with an increased risk of sporadic PCa (3.5 times), while for germline mutations in this gene only 0.44% of the cases have been

observed of PCa (220,254,255). The Breast Cancer Linkage Consortium (BCLC) described that men aged 65 with mutations in BRCA1 report an increase in the risk of PCa with a relative risk (RR) of 1.82, being linked to a series of cellular processes such as response and repair of DNA damage (220,253,255,256), transcriptional regulation and chromatin modeling.

NBN was another important gene found, which is a component of the protein complex hMRE11 / hRad50 / NBN that participates in the initiation of a response to DNA damage and is linked to the repair of double-strand rupture (260–262). This acts in the non-homologous junction pathway as a DNA damage sensor and in the homologous recombination pathway, participating in DNA repair and in the cell cycle check point in the S phase (262).

These genes are fundamental for considering the risk of familial PCA since at least 15% of patients might have this kind of condition and as a clinicians we need to consider and look for the susceptibility in our population. We need population-based studies with higher sample size and a long follow-up to determine whether these genes excerpt higher risk to develop prostate cancer.

Somatic variants

The transmembrane protease, serine 2 also called TMPRSS2, is a protease of 492 amino acids expressed on the cell surface of multiple organs and it is theorized that they are strategically located to regulate cell-cell interactions. This gene was considered a promising biomarker located at 21q22.2 and expressed in normal and malignant prostatic epithelium as an intracellular and membrane proteins, according to RNA (transcripts per million) or protein (antibodies) levels. Additionally, ERG is a member of the E-twenty six family members (ETS), which are key regulators of differentiation, apoptosis, embryonic development, cell proliferation and inflammation. It has been shown to be positively regulated by androgenic hormones in neoplastic tissue and that could modulate the inflammatory response of prostate cells through the activation of PAR-2 (311,312).

The TMPRSS2 gene has historically been associated with the malignant tumor of the prostate, which is one of the most frequent in the male gender, has been the subject of study by multiple authors, in order to link its presence with the frequency of cancer and its prognosis (276). Although there are studies that have found that this gene does not represent a worse prognosis for prostate cancer (313), an important fusion of this gene with the ERG gene was described, with increasing association with diagnosis and aggressiveness of the prostate neoplasm (Present in 50% of high risk prostate cancer) (97,314).

TMPRSS2:ERG has been assessed as a specific biomarker for PCa, since 2005 (275). There are few studies trying to look for the association between this gene, the fusion and the advanced prostate cancer, but less research for this gene as a diagnostic tool (276). Up to 53% of patients with ERG-positive HGPIN revealed progression to PCa, which needs to be addressed in other studies and systematic reviews. (275)(Figure 1). Additionally, Huang et al, determined that the fusion between the androgen regulated TMPRSS2 gene and the members of the ETS transcription factor family (ERG, ETV1, ETV4) has been identified as a genomic aberration in prostate cancer (286). The main mechanisms by which the TMPRSS2-ERG fusion genes are produced are interstitial deletion and balanced translocation (276,291).

According to the information pooled in our systematic review and meta-analysis from 15 studies, we found that TMPRSS2: ERG fusion was significantly associated with diagnosing PCa (OR 2.24 95%CI 1.29 to 3.91), mainly in urine samples (OR 2.79 95%CI 1.12 to 6.98) and DNA-based molecular templates (OR 3.55 95%CI 1.08 to 11.65). On the other side, we found a low frequency of the allelic variant associated with TMPRSS2 (4.3%) in the south-west Colombia population. Missense variants were found in 23% of data, although the most frequent variants were synonymous and introns. Only one stop variant was found in this data. These are another important data to consider when assessing the risk of prostate cancer in this population.

Because of its specificity and according to the results presented in this research, detection of these fusion gene might be used as an important diagnostic tool in the early detection of PCa and part of the comprehensive framework for understanding the pathophysiology of this condition. These rearrangements might be detected either by fluorescence in situ hybridization (FISH), reverse transcription polymerase chain reaction (RT-PCR) (292), or branched DNA (bDNA) analysis, among others techniques (291).

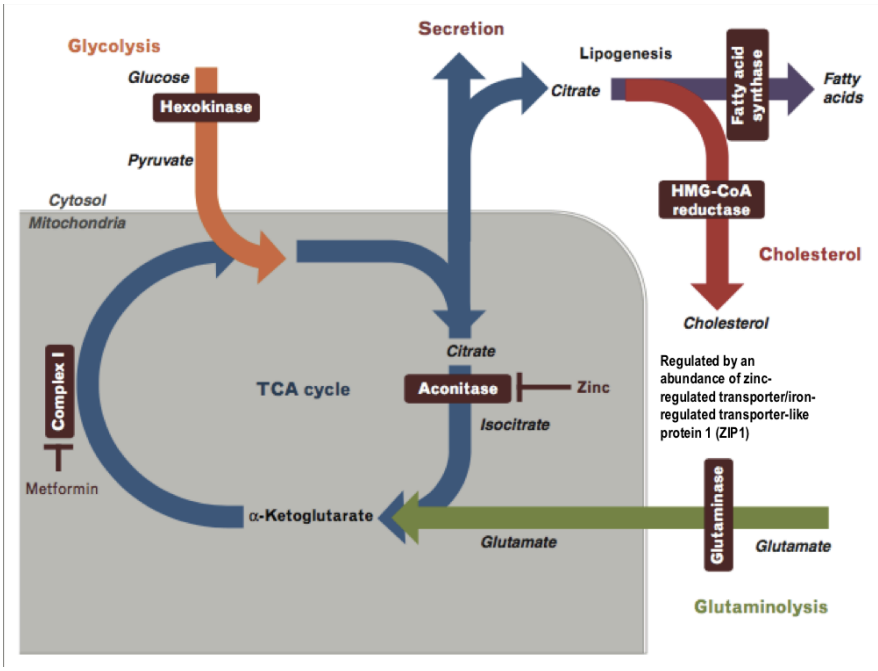
Metabolomic disturbances

According to the present, there was a consistent difference between the two groups (cancer and non-cancer patients) according to the PCA, mainly in the high risk of progression PCa patients. The two most important metabolites that discriminate cancer from non-cancer patients were lactate and lipids in plasma samples.

Cancer cells have the ability of produce ATP through anaerobic pathway (anaerobic glycolysis) instead of tricarboxylic acid cycle (Krebs cycle) even in the absence of oxygen (320). One subpopulation of these cells are capable of consume high levels of glucose and secrete high levels of lactate, whereas the others, utilizes the lactate produced by others as their main energy source (328). Therefore, tricarboxylic acid cycle and oxidative phospholylation (OXPHOS) play important roles in the metabolic study of PCa. This has been associated with mutation or loss of function of tumor-suppressor genes with the activation of tumoral pathways such as: Nuclear factor kappa-light –chain-enhancer of activated B-cells, protein kinase B (Akt), epidermal growth factor, insulin-like growth factor I, phosphoinositol 3-kinase, mammalian target of rapamycin, and HIF-1alfa (329). HIF-1alfa upregulates the following on an oxygen-independent manner: Glucose transporter 1, monocarboxylate transporter four (MCT-4) and lactate dehydrogenase A (LDH-A) (329). I will discuss this issue further.

As previously mentioned, in prostate cancer, the ZIP1 lowers, since the malignant tissue cannot accumulate zinc and the anaerobic glycolysis increases, overproducing lactate (Figure 29). This process might be a hallmark in the beginning of prostate cancer, even before of histological changes (321) and leading to two important ways that focus in a pre-cancer state such as: the chronic inflammation, lipogenesis and the energetic pathways those that might be associated or synergic to the TMPRSS2:ERG fusion gene.

Figure 29. Metabolomic pathway in prostate. Taken from Huang et al (330)



Lactic acid, for many years, was considered an inert end-product of glycolysis during hypoxia. However, during the last few years, it has been considered that under different contexts, lactate can have multiple regulatory functions and might be seen as a signal for different metabolic, physiologic and immune processes inside the prostatic cell. The acidic environment requires the adaptation of tumor cells for surviving, representing the tumor advantage over the normal cell. As a result, adjacent tissues go into a necrotic phase that allow, not competing for substrates and facilitating tumor growth (331,332).

According to this, the following must be stated: Lactate must be seen as an important marker since it has been found that promotes cancer cell survival, invasion and angiogenesis; it also plays and important regulatory position for the immune system (329). The lactate produced by the tumor, attacks the immune system lowering the pH in the stroma (ranges between 6.0 to 6.5); at these values, the cytolytic response and the cytokine secretion of tumor infiltrating T-cells are diminished, therefore regulating tumor growth. Additionally, the extracellular levels of lactate inhibit the differentiation of monocytes and dendritic cells (329).

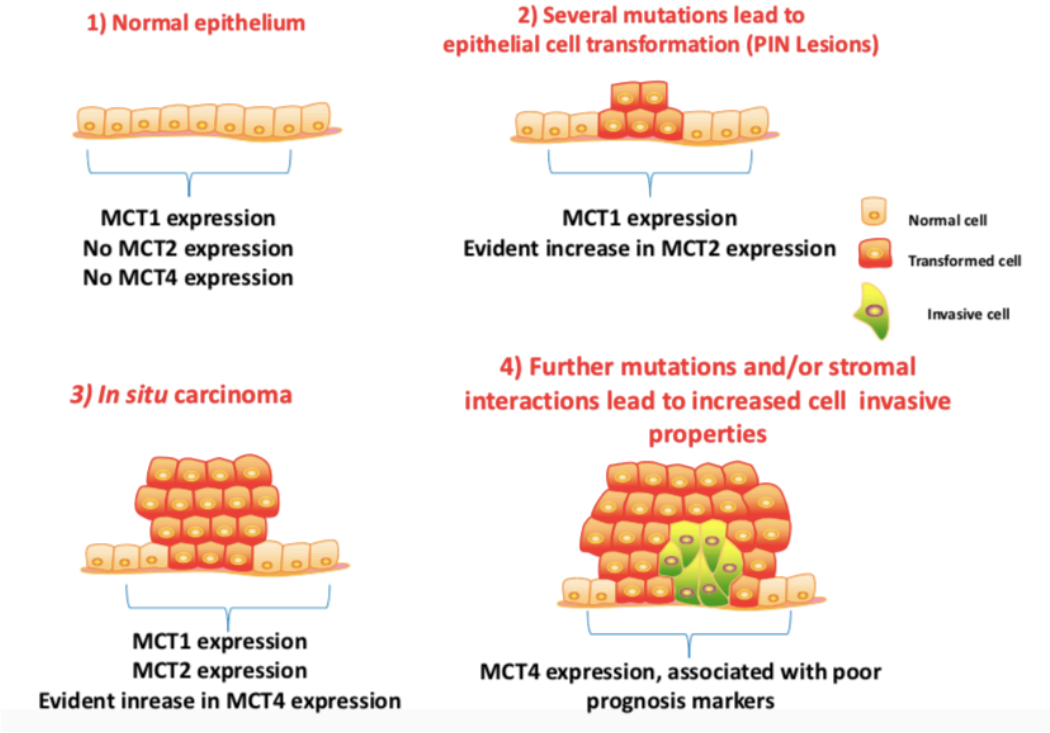
Lactate also induces the activation of HIF-1alfa and the secretion of VEGF alpha and ARG1, triggering neovascularization and immunosuppressive and remodeling processes, leading to high proportion of M2 macrophages (Tumor-associated macrophages) in the tumor stroma (329).

The fundamental role of lactate in the microenvironment of PCa led the attention to the monocarboxylate transporters (MCTs), which transport monocarboxylates across the membranes, acting as a way to communicate between tissues (328). MCTs are also important to transport another monocarboxylates such as pyruvate, the branched-chain oxoacids derived from leucine, valine and isoleucine, and the ketone bodies, acetoacetate, beta-hidroxybutyrate and acetate (333).

According to this, MCTs maintain the hyper-glycolytic and acid-resistant phenotypes, as part of the adaptation to the hypoxic microenvironment. The upregulation of MCTs is considered an adaptive mechanism by exporting the accumulating end-product (Lactate) (328).

According to Pertega-gomez et al (334), there is only MCT1 expression in the normal epithelium; there is an increase in MCT2 expression in the Prostatic intraepithelial Neoplastic lesions (PIN) and an increase in MCT4 plus MCT2 and MCT1 expression in localized PCa (Figure 30).

Figure 30. Monocarboxylate transporters in prostate tissue



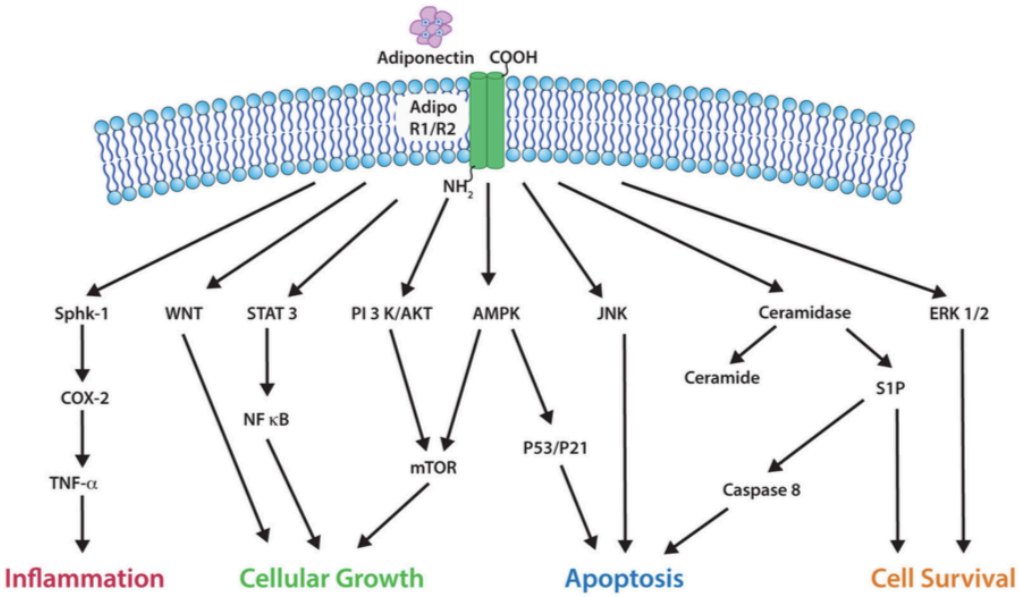
As a summary, normal prostate cells rely on glucose oxidation to provide precursors for synthesis and secretion of citrate, resulting in minimal energy production due to an incomplete krebs cycle and oxidative phosphorylation. On the other side, PCa cells no longer secrete citrate, they activate krebs cycle, however, primary and localized PCa does not increase aerobic glycolysis (328).

Another important event in PCa tumorigenesis is an increase in lipogenesis. Regarding this issue, many mechanisms have been proposed to link obesity and PCa, including the dysregulation of the insulin/insulin-like growth factor (IGF-1) axis, alteration of adipokine signaling and higher oxidative stress (335,336).
Obesity has been associated with higher levels of insulin which might be correlated with hyper-activation of intra-cellular transduction pathway that increases anabolic, antiapoptotic and mitotic activities of cancer cells. Additionally, the IGF-1 promotes mitogenesis, proangiogenesis and inhibits apoptosis facilitating the cancer progression. On the other side, adiponectin has been found to have antitumor effects by inhibiting cancer cell growth and inflammation. In obese men,

hypertrophic adipose tissue releases a disturbed profile of adipokines with elevated levels of leptin and low levels of adiponectin (337,338). Additionally, there is an obstruction of capillaries leading to hypoxia and ischemia which further creates a state of oxidative stress and an increase of reactive oxygen radicals that are able to damage cell DNA (336).

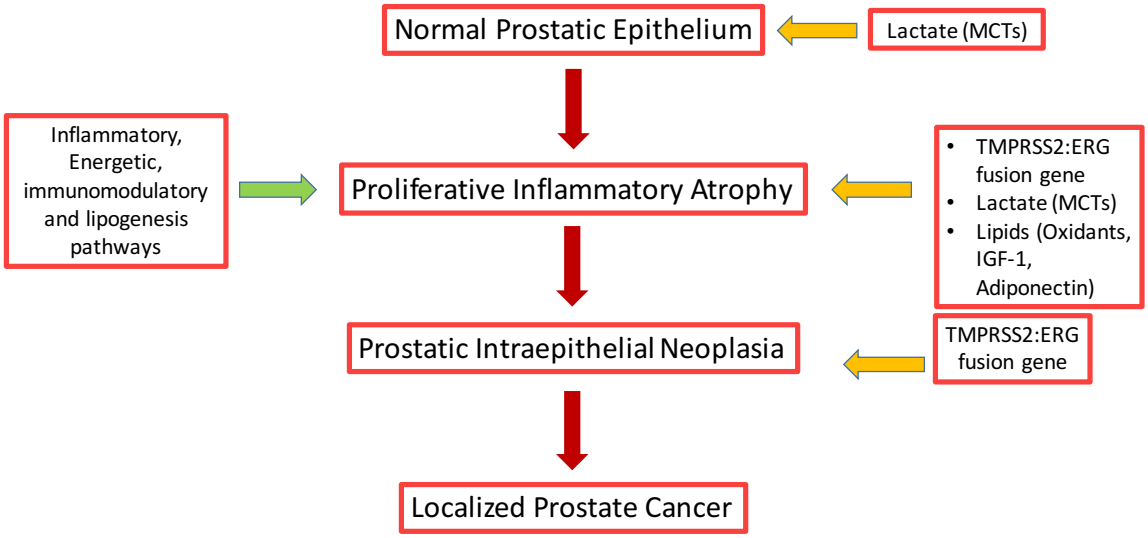
Adiponectin has been involved in different signaling pathways associated to cancer. By binding to AdipoR1 and AdipoR2, adiponectin initiates a cascade of events such as the activation of AMPK and other regulatory proteins to instigate a variety of actions. For example, anti-apoptotic actions mediated via the activation of ceramidase, Akt, and AMPK as well as via the supression of ERK1/2, PI3K/Akt, Wnt/β-catenin, NF-κB, JNK and the cell cycle regulators p53/p21, and by negatively influencing the mTOR/S6K axis and fatty acid synthase. On the other side, adiponectin can mediate inflammation by activation of sphingosine kinase-1 (Sphk1) and cyclooxygenase-2 (COX-2) (339) (Figure 31).

Figure 31. Adiponectin pathways. Taken from (339)



To conclude, there are a variety of mechanisms proposed to the pathophysiology of Prostate Cancer, therefore all those might be important elements to target in order to prevent the development of PCa or to perform therapeutic interventions in a localized PCa. According to this compendium I would like to propose the following pipeline just before the development of cancer or exactly at the very beginning of this condition (Figure 32):

Figure 32. Final model



Published papers:

Esquivel Parra LM, Caicedo Bolaños AM, Guaitarilla Soto JM **García-Perdomo HA**. Una Mirada general a los biomarcadores para la tamización y el diagnóstico temprano del cancer de próstata. Urol Colomb 2017; 26(2): 110-116

García-Perdomo HA, Zamora-Segura BD, Sanchez A. Frequency of allelic variants of the TMPRSS2 gene in a prostate cancer-free Southwestern Colombian Population. Rev Mex Urol 2018;87(5):354-358

García-Perdomo HA, Zapata-Copete JA, Sanchez A. Molecular alterations associated with prostate cancer. Cent European J Urol 2018;71:168-176

García-Perdomo HA, Zapata-Copete JA, Sanchez A. Una Mirada global y actualizada del cancer de próstata. Rev Fac Med 2018; 66(3):429-37

References

1. Ilic D, Neuberger MM, Djulbegovic M, Dahm P. Screening for prostate cancer. Cochrane database Syst Rev. 2013;1(1):CD004720.
2. Scher HI, Lu D, Schreiber NA, Louw J, Graf RP, Vargas HA, et al. Association of AR-V7 on Circulating Tumor Cells as a Treatment-Specific Biomarker With Outcomes and Survival in Castration-Resistant Prostate Cancer. JAMA Oncol. 2016;10065:1–9.
3. Antonarakis E, Lu C, Luber B, Wang H, Chen Y, Nakazawa M, et al. Androgen Receptor Splice Variant 7 and Efficacy of Taxane Chemotherapy in Patients With Metastatic Castration-Resistant Prostate Cancer. JAMA Oncol. 2015;1(5):582.
4. Antonarakis ES, Lu C, Wang H, Luber B, Nakazawa M, Roeser JC, et al. AR-V7 and Resistance to Enzalutamide and Abiraterone in Prostate Cancer. N Engl J Med. 2014;371(11):1028–38.
5. Ouyang H, Zhou Y, Zhang L, Shen G. Diagnostic Value of MicroRNAs for Urologic Cancers: A Systematic Review and Meta-Analysis. Medicine (Baltimore). 2015;94(37):e1272.
6. Luis J, Matiz P, Julián N, Reyes A, Paula M, Becerra S. Evolución de la mortalidad por cáncer de próstata en Colombia : estudio ecológico. 2014;23(1):3–10.
7. Center MM, Jemal A, Lortet-Tieulent J, Ward E, Ferlay J, Brawley O, et al. International variation in prostate cancer incidence and mortality rates. Eur Urol. 2012;61(6):1079–92.
8. Hamdy FC, Donovan JL, Lane JA, Mason M, Metcalfe C, Holding P, et al. 10-Year Outcomes after Monitoring, Surgery, or Radiotherapy for Localized Prostate Cancer. N Engl J Med. 2016;NEJMoa1606220.
9. Mottet (Chair) N, Bellmunt J, Briers (Patient Representative) E, Bolla M, Cornford (Vice-chair) P, De Santis M, et al. Guidelines on Prostate Cancer. European Association of Urology. 2017.
10. Bill-Axelson A, Holmberg L, Ruutu M, Haggman M, Andersson SO, Bratell S, et al. Radical Prostatectomy Versus Watchful Waiting in Localized Prostate Cancer. N Engl J Med. 2005;352(19):1977–1984.
11. Mancini M, Zazzara M, Zattoni F. Stem cells, biomarkers and genetic profiling: approaching future challenges in Urology. Riv Urol. 2016;83(1):4–13.
12. Restrepo JA, Bravo LE, García-Perdomo HA, García LS, Collazos P, Carbonell J. Incidencia, mortalidad y supervivencia al cáncer de próstata en Cali, Colombia, 1962-2011. Salud Publica Mex. 2014;56(5):440–7.
13. Garcia-Perdomo H. Cancer Centers of Excellence in Colombia : A fundamental way to work together. Urol Colomb. 2017;26(3):2017–8.
14. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016;66(1):7–30.
15. American Cancer Society. Cancer Facts & Figures for African Americans 2016-2018. Cancer Facts Fig 2016. 2016;
16. Esquivel Parra L, Caicedo Bolaños A, Guaitarilla J, García-Perdomo H. Una mirada general a los biomarcadores para la tamización y el diagnóstico temprano del cáncer de Próstata. Urol Colomb. 2017;26(2):110–6.
17. Hayes RB, Liff JM, Pottern LM, Greenberg RS, Schoenberg JB, Schwartz AG, et al. Prostate cancer risk in US blacks and whites with a family history of cancer. int j cancer. 1995;60(3):361–64.

18. Hoffman RM, Gilliland FD, Eley JW, Harlan LC, Stephenson RA, Stanford JL, et al. Racial and ethnic differences in advanced-stage prostate cancer: the Prostate Cancer Outcomes Study. J Natl Cancer Inst. 2001;93(5):388–95.
19. Taksler GB, Keating NL, Cutler DM. Explaining racial differences in prostate cancer mortality. Cancer. 2012;118(17):4280–9.
20. Graham-Steed T, Uchio E, Wells CK, Aslan M, Ko J, Concato J. "Race" and prostate cancer mortality in equal-access healthcare systems. Am J Med. Elsevier Inc; 2013;126(12):1084–8.
21. Sim HG, Cheng CWS. Changing demography of prostate cancer in Asia. European Journal of Cancer. 2005. p. 834–45.
22. Whittemore AS, Wu AH, Kolonel LN, John EM, Gallagher RP, Howe GR, et al. Family History and Prostate Cancer Risk in Black , White , and Asian Men in the United States and Canada. Am J Epidemiol. 1995;141(8):732–40.
23. Caini S, Gandini S, Dudas M, Bremer V, Severi E, Gherasim A. Sexually transmitted infections and prostate cancer risk: A systematic review and meta-analysis. Cancer Epidemiology. 2014. p. 329–38.
24. Dennis LK, Lynch CF, Torner JC. Epidemiologic association between prostatitis and prostate cancer. Urology. 2002;60(1):78–83.
25. Oh B, Figtree G, Costa D, Eade T, Hruby G, Lim S, et al. Oxidative stress in prostate cancer patients: A systematic review of case control studies. Prostate Int. 2016;4(3):71–8.
26. Khandrika L, Kumar B, Koul S, Maroni P, Koul HK. Oxidative stress in prostate cancer. Cancer Letters. 2009. p. 125–36.
27. Michaud JE, Billups KL, Partin AW. Testosterone and prostate cancer: an evidence-based review of pathogenesis and oncologic risk. Ther Adv Urol. 2015;7(6):378–87.
28. Nelles JL, Hu W-Y, Prins GS. Estrogen action and prostate cancer. Expert Rev Endocrinol Metab. 2011;6(3):437–51.
29. Jurado-Penagos A, García Perdomo HA. Efectividad de los micronutrientes en la prevención del cáncer de próstata. Rev la Fac Med. 2014;62(1):65–72.
30. Gennigens C, Menetrier-Caux C, Droz JP. Insulin-Like Growth Factor (IGF) family and prostate cancer. Critical Reviews in Oncology/Hematology. 2006. p. 124–45.
31. Fradet-Turcotte A, Sitz J, Grapton D, Orthwein A. BRCA2 functions: From DNA repair to replication fork stabilization. Endocr Relat Cancer. 2016;23(10):T1–17.
32. Gulati R, Cheng HH, Lange PH, Nelson PS, Etzioni R. Screening men at increased risk for prostate cancer diagnosis: Model estimates of benefits and harms. Cancer Epidemiol Biomarkers Prev. 2016;
33. Song WH, Kim SH, Joung JY, Park WS, Seo HK, Chung J, et al. Prostate Cancer in a Patient with a Family History of BRCA Mutation: a Case Report and Literature Review. J Korean Med Sci. 2017;32(2):377–81.
34. Kaaks R, Stattin P. Obesity, endogenous hormone metabolism, and prostate cancer risk: a conundrum of "highs" and "lows". Cancer Prev Res (Phila). 2010;3(3):259–62.
35. Perdana NR, Mochtar CA, Umbas R, Hamid ARAH. The Risk Factors of Prostate Cancer and Its Prevention : A Literature Review. :228–38.
36. Rota M, Scotti L, Turati F, Tramacere I, Islami F, Bellocco R, et al. Alcohol consumption and prostate cancer risk: A meta-analysis of the dose-risk relation. Eur J Cancer Prev. 2012;21(4):350–9.

37. Islami F, Moreira DM, Boffetta P, Freedland SJ. A systematic review and meta-analysis of tobacco use and prostate cancer mortality and incidence in prospective cohort studies. Eur Urol. 2014;66(6):1054–64.
38. Bell KJL, Del Mar C, Wright G, Dickinson J, Glasziou P. Prevalence of incidental prostate cancer: A systematic review of autopsy studies. Int J Cancer. 2015;137(7):1749–57.
39. Epstein JI, Egevad L, Amin MB, Delahunt B, Srigley JR, Humphrey PA, et al. The 2014 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma: Definition of Grading Patterns and Proposal for a New Grading System. Am J Surg Pathol. 2016;40(2):244–52.
40. The American Joint Committee on Cancer (AJCC). AJCC Cancer Staging Manual. 8th ed. Amin MB, Edge S, Green F, Byrd DR, Brookland RK, Washington MK, et al., editors. Springer International Publishing; 2016. 1024 p.
41. D'Amico A V., Whittington R, Malkowicz SB, Schultz D, Blank K, Broderick GA, et al. Biochemical Outcome After Radical Prostatectomy, External Beam Radiation Therapy, or Interstitial Radiation Therapy for Clinically Localized Prostate Cancer. JAMA. 1998;280(11):969.
42. National Comprehensive Cancer Network. NCCN Guidelines for Prostate Cancer. 2016.
43. Claves P. PSA (Antígeno Prostático Específico) Enfoque 2012. 2012;1–21.
44. Garcia HA, Carbonell J. Urología en pocas palabras. 2011. 261: 210-221.
45. Ortiz Sanchez JM. La eficiencia del índice de PSA libre en el diagnostico de Cáncer de Próstata. 2014.
46. Salman J., Schoots I., Carlsson S., Jenster G, Roobol M. Prostate Specific Antigen as a Tumor Marker in Prostate Cancer: Biochemical and Clinical Aspects. Adv Cancer Biomarkers. 2015;93–114.
47. Saini S. PSA and beyond: alternative prostate cancer biomarkers. Cell Oncol. 2016;
48. Chadha KC, Miller A, Nair BB, Schwartz SA, Trump DL, Underwood W. New serum biomarkers for prostate cancer diagnosis. Clin cancer Investig J. 2015;3(1):72–9.
49. Wolf AM, Wender RC, Etzioni RB, Thompson IM, Amico A Vd, Volk RJ, et al. American Cancer Society Guideline for the Early Detection of Prostate Cancer Update 2010. Cancer Journal, The. 2010;60(2):70–98.
50. Andriole GL, Crawford ED, Grubb RL, Buys SS, Chia D, Church TR, et al. Prostate cancer screening in the randomized prostate, lung, colorectal, and ovarian cancer screening trial: Mortality results after 13 years of follow-up. J Natl Cancer Inst. 2012;104(2):125–32.
51. Schroder F, Hugosson J, Roobol M, Tammela T, Ciatto S. Prostate-Cancer Mortality at 11 Years of Follow-up. N Engl J Med. 2012;366(11):981–90.
52. Ilic D, Connor DO, Green S, Wilt TJ. Screening for prostate cancer : an updated Cochrane systematic review. BJU Int. 2011;107(6):882–91.
53. Kontos CK, Adamopoulos PG, Scorilas A, Kontos CK. cancer Prognostic and predictive biomarkers in prostate cancer. 2015;7159(November).
54. García P HA, Varela R R. Validez diagnóstica del antígeno prostático específico para la presencia de metástasis en pacientes con cáncer de próstata. Urol Colomb. 2010;XIX(3):13–8.
55. Mottet N, Bellmunt J, Patient EB, Associate RCNVDBG, Culine S, Joniau S, et al. Guidelines on Prostate Cancer. Eur Assoc Urol. 2015;

56. Albert JM, Swanson DA, Pugh TJ, Zhang M, Bruno TL, Kudchadker RJ, et al. Magnetic resonance imaging-based treatment planning for prostate brachytherapy. Brachytherapy. 2013;12(1):30–7.
57. von Eyben FE, Kairemo K. Meta-analysis of (11)C-choline and (18)F-choline PET/CT for management of patients with prostate cancer. Nucl Med Commun. 2014;35(3):221–30.
58. Wallis CJD, Saskin R, Choo R, Herschorn S, Kodama RT, Satkunasivam R, et al. Surgery Versus Radiotherapy for Clinically-localized Prostate Cancer: A Systematic Review and Meta-analysis. Eur Urol. European Association of Urology; 2016;70(1):21–30.
59. Gonzalgo M, Stefanos K, Meeker A. Part IV. Molecular and cellular biology. In: Wein A, Kavoussi L, Partin A, Peters C, editors. Campbell Walsh Urology. Eleventh. Philadelphia: Elsevier Inc.; 2016. p. 459.
60. Feitelson MA, Arzumanyan A, Kulathinal RJ, Blain SW, Holcombe RF, Mahajna J, et al. Sustained proliferation in cancer: Mechanisms and novel therapeutic targets. Semin Cancer Biol. 2015;35:S25–54.
61. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. Cell. 2011;144(5):646–74.
62. Luo J, Solimini NL, Elledge SJ. Principles of cancer therapy: oncogene and non-oncogene addiction. Cell. 2009;136(5):823–37.
63. Netto GJ. Molecular Updates in Prostate Cancer. Surg Pathol Clin. 2015;8(4):561–80.
64. Netto GJ, Cheng L. Emerging critical role of molecular testing in diagnostic genitourinary pathology. Arch Pathol Lab Med. 2012;136(4):372–90.
65. Gil J, Kerai P, Lleonart M, Bernard D, Cigudosa JC, Peters G, et al. Immortalization of primary human prostate epithelial cells by c-Myc. Cancer Res. 2005;65(6):2179–85.
66. Schmidt L, Duh FM, Chen F, Kishida T, Glenn G, Choyke P, et al. Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. Nat Genet. 1997;16(1):68–73.
67. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun X-W, et al. Recurrent Fusion of TMPRSS2 and ETS Transcription Factor Genes in Prostate Cancer. Science (80-). 2005;310(5748):644–8.
68. Bartek J, Lukas J, Bartkova J. Perspective: defects in cell cycle control and cancer. J Pathol. 1999;187(1):95–9.
69. Schulz W, Burchardt M, Cronauer M. Molecular biology of prostate cancer. Mol Hum Reprod. 2003;9(8):437–48.
70. Kubota Y, Fujinami K, Uemura H, Dobashi Y, Miyamoto H, Iwasaki Y, et al. Retinoblastoma gene mutations in primary human prostate cancer. Prostate. 1995;27(6):314–20.
71. Logothetis CJ, Xu HJ, Ro JY, Hu SX, Sahin A, Ordonez N, et al. Altered expression of retinoblastoma protein and known prognostic variables in locally advanced bladder cancer. J Natl Cancer Inst. 1992;84(16):1256–61.
72. Sherr CJ, Roberts JM. Inhibitors of mammalian G1 cyclin-dependent kinases. Genes Dev. 1995;9(10):1149–63.
73. Serrano M, Hannon GJ, Beach D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature. 1993;366(6456):704–7.
74. Merlo A, Herman J, Mao L, Lee D, Gabrielson E, Burger P, et al. 5′ CpG island methylation is associated with transcriptional silencing of the tumour suppressor p16/CDKN2/ MTS1 in human cancers. Nat Med. 1995;1:686–92.

75. Herman JG, Merlo A, Mao L, Herman G, Lapidus G, Issa J, et al. Inactivation of the CDKN2 / p16 / MTS1 Gene Is Frequently Associated with Aberrant DNA Methylation in All Common Human Cancers DNA Methylation in All Common Human Cancers. Cancer. 1995;55(1):4525–30.
76. Freedland SJ, DeGregorio F, Sacoolidge JC, Elshimali YI, Csathy GS, Dorey F, et al. Preoperative p27 status is an independent predictor of prostate specific antigen failure following radical prostatectomy. J Urol. 2003;169(4):1325–30.
77. Cordon-Cardo C, Koff A, Drobnjak M, Capodieci P, Osman I, Millard SS, et al. Distinct altered patterns of p27KIP1 gene expression in benign prostatic hyperplasia and prostatic carcinoma. J Natl Cancer Inst. 1998;90(17):1284–91.
78. Oda E, Ohki R, Murasawa H, Nemoto J, Shibue T, Yamashita T, et al. Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. Science (80-). 2000;288:1053–8.
79. Jerónimo C, Usadel H, Henrique R, Oliveira J, Lopes C, Nelson WG, et al. Quantitation of GSTP1 methylation in non-neoplastic prostatic tissue and organ-confined prostate adenocarcinoma. J Natl Cancer Inst. 2001;93(22):1747–52.
80. Maruyama R, Toyooka S, Toyooka KO, Virmani AK, Zöchbauer-Müller S, Farinas AJ, et al. Aberrant promoter methylation profile of prostate cancers and its relationship to clinicopathological features. Clin cancer Res. 2002;8(2):514–9.
81. Liu L, Yoon J-H, Dammann R, Pfeifer GP. Frequent hypermethylation of the RASSF1A gene in prostate cancer. Oncogene. 2002;21(44):6835–40.
82. Kuzmin I, Gillespie JW, Protopopov A, Geil L, Dreijerink K, Yang Y, et al. The RASSF1A tumor suppressor gene is inactivated in prostate tumors and suppresses growth of prostate carcinoma cells. Cancer Res. 2002;62(12):3498–502.
83. Yegnasubramanian S, Wu Z, Haffner MC, Esopi D, Aryee MJ, Badrinath R, et al. Chromosome-wide mapping of DNA methylation patterns in normal and malignant prostate cells reveals pervasive methylation of gene-associated and conserved intergenic sequences. BMC Genomics. 2011;12(1):313.
84. Aryee MJ, Liu W, Engelmann JC, Nuhn P, Gurel M, Haffner MC, et al. DNA methylation alterations exhibit intraindividual stability and interindividual heterogeneity in prostate cancer metastases. Sci Transl Med. 2013;5:169.
85. Nock NL, Bock C, Neslund-Dudas C, Beebe-Dimmer J, Rundle A, Tang D, et al. Polymorphisms in glutathione S-transferase genes increase risk of prostate cancer biochemical recurrence differentially by ethnicity and disease severity. Cancer Causes Control. 2009;20(10):1915–26.
86. Kumar-Sinha C, Tomlins SA, Chinnaiyan AM. Recurrent gene fusions in prostate cancer. Nat Rev Cancer. 2008;8(7):497–511.
87. Perner S, Mosquera J-M, Demichelis F, Hofer MD, Paris PL, Simko J, et al. TMPRSS2-ERG fusion prostate cancer: an early molecular event associated with invasion. Am J Surg Pathol. 2007;31(6):882–8.
88. Falzarano SM, Zhou M, Carver P, Tsuzuki T, Simmerman K, He H, et al. ERG gene rearrangement status in prostate cancer detected by immunohistochemistry. Virchows Arch. 2011;459(4):441–7.
89. Park K, Tomlins SA, Mudaliar KM, Chiu Y-L, Esgueva R, Mehra R, et al. Antibody-based detection of ERG rearrangement-positive prostate cancer. Neoplasia. 2010;12(7):590–8.

90. Chaux A, Albadine R, Toubaji A, Hicks J, Meeker A, Platz EA, et al. Immunohistochemistry for ERG expression as a surrogate for TMPRSS2-ERG fusion detection in prostatic adenocarcinomas. Am J Surg Pathol. 2011;35(7):1014–20.
91. Fine SW, Gopalan A, Leversha MA, Al-Ahmadie HA, Tickoo SK, Zhou Q, et al. TMPRSS2-ERG gene fusion is associated with low Gleason scores and not with high-grade morphological features. Mod Pathol. 2010;23(10):1325–33.
92. Leyten G, Hessels D, Jannink SA, Smit FP, de Jong H, Cornel EB, et al. Prospective Multicentre Evaluation of PCA3 and TMPRSS2-ERG Gene Fusions as Diagnostic and Prognostic Urinary Biomarkers for Prostate Cancer. Eur Urol. 2014;65(3):534–42.
93. Helgeson BE, Tomlins SA, Shah N, Laxman B, Cao Q, Prensner JR, et al. Characterization of TMPRSS2:ETV5 and SLC45A3:ETV5 gene fusions in prostate cancer. Cancer Res. 2008;68(1):73–80.
94. Tomlins SA, Mehra R, Rhodes DR, Smith LR, Roulston D, Helgeson BE, et al. TMPRSS2:ETV4 gene fusions define a third molecular subtype of prostate cancer. Cancer Res. 2006;66(7):3396–400.
95. Pflueger D, Terry S, Sboner A, Habegger L, Esgueva R, Lin P-C, et al. Discovery of non-ETS gene fusions in human prostate cancer using next-generation RNA sequencing. Genome Res. 2011;21(1):56–67.
96. Sarker D, Reid AHM, Yap TA, de Bono JS. Targeting the PI3K/AKT pathway for the treatment of prostate cancer. Clin cancer Res. 2009;15(15):4799–805.
97. Bismar TA, Yoshimoto M, Vollmer RT, Duan Q, Firszt M, Corcos J, et al. PTEN genomic deletion is an early event associated with ERG gene rearrangements in prostate cancer. BJU Int. 2011;107(3):477–85.
98. Han B, Mehra R, Lonigro RJ, Wang L, Suleman K, Menon A, et al. Fluorescence in situ hybridization study shows association of PTEN deletion with ERG rearrangement during prostate cancer progression. Mod Pathol. 2009;22(8):1083–93.
99. Fordyce CA, Heaphy CM, Joste NE, Smith AY, Hunt WC, Griffith JK. Association between cancer-free survival and telomere DNA content in prostate tumors. J Urol. 2005;173(2):610–4.
100. Heaphy CM, Yoon GS, Peskoe SB, Joshu CE, Lee TK, Giovannucci E, et al. Prostate cancer cell telomere length variability and stromal cell telomere length as prognostic markers for metastasis and death. Cancer Discov. 2013;3(10):1130–41.
101. Fadok VA, Savill JS, Haslett C, Bratton DL, Doherty DE, Campbell PA, et al. Different populations of macrophages use either the vitronectin receptor or the phosphatidylserine receptor to recognize and remove apoptotic cells. J Immunol. 1992;149(12):4029–35.
102. Wolf BB, Green DR. Suicidal tendencies: apoptotic cell death by caspase family proteinases. J Biol Chem. 1999;274(29):20049–52.
103. Koivisto P, Visakorpi T, Rantala I, Isola J. Increased cell proliferation activity and decreased cell death are associated with the emergence of hormone-refractory recurrent prostate cancer. J Pathol. 1997;183(1):51–6.
104. Adams JM, Cory S. The Bcl-2 protein family: arbiters of cell survival. Science (80-). 1998;281(5381):1322–6.
105. Kroemer G, Reed JC. Mitochondrial control of cell death. Nat Med. 2000;6(5):513–9.
106. Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. Science (80-). 1998;281(5381):1305–8.
107. Grimberg A, Cohen P. Role of insulin-like growth factors and their binding proteins in

growth control and carcinogenesis. J Cell Physiol. 2000;183(1):1–9.
108. Chan JM, Stampfer MJ, Ma J, Gann P, Gaziano JM, Pollak M, et al. Insulin-like growth factor-I (IGF-I) and IGF binding protein-3 as predictors of advanced-stage prostate cancer. J Natl Cancer Inst. 2002;94(14):1099–106.
109. Srikantan V, Valladares M, Rhim JS, Moul JW, Srivastava S. HEPSIN inhibits cell growth/invasion in prostate cancer cells. Cancer Res. 2002;62(23):6812–6.
110. Jiang Z, Woda BA, Rock KL, Xu Y, Savas L, Khan A, et al. P504S: a new molecular marker for the detection of prostate carcinoma. Am J Surg Pathol. 2001;25(11):1397–404.
111. Rubin MA, Zhou M, Dhanasekaran SM, Varambally S, Barrette TR, Sanda MG, et al. Alpha-Methylacyl coenzyme A racemase as a tissue biomarker for prostate cancer. JAMA. 2002;287(13):1662–70.
112. Sfanos KS, Hempel HA, De Marzo AM. The role of inflammation in prostate cancer. Adv Exp Med Biol. 2014;816:153–81.
113. De Marzo AM, Platz EA, Sutcliffe S, Xu J, Grönberg H, Drake CG, et al. Inflammation in prostate carcinogenesis. Nat Rev Cancer. 2007;7(4):256–69.
114. Elkahwaji JE. The role of inflammatory mediators in the development of prostatic hyperplasia and prostate cancer. Res reports Urol. 2012;5:1–10.
115. Sutcliffe S, Neace C, Magnuson NS, Reeves R, Alderete JF. Trichomonosis, a common curable STI, and prostate carcinogenesis--a proposed molecular mechanism. PLoS Pathog. 2012;8(8):e1002801.
116. Sutcliffe S, Platz EA. Inflammation and prostate cancer: a focus on infections. Curr Urol Rep. 2008;9(3):243–9.
117. Survival rates for prostate cancer. American Cancer Society.
118. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Int Agency Res Cancer. 2013;
119. Hoffman RM. Screening for Prostate Cancer. N Engl J Med. 2011;1–7.
120. Balk SP. Biology of Prostate-Specific Antigen. J Clin Oncol. 2003;21(2):383–91.
121. Mikolajczyk SD, Millar LS, Wang TJ, Rittenhouse HG, Wolfert RL, Marks LS, et al. "BPSA," a specific molecular form of free prostate-specific antigen, is found predominantly in the transition zone of patients with nodular benign prostatic hyperplasia. Urology. 2000;55(1):41–5.
122. Mikolajczyk SD, Millar LS, Wang TJ, Rittenhouse HG, Marks LS, Song W, et al. A precursor form of prostate-specific antigen is more highly elevated in prostate cancer compared with benign transition zone prostate tissue. Cancer Res. 2000;60(3):756–9.
123. Djulbegovic M, Beyth RJ, Neuberger MM, Stoffs TL, Vieweg J, Djulbegovic B. Screening for prostate cancer: systematic review and meta-analysis of randomised controlled trials. BMJ. 2010;341:c4543:1–9.
124. Wei W, Leng J, Shao H, Wang W. High PCA3 scores in urine correlate with poor-prognosis factors in prostate cancer patients. 2015;8(9):16606–12.
125. Jedinak A, Curatolo A, Zurakowski D, Dillon S, Bhasin MK, Libermann TA, et al. Novel non-invasive biomarkers that distinguish between benign prostate hyperplasia and prostate cancer. BMC Cancer. ???; 2015;15:1–9.
126. Pedersen LO, Hansen AS, Olsen AC, Gerwien J, Nissen MH, Buus S. The interaction between beta 2-microglobulin (??2m) and purified class-I major histocompatibility (MHC) antigen. Scand J Immunol. 1994;39(1):64–72.

127. Berggard I, Bearn AG. Isolation and properties of a low molecular weight B2-globulin occurring in human biological fluids. J Biol Chem. 1968;243:4095–103.
128. Klein T, Levin I, NISKA A, JOREN R, GAL R, SCHATER J. CORRELATION B E T W E E N T U M O U R AND S E R U M P2m. Eur J Immunogenet. 1996;(1 23):417–23.
129. Auer I, Watzel C, Greulich M. The plasma concentration of b2-microglobulin in the diagnosis of malignancy of the gastrointestinal tract. Med Klin. 1979;(74):1581–3.
130. Huang W, Wu D, Xie Z, Pohl J, Hsieh C, Weitzmann MN, et al. β 2-Microglobulin Is a Signaling and Growth-Promoting Factor for Human Prostate Cancer Bone Metastasis. Cancer Res. 2006;66(18):9108–16.
131. Rowley DR, Dang TD, Mcbride L, Gerdes MJ, Lu B. β -2 Microglobulin Is Mitogenic to PC-3 Prostatic Carcinoma Cells and Antagonistic to Transforming Growth Factor β 1 Action Microglobulin Is Mitogenic to PC-3 Prostatic Carcinoma Cells and Antagonistic to Transforming Growth Factor flu Action1. Cancer Res. 1995;55:781–6.
132. Yang J, Qian J, Wezeman M, Wang S, Lin P, Wang M, et al. Targeting B2-microglobulin for induction of tumor apoptosis in human hematological malignancies. Cancer Cell. 2006;10(4):295–307.
133. Josson S, Nomura T, Lin J-T, Huang W-C, Wu D. B2-Microglobulin induces epitelial to mesenchymal transition and confers cancer lethality and bone metástasis in human cancer cells. cancer res. 2011;(7):2600–10.
134. Zhang Y xin, Wang L, Ji P yu, Zhao G gang, Zhong G ping, Wang Z ping. Correlation of serum β2-microglobulin levels with prostate-specific antigen, gleason score, clinical stage, tumor metastasis and therapy efficacy inprostate cancer. Arch Med Res. Elsevier Inc; 2013;44(4):259–65.
135. Huang WC, J H, Zhau H. B2-Microglobulin signaling blockade inhibited androgen receptor axis and caused apoptosis in human prostate cancer cells. Clin Cancer Res. 2008;14(17):5341–7.
136. Mcdonald ML, Parsons JK. 4-Kallikrein Test a nd Ka llik rein Mar ker s i n P ro s t a t e C a n c e r S c re e n i n g. Urol Clin NA. Elsevier Inc; 2016;43(1):39–46.
137. Vickers AJ, Cronin AM, Aus G, Puihl C-G, Becker C, Pettersson K, et al. Impact of recent screening on predicting the outcome of prostate cancer biopsy in men with elevated PSA: data from the European Randomized Study of Prostate Cancer Screening in Gothenburg, Sweden. 2010;116(11):2612–20.
138. Bryant RJ, Sjoberg DD, Vickers AJ, Robinson MC, Kumar R, Marsden L, et al. Predicting High-Grade Cancer at Ten-Core Prostate Biopsy Using Four Kallikrein Markers Measured in Blood in the ProtecT Study. 2015;107:1–6.
139. Vickers AJ, Cronin AM, Roobol MJ, Savage CJ, Pettersson K, Scardino PT, et al. A four-kallikrein panel predicts prostate cancer in men with recent screening: data from the European Randomized Study of Prostate Cancer Screening, Rotterdam. 2010;16(12):3232–9.
140. Braun K, Sjoberg DD, Vickers AJ, Lilja H, Bjartell AS, Catto J. A Four-kallikrein Panel Predicts High-grade Cancer on Biopsy : Independent Validation in a Community Cohort. Eur Urol. European Association of Urology; 2015;1–7.
141. Parekh DJ, Punnen S, Sjoberg DD, Asroff SW, Bailen JL, Cochran JS, et al. A Multi-institutional Prospective Trial in the USA Confirms that the 4Kscore Accurately Identifies Men with High-grade Prostate Cancer. Eur Urol. European Association of Urology; 2014;1–7.

142. O'Connor JC, Julian J, Lim SD, Carson DD. MUC1 expression in human prostate cancer cell lines and primary tumors. Prostate Cancer Prostatic Dis. 2005;8(1):36–44.
143. Cozzi PJ, Wang J, Delprado W, Perkins AC, Allen BJ, Russell J, et al. MUC1 , MUC2 , MUC4 , MUC5AC and MUC6 expression in the progression of prostate cancer. Springer. 2006;22:565–73.
144. Li Y, Cozzi PJ. MUC1 is a promising therapeutic target for prostate cancer therapy. Curr Cancer Drug Targets. 2007;7(3):259–71.
145. Ananthanarayanan V, Deaton RJ, Yang XJ, Pins MR, Gann PH. Alpha-methylacyl-CoA racemase (AMACR) expression in normal prostatic glands and high-grade prostatic intraepithelial neoplasia (HGPIN): Association with diagnosis of prostate cancer. Prostate. 2005;63(4):341–6.
146. Alinezhad S, Väänänen R-M, Ochoa NT, Vertosick EA, Bjartell A, Boström PJ, et al. Global expression of AMACR transcripts predicts risk for prostate cancer – a systematic comparison of AMACR protein and mRNA expression in cancerous and noncancerous prostate. BMC Urol. BMC Urology; 2016;16(1):10.
147. Jiang Z, Woda BA, Wu C-L, Yang XJ. Discovery and clinical application of a novel prostate cancer marker: alpha-methylacyl CoA racemase (P504S). Am J Clin Pathol. 2004;122(2):275–89.
148. Markley JL, Brüschweiler R, Edison AS, Eghbalnia HR, Powers R, Raftery D, et al. The future of NMR-based metabolomics. Curr Opin Biotechnol. 2017;43:34–40.
149. Robinette SL, Brüschweiler R, Schroeder FC, Edison AS. NMR in Metabolomics and Natural Products Research: Two Sides of the Same Coin. Acc Chem Res. American Chemical Society; 2012 Feb 21;45(2):288–97.
150. Roux A, Lison D, Junot C, Heilier J-F. Applications of liquid chromatography coupled to mass spectrometry-based metabolomics in clinical chemistry and toxicology: A review. Clin Biochem. 2011 Jan;44(1):119–35.
151. Smolinska A, Blanchet L, Buydens LMC, Wijmenga SS. NMR and pattern recognition methods in metabolomics: From data acquisition to biomarker discovery: A review. Anal Chim Acta. Elsevier B.V.; 2012;750:82–97.
152. Gowda N, Zhang S, Gu H, Asiago V, Shanaiah N, Raftery D. Metabolomics-Based Methods for Early Disease Diagnostics: A Review. Expert Rev Mol Diagn. 2008;8(5):617–33.
153. Nicholson J, Lindon J, Holmes E. "Metabonomics": understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica. 1999;29:1181–1189.
154. Bollard M, Stanley E, Lindon J, Nicholson J, Holmes E. NMR-based metabonomic approaches for evaluating physiological influences on biofluid composition. NMR Biomed. 2005;18:143–162.
155. Kemperman RFJ, Horvatovich PL, Hoekman B, Reijmers TH, Muskiet FAJ, Bischoff R. Comparative urine analysis by liquid chromatography-mass spectrometry and multivariate statistics: method development, evaluation, and application to proteinuria. J Proteome Res. 2007 Jan;6(1):194–206.
156. Zhang A, Sun H, Wang P, Han Y, Wang X. Recent and potential developments of biofluid analyses in metabolomics. J Proteomics. 2012 Feb 2;75(4):1079–88.
157. Griffin JL, Kauppinen RA. Tumour metabolomics in animal models of human cancer. J Proteome Res. 2007 Feb;6(2):498–505.

158. Saude E, Slupsky C, Sykes B. Optimization of NMR analysis of biological fluids for quantitative accuracy. Metabolomics. 2006;2:113–123.
159. Mo H, Raftery D. Pre-SAT180, a simple and effective faraway water suppression method. J Magn Reson. 2008;190:1–6.
160. Tang H, Wang Y, Nicholson JK, Lindon JC. Use of relaxation-edited one-dimensional and two dimensional nuclear magnetic resonance spectroscopy to improve detection of small metabolites in blood plasma. Anal Biochem. 2004 Feb 15;325(2):260–72.
161. Xi Y, de Ropp JS, Viant MR, Woodruff DL, Yu P. Improved identification of metabolites in complex mixtures using HSQC NMR spectroscopy. Anal Chim Acta. 2008 May 5;614(2):127–33.
162. Espinosa JF. High resolution magic angle spinning NMR applied to the analysis of organic compounds bound to solid supports. Curr Top Med Chem. 2011;11(1):74–92.
163. Zhang A, Wang P, Sun H, Yan G, Han Y, Wang X. High-throughput ultra-performance liquid chromatography-mass spectrometry characterization of metabolites guided by a bioinformatics program. Mol Biosyst. 2013 Sep;9(9):2259.
164. Granger J, Bake A, Plumb R, Perez J, Wilson I. Ultra performance liquid chromatography- MS(TOF): New separations technology for high throughput metabonomics. Drug Met Rev. 2004;36:252–252.
165. Wilson ID, Plumb R, Granger J, Major H, Williams R, Lenz EM. HPLC-MS-based methods for the study of metabonomics. J Chromatogr B Analyt Technol Biomed Life Sci. 2005 Mar 5;817(1):67–76.
166. Wilson ID, Nicholson JK, Castro-Perez J, Granger JH, Johnson KA, Smith BW, et al. High resolution "ultra performance" liquid chromatography coupled to oa-TOF mass spectrometry as a tool for differential metabolic pathway profiling in functional genomic studies. J Proteome Res. 2005 Apr;4(2):591–8.
167. Ryan D, Robards K, Prenzler PD, Kendall M. Recent and potential developments in the analysis of urine: a review. Anal Chim Acta. 2011 Jan 17;684(1–2):8–20.
168. Pasikanti KK, Ho PC, Chan ECY. Development and validation of a gas chromatography/mass spectrometry metabonomic platform for the global profiling of urinary metabolites. Rapid Commun Mass Spectrom. 2008 Oct 15;22(19):2984–92.
169. Bingol K, Bruschweiler-Li L, Yu C, Somogyi A, Zhang F, Brüschweiler R. Metabolomics beyond spectroscopic databases: a combined MS/NMR strategy for the rapid identification of new metabolites in complex mixtures. Anal Chem. 2015 Apr 7;87(7):3864–70.
170. Bingol K, Brüschweiler R. NMR/MS Translator for the Enhanced Simultaneous Analysis of Metabolomics Mixtures by NMR Spectroscopy and Mass Spectrometry: Application to Human Urine. J Proteome Res. 2015 Jun 5;14(6):2642–8.
171. Gu H, Gowda GAN, Neto FC, Opp MR, Raftery D. RAMSY: Ratio Analysis of Mass Spectrometry to Improve Compound Identification. Anal Chem. American Chemical Society; 2013 Nov 19;85(22):10771–9.
172. Wishart DS, Knox C, Guo AC, Eisner R, Young N, Gautam B, et al. HMDB: a knowledgebase for the human metabolome. Nucleic Acids Res. Oxford University Press; 2009 Jan 1;37(Database):D603–10.
173. Ulrich EL, Akutsu H, Doreleijers JF, Harano Y, Ioannidis YE, Lin J, et al. BioMagResBank. Nucleic Acids Res. 2008;36:402–8.
174. Sud M, Fahy E, Cotter D, Azam K, Vadivelu I, Burant C, et al. Metabolomics Workbench: An international repository for metabolomics data and metadata, metabolite

standards, protocols, tutorials and training, and analysis tools. Nucleic Acids Res. 2016 Jan 4;44(D1):D463-70.
175. Salek RM, Neumann S, Schober D, Hummel J, Billiau K, Kopka J, et al. COordination of Standards in MetabOlomicS (COSMOS): facilitating integrated metabolomics data access. Metabolomics. 2015 Dec 26;11(6):1587–97.
176. Dona AC, Jiménez B, Schäfer H, Humpfer E, Spraul M, Lewis MR, et al. Precision High-Throughput Proton NMR Spectroscopy of Human Urine, Serum, and Plasma for Large-Scale Metabolic Phenotyping. Anal Chem. American Chemical Society; 2014 Oct 7;86(19):9887–94.
177. Emwas A-H, Roy R, McKay RT, Ryan D, Brennan L, Tenori L, et al. Recommendations and Standardization of Biomarker Quantification Using NMR-Based Metabolomics with Particular Focus on Urinary Analysis. J Proteome Res. 2016 Feb 5;15(2):360–73.
178. Chiaradonna F, Moresco RM, Airoldi C, Gaglio D, Palorini R, Nicotra F, et al. From cancer metabolism to new biomarkers and drug targets. Biotechnol Adv. 2012 Jan;30(1):30–51.
179. Issaq HJ, Nativ O, Waybright T, Luke B, Veenstra TD, Issaq EJ, et al. Detection of bladder cancer in human urine by metabolomic profiling using high performance liquid chromatography/mass spectrometry. J Urol. 2008 Jun;179(6):2422–6.
180. Cheng Y, Yang X, Deng X, Zhang X, Li P, Tao J, et al. Metabolomics in bladder cancer: a systematic review. Int J Clin Exp Med. 2015;8(7):11052–63.
181. Putluri N, Shojaie A, Vasu VT, Vareed SK, Nalluri S, Putluri V, et al. Metabolomic profiling reveals potential markers and bioprocesses altered in bladder cancer progression. Cancer Res. 2011 Dec 15;71(24):7376–86.
182. Giskeødegård GF, Hansen AF, Bertilsson H, Gonzalez SV, Kristiansen KA, Bruheim P, et al. Metabolic markers in blood can separate prostate cancer from benign prostatic hyperplasia. Br J Cancer. 2015;113:1712–9.
183. Rantalainen M, Cloarec O, Beckonert O, Wilson ID, Jackson D, Tonge R, et al. Statistically integrated metabonomic-proteomic studies on a human prostate cancer xenograft model in mice. J Proteome Res. 2006 Oct;5(10):2642–55.
184. Swanson MG, Zektzer AS, Tabatabai ZL, Simko J, Jarso S, Keshari KR, et al. Quantitative analysis of prostate metabolites using 1H HR-MAS spectroscopy. Magn Reson Med. 2006 Jun;55(6):1257–64.
185. Burns MA, He W, Wu C-L, Cheng LL. Quantitative pathology in tissue MR spectroscopy based human prostate metabolomics. Technol Cancer Res Treat. 2004 Dec;3(6):591–8.
186. Cheng LL, Burns MA, Taylor JL, He W, Halpern EF, McDougal WS, et al. Metabolic characterization of human prostate cancer with tissue magnetic resonance spectroscopy. Cancer Res. 2005 Apr 15;65(8):3030–4.
187. Sreekumar A, Poisson LM, Rajendiran TM, Khan AP, Cao Q, Yu J, et al. Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. Nature. 2009 Feb 12;457(7231):910–4.
188. Lokhov PG, Dashtiev MI, Moshkovskii SA, Archakov AI. Metabolite profiling of blood plasma of patients with prostate cancer. Metabolomics. 2010;6(1):156–63.
189. Serkova NJ, Gamito EJ, Jones RH, O'Donnell C, Brown JL, Green S, et al. The metabolites citrate, myo-inositol, and spermine are potential age-independent markers of prostate cancer in human expressed prostatic secretions. Prostate. 2008;68(6):620–8.
190. Osl M, Dreiseitl S, Pfeifer B, Weinberger K, Klocker H, Bartsch G, et al. A new rule-

based algorithm for identifying metabolic markers in prostate cancer using tandem mass spectrometry. Bioinformatics. 2008;24(24):2908–14.
191. Miyagi Y, Higashiyama M, Gochi A, Akaike M, Ishikawa T, Miura T, et al. Plasma free amino acid profiling of five types of cancer patients and its application for early detection. PLoS One. 2011;6(9):e24143.
192. Zang X, Jones CM, Long TQ, Monge ME, Zhou M, Walker LD, et al. Feasibility of detecting prostate cancer by ultraperformance liquid chromatography-mass spectrometry serum metabolomics. J Proteome Res. 2014;13(7):3444–54.
193. Struck-Lewicka W, Kordalewska M, Bujak R, Yumba Mpanga A, Markuszewski M, Jacyna J, et al. Urine metabolic fingerprinting using LC-MS and GC-MS reveals metabolite changes in prostate cancer: A pilot study. J Pharm Biomed Anal. 2015;111:351–61.
194. Wu H, Liu T, Ma C, Xue R, Deng C, Zeng H, et al. GC/MS-based metabolomic approach to validate the role of urinary sarcosine and target biomarkers for human prostate cancer by microwave-assisted derivatization. Anal Bioanal Chem. 2011 Aug 29;401(2):635–46.
195. Hahn P, Smith IC, Leboldus L, Littman C, Somorjai RL, Bezabeh T. The classification of benign and malignant human prostate tissue by multivariate analysis of 1H magnetic resonance spectra. Cancer Res. 1997;57(16):3398–401.
196. Halliday KR, Fenoglio-Preiser C, Sillerud LO. Differentiation of human tumors from nonmalignant tissue by natural-abundance 13C NMR spectroscopy. Magn Reson Med. 1988;7(4):384–411.
197. Kami K, Fujimori T, Sato H, Sato M, Yamamoto H, Ohashi Y, et al. Metabolomic profiling of lung and prostate tumor tissues by capillary electrophoresis time-of-flight mass spectrometry. Metabolomics. 2013;9(2):444–53.
198. Mondul AM, Moore SC, Weinstein SJ, Karoly ED, Sampson JN, Albanes D. Metabolomic analysis of prostate cancer risk in a prospective cohort: The alpha-tocolpherol, beta-carotene cancer prevention (ATBC) study. Int J Cancer. 2015;137(9):2124–32.
199. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg Effect: The Metabolic Requirements of Cell Proliferation. Science (80-). 2009;324(5930):1029–33.
200. Shuster JR, Lance RS, Troyer D a. Molecular preservation by extraction and fixation, mPREF: a method for small molecule biomarker analysis and histology on exactly the same tissue. BMC Clin Pathol. 2011;11(1):14.
201. Li T, Apte U. Bile Acid Metabolism and Signaling in Cholestasis, Inflammation, and Cancer. In: Advances in Pharmacology. 2015. p. 263–302.
202. Swanson MG, Vigneron DB, Tabatabai ZL, Males RG, Schmitt L, Carroll PR, et al. Proton HR-MAS spectroscopy and quantitative pathologic analysis of MRI/3D-MRSI-targeted postsurgical prostate tissues. Magn Reson Med. 2003 Nov;50(5):944–54.
203. Mondul AM, Moore SC, Weinstein SJ, Männistö S, Sampson JN, Albanes D. 1-Stearoylglycerol is associated with risk of prostate cancer: results from a serum metabolomic profiling analysis. Metabolomics. 2014;10:1036–41.
204. Huang G, Liu X, Jiao L, Xu C, Zhang Z, Wang L, et al. Metabolomic evaluation of the response to endocrine therapy in patients with prostate cancer. Eur J Pharmacol. 2014;729(1):132–7.
205. Currie E, Schulze A, Zechner R, Walther TC, Farese R V. Cellular fatty acid metabolism and cancer. Cell Metabolism. 2013. p. 153–61.

206. Griffin J, Shockcor J. Metabolic profiles of cancer cells. Nat Rev Cancer. 2004;4(7):551–61.
207. Soronen P, Laiti M, Törn S, Härkönen P, Patrikainen L, Li Y, et al. Sex steroid hormone metabolism and prostate cancer. J Steroid Biochem Mol Biol. 2004;92(4):281–6.
208. Cho HJ, Kim JD, Lee WY, Chung BC, Choi MH. Quantitative metabolic profiling of 21 endogenous corticosteroids in urine by liquid chromatography-triple quadrupole-mass spectrometry. Anal Chim Acta. 2009;632(1):101–8.
209. Moreno-Smith M, Lutgendorf S, Sood A. Impact of stress on cancer metastasis. Futur Oncol. 2011;6(12):1863–81.
210. Schiebler ML, Miyamoto KK, White M, Maygarden SJ, Mohler JL. In vitro high resolution 1h-spectroscopy of the human prostate: Benign prostatic hyperplasia, normal peripheral zone and adenocarcinoma. Magn Reson Med. 1993;29(3):285–91.
211. Komoroski R a, Holder JC, Pappas A a, Finkbeiner AE. 31P NMR of phospholipid metabolites in prostate cancer and benign prostatic hyperplasia. Magn Reson Med. 2011;65(4):911–3.
212. Rodrigues D, Monteiro M, Jerónimo C, Henrique R, Belo L, Bastos M de L, et al. Renal cell carcinoma: a critical analysis of metabolomic biomarkers emerging from current model systems. Transl Res. 2017;180:1–11.
213. Ari Hakimi A, Reznik E, Lee C, Creighton C, Rose Brannon A, Luna A, et al. An integrated metabolomic atlas of clear cell renal cell carcinoma. Cancer Cell. 2016;29(1):104–16.
214. Kim K, Aronov P, Zakharkin SO, Anderson D, Perroud B, Thompson IM, et al. Urine metabolomics analysis for kidney cancer detection and biomarker discovery. Mol Cell Proteomics. 2009 Mar 1;8(3):558–70.
215. Kim K, Taylor SL, Ganti S, Guo L, Osier M V, Weiss RH. Urine metabolomic analysis identifies potential biomarkers and pathogenic pathways in kidney cancer. OMICS. 2011 May;15(5):293–303.
216. Wu X, Gu J. Heritability of prostate cancer : a tale of rare variants and common single nucleotide polymorphisms. Ann Transl Med. 2016;4(10):10–3.
217. Packer JR, Maitland NJ. The molecular and cellular origin of human prostate cancer. Biochim Biophys Acta - Mol Cell Res. 2016;1863:1238–60.
218. Rand KA, Rohland N, Tandon A, Stram A, Sheng X, Do R, et al. Whole-exome sequencing of over 4100 men of African ancestry and prostate cancer risk. Hum Mol Genet. 2016;25(2):371–81.
219. Nakagawa H. Prostate cancer genomics by high-throughput technologies: Genome-wide association study and sequencing analysis. Endocr Relat Cancer. 2013;20(4).
220. Castro E, Eeles R. The role of BRCA1 and BRCA2 in prostate cancer. Asian J Androl. 2012;14(3):409–14.
221. Alvarez-cubero MJ, Ph D, Saiz M, S M, Martinez-gonzalez LJ, Ph D, et al. Genetic analysis of the principal genes related to prostate cancer : A review. Urolo. 2013;31(8):1419–29.
222. Valencia O, Lopes G, Sánchez P, Acuña L, Uribe D, González J. Incidence and Prevalence of Cancer in Colombia: The Methodology Used Matters. J Glob Oncol. 2017;JGO.17.00008.
223. Antczak A, Wokolorczyk D, Kluzniak W, Kashyap A, Jakubowska A, Gronwald J, et al. The variant allele of the rs188140481 polymorphism confers a moderate increase in the

risk of prostate cancer in Polish men. Eur J Cancer Prev. 2015;24(2):122–7.
224. Smith RA, Andrews KS, Brooks D, Fedewa SA, Manassaram-Baptiste D, Saslow D, et al. Cancer screening in the United States, 2017: A review of current American Cancer Society guidelines and current issues in cancer screening. CA Cancer J Clin. 2017 Mar;67(2):100–21.
225. Choudhury AD, Eeles R, Freedland SJ, Isaacs WB, Pomerantz MM, Schalken JA, et al. The Role of Genetic Markers in the Management of Prostate Cancer. Eur Urol. 2012;62:577–87.
226. Pritchard CC, Mateo J, Walsh MF, Sarkar N De, Abida W, Beltran H, et al. Inherited DNA-Repair Gene Mutations in Men with Metastatic Prostate Cancer. N Engl J Med. 2016;375:443–53.
227. Seppälä EH, Ikonen T, Mononen N, Autio V, Rökman a, Matikainen MP, et al. CHEK2 variants associate with hereditary prostate cancer. Br J Cancer. 2003;89(10):1966–70.
228. Smits M, Mehra N, Sedelaar M, Gerritsen W, Jack A. Expert Review of Molecular Diagnostics Molecular biomarkers to guide precision medicine in localized prostate cancer. Expert Rev Mol Diagn. 2017;17(8):791–804.
229. Shui IM, Lindström S, Kibel AS, Berndt SI, Campa D, Gerke T, et al. Prostate cancer (PCa) risk variants and risk of fatal PCa in the national cancer institute breast and prostate cancer cohort consortium. Eur Urol. 2014;65(6):1069–75.
230. GENEDx. Hereditary Prostate Cancer [Internet]. 2017 [cited 2017 May 1]. Available from: www.genedx.com
231. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2017.
232. Exome Aggregation Consortium (ExAC). ExAC Browser [Internet]. [cited 2017 Nov 11]. Available from: http://exac.broadinstitute.org/
233. PharmGKB. PharmGKB [Internet]. [cited 2017 Nov 11]. Available from: https://www.pharmgkb.org/
234. NCBI. ClinVar [Internet]. [cited 2017 Nov 11]. Available from: https://www.ncbi.nlm.nih.gov/clinvar/
235. Ensemble. Emsembl [Internet]. [cited 2017 Nov 11]. Available from: https://www.ensembl.org/info/website/tutorials/gene_snps.html
236. Hsu F, Sun J, Zhu Y, Kim S, Jin T, Wiklund F, et al. Comparison of two methods for estimating absolute risk of prostate cancer based on single nucleotide polymorphisms and family history. Cancer Epidemiol Biomarkers Prev. 2010;19(4):1083–8.
237. Parkin D, Ferlay J, Curado M, Bray F, Edwards B, Shin H. Cancer Incidence in Five Continents Volume IX InternatIonal agency for research on cancer InternatIonal assocIatIon of cancer regIstrIes Fifty years of cancer incidence: CI5 I-IX. Int J Cancer. 2010;127.
238. Pilié PG, Johnson AM, Hanson KL, Dayno ME, Kapron AL, Stoffel EM, et al. Germline genetic variants in men with prostate cancer and one or more additional cancers. Cancer. 2017;123(20):3925–32.
239. Bratt O, Loman N. Clinical Management of Prostate Cancer in Men with BRCA Mutations. Eur Urol. 2015;68(2):194–5.
240. Rebbeck TR. Prostate Cancer Genetics: Variation by Race, Ethnicity, and Geography. Semin Radiat Oncol. Elsevier; 2016;27(1):3–10.
241. Foulkes WD, Sugano K. BRCA2 : a grown-up cancer susceptibility gene. Endocr Relat

Cancer. 2016;23:10–2.
242. Li Q, Guan R, Qiao Y, Liu C, He N. Association between the BRCA2 rs144848 polymorphism and cancer susceptibility : a meta-analysis Study characteristics. Oncotarget. 2017;8(24):39818–32.
243. Na R, Zheng SL, Han M, Yu H, Jiang D, Shah S, et al. Germline Mutations in ATM and BRCA1/2 Distinguish Risk for Lethal and Indolent Prostate Cancer and are Associated with Early Age at Death [figure presented]. Eur Urol. 2017;71(5):740–7.
244. Cherbal F, Salhi N, Bakour R, Adane S, Boualga K, Maillet P. BRCA1 and BRCA2 unclassified variants and missense polymorphisms in Algerian breast/ovarian cancer families. Dis Markers. 2012;32(6):343–53.
245. Miao H, Chen L, Cai D, Kong W, Xiao L, Lin J. MSH3 rs26279 polymorphism increases cancer risk: a meta-analysis. Int J Clin Exp Pathol. 2015;8(9):11060–7.
246. Chen P, Zhang W, Wang X, Zhao K, Negi DS, Zhuo L, et al. Lycopene and Risk of Prostate Cancer: A Systematic Review and Meta-Analysis. Medicine (Baltimore). 2015;94(33):e1260.
247. D'Argenio V, Esposito MV, Telese A, Precone V, Starnone F, Nunziato M, et al. The molecular analysis of BRCA1 and BRCA2: Next-generation sequencing supersedes conventional approaches. Clin Chim Acta. Elsevier B.V.; 2015;446:221–5.
248. De Silva S, Tennekoon KH, Dissanayake A, De Silva K, Jayasekara L. Novel and reported pathogenic variants in exon 11 of BRCA2 gene in a cohort of Sri Lankan young breast cancer patients. Fam Cancer. 2017;16(3):329–38.
249. Arias-blanco JF, Ospino-durán EA, Restrepo-fernández CM, Guzmán-abisaab L. Frequency of sequence mutations and variants for the BRCA1 and BRCA2 genes in a sample of Colombian. Rev Colomb Obs y Ginecol. 2015;66(4):287–96.
250. Ruiz de Garibay G. Síndromes Hereditarios De Cáncer De Mama Familiar: Variantes De Significado Clínico Incierto Y Consejo Genético. Universidad Complutense de Madrid; 2014.
251. Cui M, Gao X, Gu X, Guo W, Li X, Ma M. BRCA2 mutations should be screened early and routinely as markers of poor prognosis : evidence from 8988 patients with prostate cancer. Oncotarget. 2017;8(25):40222–32.
252. Yang H, Spitz MR, Stewart DJ, Lu C, Gorlov IP, Wu X. ATM sequence variants associate with susceptibility to non-small cell lung cancer. Int J Cancer. 2007;121(10):2254–9.
253. Li D, Harlan-williams LM, Jensen RA. The role of BRCA1 and BRCA2 in prostate cancer. Front Biosci (Landmark Ed). 2013;1(18):1445–59.
254. Choi M, Kipps T, Kurzrock R. ATM Mutations in Cancer: Therapeutic Implications. Mol Cancer Ther. 2016;15(8):1781–91.
255. Cavanagh H, Rogers KMA. The role of BRCA1 and BRCA2 mutations in prostate , pancreatic and stomach cancers. Hered Cancer Clin Pract. 2015;1–7.
256. Buleje J, Guevara-Fujita M, Acosta O, Huaman FDP, Danos P, Murillo A, et al. Mutational analysis of *BRCA1* and *BRCA2* genes in Peruvian families with hereditary breast and ovarian cancer. Mol Genet Genomic Med. 2017;2:1–14.
257. Nicoloso M, Sun H, Spizzo R, Kim H, Wickramasinghe P, Shimizu M, et al. Single-nucleotide polymorphisms inside microRNA target sites influence tumor susceptibility. Cancer. 2011;70(7):2789–98.
258. Huo X, Lu C, Huang X, Hu Z, Jin G, Ma H, et al. Polymorphisms in BRCA1, BRCA1-interacting genes and susceptibility of breast cancer in Chinese women. J Cancer Res Clin

Oncol. 2009;135(11):1569–75.
259. Wang Q, Lu Q, Zhao H. A review of study designs and statistical methods for genomic epidemiology studies using next generation sequencing. Front Genet. 2015;6:1–12.
260. Berardinelli F, Masi A, Antoccia A. NBN Gene Polymorphisms and Cancer Susceptibility: A Systemic Review. Curr Genomics. 2013;14(7):425–40.
261. Mehdinejad M, Sobhan MR, Mazaheri M, Shehneh Z, Neamatzadeh H, Kalantar SM. Genetic Association between ERCC2 , NBN , RAD51 Gene Variants and Osteosarcoma Risk : a Systematic Review and. 2017;18:1315–21.
262. Park SL, Bastani D, Goldstein BY, Chang SC, Cozen W, Cai L, et al. Associations between NBS1 polymorphisms, haplotypes and smoking-related cancers. Carcinogenesis. 2010;31(7):1264–71.
263. Vineis P, Manuguerra M, Kavvoura FK, Guarrera S, Allione A, Rosa F, et al. A field synopsis on low-penetrance variants in DNA repair genes and cancer susceptibility. J Natl Cancer Inst. 2009;101(1):24–36.
264. Lu M, Lu J, Yang X, Yang M, Tan H, Yun B, et al. Association between the NBS1E185Q polymorphism and cancer risk: a meta-analysis. BMC Cancer. 2009;9(1):124.
265. Xu J-L, Hu L-M, Huang M-D, Zhao W, Yin Y-M, Hu Z-B, et al. Genetic variants of NBS1 predict clinical outcome of platinum-based chemotherapy in advanced non-small cell lung cancer in Chinese. Asian Pac J Cancer Prev. 2012;13(3):851–6.
266. Medina PP, Ahrendt S a, Pollan M, Fernandez P, Sidransky D, Sanchez-Cespedes M. Screening of homologous recombination gene polymorphisms in lung cancer patients reveals an association of the NBS1-185GIn variant and p53 gene mutations. Cancer Epidemiol Biomarkers Prev. 2003;12(8):699–704.
267. Stern MC, Lin J, Figueroa JD, Kelsey KT, Kiltie AE, Yuan M, et al. Polymorphisms in DNA repair genes, smoking, and bladder cancer risk: findings from the international consortium of bladder cancer. Cancer Res. 2009;69(17):6857–64.
268. Zheng J, Zhang C, Jiang L, You Y, Liu Y, Lu J, et al. Functional NBS1 polymorphism is associated with occurrence and advanced disease status of nasopharyngeal carcinoma. Mol Carcinog. 2011;50(9):689–96.
269. Jin G, Wang M, Chen W, Shi W, Yin J, Gang W. Single nucleotide polymorphisms of nucleotide excision repair and homologous recombination repair pathways and their role in the risk of osteosarcoma. Pakistan J Med Sci. 2015;31(2):269–73.
270. Wang Z-Y, Li H-Y, Jiang Z, Zhou T-B, Drummen GPC. *GSTM1* Gene Polymorphism is Implicated in Increased Susceptibility to Prostate Cancer in Caucasians and Asians. Technol Cancer Res Treat. SAGE PublicationsSage CA: Los Angeles, CA; 2016 Dec 9;15(6):NP69-NP78.
271. Helfand B, Kearns J, Conran C, Xu J. Clinical validity and utility of genetic risk scores in prostate cancer. Asian J Androl. 2016;18(4):509.
272. Attard G, Parker C, Eeles RA, Schröder F, Tomlins SA, Tannock I, et al. Prostate cancer. Lancet. Elsevier; 2016 Jan 2;387(10013):70–82.
273. Dimitriadis E, Kalogeropoulos T, Velaeti S, Sotiriou S, Vassiliou E, Fasoulis L, et al. Study of genetic and epigenetic alterations in urine samples as diagnostic markers for prostate cancer. Anticancer Res. 2013;33(1):191–7.
274. Salami S, Schmidt F, Laxman B, Regan M, Rickman D, Scherr D, et al. Combining urinary detection of TMPRSS2: ERG and PCA3 with serum PSA to predict diagnosis of prostate cancer. Urol Oncol. 2013;31(5):566–71.

275. Park K, Dalton JT, Narayanan R, Barbieri CE, Hancock ML, Bostwick DG, et al. TMPRSS2 : ERG Gene Fusion Predicts Subsequent Detection of Prostate Cancer in Patients With High-Grade Prostatic Intraepithelial Neoplasia. J Clin Oncol. 2014;32(3):206–12.
276. Fernández-Serra A, Rubio-Briones J, García-Casado Z, Solsona E, López-Guerrero JA. Cáncer de próstata: la revolución de los genes de fusión. Actas Urológicas Españolas. Elsevier Doyma; 2011 Jul;35(7):420–8.
277. Maekawa S, Suzuki M, Arai T, Suzuki M, Kato M, Morikawa T, et al. TMPRSS2 Met160Val polymorphism: Significant association with sporadic prostate cancer, but not with latent prostate cancer in Japanese men. Int J Urol. 2014;21(12):1234–8.
278. Nguyen PN, Violette P, Chan S, Tanguay S, Kassouf W, Aprikian A, et al. A panel of TMPRSS2:ERG fusion transcript markers for urine-based prostate cancer detection with high specificity and sensitivity. Eur Urol. 2011;59(3):407–14.
279. Sanda M, Feng Z, Wei J, Howard D, Rubin M, Groskopf J, et al. Pi-08 Combining Urine Pca3 and Tmprss2: Erg Tests To Refine Prostate Cancer Detection – Validation Study and Health Economic Analysis. J Urol. 2015;193(4):e982–3.
280. Robert G, Jannink S, Smit F, Aalders T, Hessels D, Cremers R, et al. Rational basis for the combination of PCA3 and TMPRSS2:ERG gene fusion for prostate cancer diagnosis. Prostate. 2013;73(2):113–20.
281. Penney KL, Pettersson A, Shui IM, Graff RE, Kraft P, Lis RT, et al. Association of Prostate Cancer Risk Variants with TMPRSS2:ERG Status: Evidence for Distinct Molecular Subtypes. Cancer Epidemiol Biomarkers Prev. 2016;25(5):745–9.
282. Cornu JN, Cancel-Tassin G, Egrot C, Gaffory C, Haab F, Cussenot O. Urine TMPRSS2: ERG fusion transcript integrated with PCA3 score, genotyping, and Biological features are correlated to the Results of prostatic biopsies in men at risk of prostate cancer. Prostate. 2013;73(3):242–9.
283. Tomlins S, Aubin S, Siddiqui J, Lonigro R, Sefton-Miller L, Miick S, et al. Urine TMPRSS2:ERG for prostate cancer risk stratification in men with elevated serum PSA. Cancer Res. 2011;71(8 SUPPL. 1).
284. Tavukcu HH, Mangir N, Ozyurek M, Turkeri L. Preliminary Results of Noninvasive Detection of TMPRSS2 : ERG Gene Fusion in a Cohort of Patients With Localized Prostate Cancer. Korean J Urol. 2013;54(6):359–63.
285. Mosquera J, Mehra R, Regan MM, Perner S, Genega EM, Bueti G, et al. Prevalence of TMPRSS2-ERG Fusion Prostate Cancer among Men Undergoing Prostate Biopsy in the United States. Clin Cancer Res. 2009;15(14):4706–12.
286. Huang L, Schauer IG, Zhang J, Mercado-uribe I, Deavers MT. The oncogenic gene fusion TMPRSS2 : ERG is not a diagnostic or prognostic marker for ovarian cancer. Int J Clin Exp Pathol. 2011;4(7):644–50.
287. Albadine R, Latour M, Platz E, Meeker A, Demazo A, Netto G. Tmprss2-Erg Gene Fusions in Minimal Prostatic Carcinoma. J Urol. 2009;181(4):814.
288. Chan SW, Nguyen P-N, Violette P, Brimo F, Taguchi Y, Aprikian A, et al. Early detection of clinically significant prostate cancer at diagnosis: a prospective study using a novel panel of TMPRSS2:ETS fusion gene markers. Cancer Med. 2013;2(1):63–75.
289. Laxman B, Morris DS, Yu J, Siddiqui J, Cao J, Mehra R, et al. A first-generation multiplex biomarker analysis of urine for the early detection of prostate cancer. Cancer Res. 2008;68(3):645–9.

290. Lin DW, Newcomb LF, Brown EC, Brooks JD, Carroll PR, Feng Z, et al. Urinary TMPRSS2:ERG and PCA3 in an active surveillance cohort: results from a baseline analysis in the canary prostate active surveillance study. Clin Cancer Res. 2013;19(9):2442–50.
291. Fernandez-Serra A, Rubio L, Calatrava A, Rubio-Briones J, Salgado R, Gil-Benso R, et al. Molecular characterization and clinical impact of TMPRSS2-ERG rearrangement on prostate cancer: comparison between FISH and RT-PCR. Biomed Res Int. 2013;2013:1–10.
292. Perner S, Demichelis F, Beroukhim R, Schmidt FH, Mosquera J-M, Setlur S, et al. TMPRSS2:ERG Fusion-Associated Deletions Provide Insight into the Heterogeneity of Prostate Cancer. Cancer Res. 2006;66(17):8337–41.
293. Attard G, Clark J, Ambroisine L, Fisher G, Kovacs G, Flohr P, et al. Duplication of the fusion of TMPRSS2 to ERG sequences identifies fatal human prostate cancer. Oncogene. Nature Publishing Group; 2008 Jan;27(3):253–63.
294. Yoshimoto M, Joshua AM, Chilton-Macneill S, Bayani J, Selvarajah S, Evans AJ, et al. Three-Color FISH Analysis of TMPRSS2/ERG Fusions in Prostate Cancer Indicates That Genomic Microdeletion of Chromosome 21 Is Associated with Rearrangement 1. Neoplasia. 2006;8(6):465–9.
295. Machado I, Noguera R, Pellin A, Lopez-guerrero JA, Piqueras M, Navarro S, et al. Molecular Diagnosis of Ewing Sarcoma Family of Tumors: A Comparative Analysis of 560 Cases With Fish and Rt-pcr. Diagnostic Mol Pathol. Diagnostic Molecular Pathology; 2009 Dec;18(4):189–99.
296. Salgado R, Llombart B, M. Pujol R, Fernández-Serra A, Sanmartín O, Toll A, et al. Molecular diagnosis of dermatofibrosarcoma protuberans: A comparison between reverse transcriptase-polymerase chain reaction and fluorescence in situ hybridization methodologies. Genes, Chromosom Cancer. Wiley Subscription Services, Inc., A Wiley Company; 2011 Jul;50(7):510–7.
297. Hermans KG, Boormans JL, Gasi D, Van Leenders GJHL, Jenster G, Verhagen PCMS, et al. Overexpression of prostate-specific TMPRSS2(exon 0)-ERG fusion transcripts corresponds with favorable prognosis of prostate cancer. Clin Cancer Res. 2009;15(20):6398–403.
298. Wang J, Cai Y, Ren C, Ittmann M. Expression of variant TMPRSS2/ERG fusion messenger RNAs is associated with aggressive prostate cancer. Cancer Res. 2006;66(17):8347–51.
299. Weier C, Haffner MC, Mosbruger T, Esopi DM, Hicks J, Zheng Q, et al. Nucleotide resolution analysis of TMPRSS2 and ERG rearrangements in prostate cancer. J Pathol. 2013;230(2):174–83.
300. Lu B, Maqsodi B, Yang W, McMaster GK, Perner S, Regan M, et al. Detection of TMPRSS2-ERG Fusion Gene Expression in Prostate Cancer Specimens by a Novel Assay Using Branched DNA. Urology. Elsevier Inc.; 2009;74(5):1156–61.
301. Bray F, Ren J-S, Masuyer E, Ferlay J. Global estimates of cancer prevalence for 27 sites in the adult population in 2008 GLOBOCAN. Int J Cancer. 2013 Mar;132(5):1133–45.
302. Valencia O, Lopes G, Sánchez P, Acuña L, Uribe D, González J. Incidence and Prevalence of Cancer in Colombia: The Methodology Used Matters. J Glob Oncol. American Society of Clinical Oncology; 2018 Jan;(4):1–7.
303. Mihammad Naeem Bashir. Epidemiology of Prostate Cancer. Asian Pacific J Cancer Prev.

2015;Vol 16.
304. Parkin DM, Ferlay J, Curado M-P, Bray F, Edwards B, Shin H-R, et al. Fifty years of cancer incidence: CI5 I-IX. UICC Int J Cancer IJC. 2010;127:2918–27.
305. Antczak A, Wokołorczyk D, Kluźniak W, Kashyap A, Jakubowska A, Gronwald J, et al. The variant allele of the rs188140481 polymorphism confers a moderate increase in the risk of prostate cancer in Polish men. Eur J Cancer Prev. 2015 Mar;24(2):122–7.
306. Antalis T, Bugge T, Wu Q. Membrane-anchored serine proteases in health and disease. Prog Mol Biol Transl Sci. 2011;99:1–50.
307. Fernández-Serra A, Rubio L, Calatrava A, Rubio-Briones J, Salgado R, Gil-Benso R, et al. Molecular characterization and clinical impact of TMPRSS2-ERG rearrangement on prostate cancer: comparison between FISH and RT-PCR. Biomed Res Int. Hindawi Limited; 2013;2013:465179.
308. Whirl-Carrillo M, McDonagh E, Hebert J, Gong L, Sangkuhl K, Thorn C, et al. Pharmacogenomics knowledge for personalized medicine. Clin Pharmacol Ther. 2012;92((4)):414–7.
309. Landrum M, Lee J, Benson M, Brown G, Chao C, Chitipiralla S, et al. ClinVar: Improving access to variant interpretations and supporting evidence. Nucleic Acids Res. 2018;
310. Sherry S, Ward M, Kholodov M, Barker J, Phan L, Smigielski E, et al. dbSNP: The NCBI database of genetic variation. Nucleic acids research. 2001;29(1):308-11.
311. Blee AM, He Y, Yang Y, Ye Z, Yan Y, Pan Y, et al. TMPRSS2-ERG controls luminal epithelial lineage and antiandrogen sensitivity in PTEN and TP53 -mutated prostate cancer. Clin Cancer Res. 2018 May;clincanres.0653.2018.
312. Knuuttila M, Mehmood A, Mäki-Jouppila J, Ryberg H, Taimen P, Knaapila J, et al. Intratumoral androgen levels are linked to TMPRSS2-ERG fusion in prostate cancer. Endocr Relat Cancer. 2018 Sep;25(9):807–19.
313. Tomlins SA, Laxman B, Varambally S, Cao X, Yu J, Helgeson BE, et al. Role of the TMPRSS2-ERG gene fusion in prostate cancer. Neoplasia. Neoplasia Press; 2008 Feb;10(2):177–88.
314. Hsu F-C, Sun J, Zhu Y, Kim S-T, Jin T, Zhang Z, et al. Comparison of two methods for estimating absolute risk of prostate cancer based on single nucleotide polymorphisms and family history. Cancer Epidemiol Biomarkers Prev. American Association for Cancer Research; 2010 Apr;19(4):1083–8.
315. Karczewski KJ, Weisburd B, Thomas B, Solomonson M, Ruderfer DM, Kavanagh D, et al. The ExAC browser: displaying reference data information from over 60 000 exomes. Nucleic Acids Res. Oxford University Press; 2017 Jan;45(D1):D840–5.
316. Lek M, Karczewski KJ, Minikel E V., Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature. Nature Publishing Group; 2016 Aug;536(7616):285–91.
317. Kelly RS, Heiden MGV, Giovannucci E, Mucci LA. Metabolomic biomarkers of prostate cancer: Prediction, diagnosis, progression, prognosis, and recurrence. Cancer Epidemiol Biomarkers Prev. 2016;25(6):887–906.
318. Hsing AW, Sakoda LC, Chua Jr. S. Obesity, metabolic syndrome, and prostate cancer. Am J Clin Nutr. 2007;86(3):s843-57.
319. Spratlin JL, Serkova NJ, Eckhardt SG. Clinical applications of metabolomics in oncology: A review. Clinical Cancer Research. 2009. p. 431–40.
320. Hsu PP, Sabatini DM. Cancer cell metabolism: Warburg and beyond. Cell.

2008;134(5):703–7.
321. Lima AR, Bastos M de L, Carvalho M, Guedes de Pinho P. Biomarker discovery in human prostate cancer: An update in metabolomics studies. Transl Oncol. The Authors; 2016;9(4):357–70.
322. Gonzalez-Menendez P, Hevia D, Mayo J, Sainz R. The dark side of glucose transporters in prostate cancer: Are they a new feature to characterize carcinomas? Int J Cancer. 2017;00(00):00–00.
323. Vaz C, Alves M, Marques R, Moreira P, Oliveira P, Maia C, et al. Androgen-responsive and nonresponsive prostate cancer cells present a distinct glycolytic metabolism profile. Int J Biochem Cell Biol. 2012;44(11):2077–84.
324. Kumar D, Gupta A, Mandhani A, Sankhwar S. Metabolomics-derived prostate cancer biomarkers: fact or fiction? J Proteome Res. 2015;14(3):1455–64.
325. Johansson M, Van Guelpen B, Vollset S, Hultdin J, Bergh A, Key T, et al. One-carbon metabolism and prostate cancer risk: prospective investigation of seven circulating B vitamins and metabolites. Cancer Epidemiol Biomarkers Prev. 2009;18(5):1538–43.
326. Crowe F, Allen N, Appleby P, Overvad K, Aardestrup I, Johnsen N, et al. Fatty acid composition of plasma phospholipids and risk of prostate cancer in a case-control analysis nested within the European Prospective Investigation into Cancer and Nutrition. Am J Clin Nutr. 2008;88(5):1353–63.
327. Saylor P, Karoly E, Smith M. Prospective study of changes in the metabolomic profiles of men during their first three months of androgen deprivation therapy for prostate cancer. Clin Cancer Res. 2012;18(13):3677–85.
328. Pértega-Gomes N, Baltazar F. Lactate transporters in the context of prostate cancer metabolism: What do we know? Int J Mol Sci. 2014;15(10):18333–48.
329. Nenu I, Gafencu G-A, Popescu T, Kacso G. Lactate - A new frontier in the immunology and therapy of prostate cancer. J Cancer Res Ther. 2015;13(3):406–11.
330. Huang J, Mondul AM, Weinstein SJ, Karoly ED, Sampson JN, Albanes D. Prospective serum metabolomic profile of prostate cancer by size and extent of primary tumor. Oncotarget. 2017;8(28):45190–9.
331. Sola-Penna M. Metabolic Regulation by Lactate. IUBMB Life. 2008;60(9):605–608.
332. Sun S, Li H, Chen J, Qian Q. Lactic Acid: No Longer an Inert and End-Product of Glycolysis. Physiology. 2017;32(6):453–63.
333. Halestrap A., Price NT. The proton-linked monocarboxylate transporter (MCT) family: Structure, function and regulation. Biochem J. 1999;343:281–299.
334. Pertega-Gomes N, Vizcaino J., Miranda-Goncalves V, Pinheiro C, Silva J, Pereira H, et al. Monocarboxylate transporter 4 (MCT4) and CD147 overexpression is associated with poor prognosis in prostate cancer. BMC Cancer. 2011;11:312.
335. Divella R, De Luca R, Abbate I. Obesity and cancer: the role of adipose tissue and adipo-cytokines-induced chronic inflammation. J Cancer. 2016;7:2346 – 2359.
336. Bandini M, Gandaglia G, Briganti A. Obesity and prostate cancer. Curr Opin Urol. 2017;27(5):415–21.
337. Grossmann M, Mizuno N, Bonorden M. Role of the adiponectin leptin ratio in prostate cancer. Oncol Res. 2009;18(5–6):269–277.
338. Booth A, Magnuson A, Fouts J, Foster M. Adipose tissue, obesity and adipokines: role in cancer promotion. Horm Mol Biol Clin Investig. 2015;21:57 – 74.
339. Curtis B, Grassel C, Laufer R, Sears K, Marcela F, Barry EM, et al. Adiponectin as a

potential therapeutic target for prostate cancer. Curr Pharm Des. 2017;23(28):130–5.

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ARTÍCULO DE REVISIÓN

Una mirada general a los biomarcadores para la tamización y el diagnóstico temprano del cáncer de próstata

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PALABRAS CLAVE
Biomarcadores;
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Revisión

Resumen El antígeno prostático específico y el tacto rectal son herramientas diagnósticas que en la actualidad se quedan cortas al momento de diferenciar entre procesos malignos como el cáncer prostático y procesos benignos como la hiperplasia prostática.

Nuevos biomarcadores con altas sensibilidades en el diagnóstico de procesos malignos prostáticos han entrado a desempeñar un papel significativo en la determinación temprana de cáncer de próstata (CP).

El gen de cáncer de próstata 3 está implicado en la supervivencia de la célula cancerígena; puede identificarse en orina y permite diferenciar entre cáncer y prostatitis crónica/hiperplasia prostática. Por otro lado, la b2-microglobulina es una proteína que forma complejos con el complejo mayor de histocompatibilidad tipo I de la célula cancerígena prostática, y gracias a esta interacción hace parte de procesos moleculares que terminan generando metástasis ósea; sus niveles séricos pueden ser determinados por medio de exámenes de laboratorio, y de esta forma se puede hacer una diferenciación entre procesos benignos y cáncer. Las calicreínas (test 4K score), las mucinas (MUC1), la proteína LRPPRC, el ADN circulante del tumor, la alfa metilacil coenzima a racemasa y las células madre prostáticas son moléculas prometedoras, tanto en el diagnóstico temprano del CP como en el pronóstico y respuesta al tratamiento.

La importancia del hallazgo de nuevos biomarcadores con especificidades y sensibilidades altas para el diagnóstico precoz del CP no solo podrá reducir la mortalidad por esta enfermedad, sino también propiciará nuevos campos de investigación en busca de dianas terapéuticas más efectivas en el tratamiento del mismo.

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Review

A general overview of biomarkers for the screening and early diagnosis of prostate cancer

Abstract Prostate specific antigen and digital rectal examination are diagnostic tools that currently fall short when differentiating between malignant processes, such as prostate cancer and benign prostatic hyperplasia. New biomarkers with high sensitivity in the diagnosis of prostate malignancies have come to play a significant role in the early identification of prostate cancer (PC). Prostate cancer gene 3 is involved in cancer cell survival. It can be identified in urine and used to differentiate between cancer and chronic prostatitis/. Furthermore, b2-microglobulin is a protein that complexes with major histocompatibility complex type 1 prostate cancer cell, and through this interaction is part of the molecular processes that end up generating bone metastases. Serum levels can be determined by laboratory tests and as such can be used to differentiate between benign processes and cancer. Kallikreins (4K score test), mucins, the leucine rich pentatricopeptide repeat containing protein, circulating tumour DNA, the alpha methyl acyl coenzyme A racemase, and Stem Cells are promising molecules, both for the early diagnosis of PC, as well as for the prognosis and response to treatment. The importance of the discovery of new biomarkers with high sensitivity and specificity for early diagnosis of PC not only can reduce mortality from this disease, but will also foster new fields of research for more effective treatment in the same therapeutic targets.

Introducción

En la actualidad, el antígeno prostático específico en conjunto con el tacto rectal constituyen los métodos de tamización principalmente usados en la clínica para detectar el cáncer de próstata, sin embargo, estos tienen bajo rendimiento diagnóstico, tanto individualmente como en conjunto.

La importancia de encontrar nuevos biomarcadores, que sean más sensibles y específicos para tamización y diagnóstico temprano del cáncer de próstata, se ha visto especialmente reforzada por la falencia del PSA. El hecho que este último se eleve en una condición benigna como la hiperplasia prostática y también en condiciones de malignidad[1], ha generado que se soliciten biopsias costosas e innecesarias a pacientes que no lo requerían desde un comienzo[1]. Como consecuencia de esto, se han explorado otras técnicas y moléculas para hacer un diagnóstico más específico, tales como el PCA3, la microglobulina y las mucinas, entre otros.

El objetivo del presente trabajo fue describir algunos de los nuevos biomarcadores involucrados en la tamización y el diagnóstico temprano del cáncer de próstata.

Epidemiología

El cáncer de próstata (CP) es el segundo tipo de cáncer más común en la población masculina del mundo[2]. Se estima que uno de cada 7 hombres será diagnosticado a lo largo de su vida con CP, y uno de cada 38 hombres morirá como consecuencia de este. De igual manera, en Estados Unidos 6 de cada 10 hombres diagnosticados con cáncer de próstata son mayores de 65 años[3]. Es el tipo de cáncer que con mayor frecuencia se diagnostica como consecuencia de la introducción en 1980 del test de antígeno prostático específico (PSA) como herramienta diagnóstica[3]. Se estima que para el año 2030 haya 1,7 millones de casos nuevos de CP en el mundo, con una mortalidad esperada de 499.000 casos (29,3%)[4].

Durante la última década se ha observado una notable disminución en la mortalidad por CP en países desarrollados. El estudio GLOBOCAN 2012 notifica que la incidencia del CP es variable en el contexto mundial: las tasas son más altas en países como Australia/Nueva Zelanda, América del Norte (ASR -—por sus siglas en inglés— 111,6 y 97,2 por 100.000 respectivamente) y en Europa Occidental y del Norte, debido a que la tamización con PSA y posterior biopsia se realizan de forma rutinaria[5]. En países de América del Norte (Estados Unidos y Canadá) la mortalidad por CP ha disminuido a 4,3% y 3,1% respectivamente, y en países como Dinamarca, Noruega y Suecia (Europa del Norte) las tasas de mortalidad han venido en descenso desde el año 2000 hasta 3,1% por año[6]; sin embargo, en países en vías de desarrollo la mortalidad por este se ha visto en aumento (aunque hay tendencias hacia el incremento en el diagnóstico, la mortalidad ha aumentado en países como Colombia —3,4% por año—, Costa Rica —3,4% por año—, Chile —2,8% por año— y Cuba —5,5% por año—)[4,6]. Con respecto al contexto mundial, Colombia cuenta con una de las incidencias de CP más bajas de Latinoamérica, y una proporción de 28% entre incidencia y mortalidad por el mismo, muy cercano al promedio mundial de 28,6% e inferior a países como Ecuador (40,41%), Perú (37,74%) y Cuba (46,65%)[6].

Finalmente, en Colombia la tasa de mortalidad por CP ha disminuido en los últimos 4 años[4] y el mayor número de casos reportados se origina en ciudades como Bogotá, Valle y Antioquia (las regiones más pobladas y con mayor cantidad de urólogos)[4].

Biomarcadores en el diagnóstico del cáncer de próstata

¿Qué teníamos?

Antígeno prostático específico

Los antecedentes de uso del PSA *(prostatic specific antigen)* datan de la década de 1980, cuando era utilizado en el seguimiento de pacientes diagnosticados con CP[7], posteriormente, para el año 1994 la *Food and Drug Administration* de Estados Unidos aprobó el uso del PSA junto con el tacto rectal como métodos de tamización para CP[8].

El PSA, también llamado calicreína III, es una glucoproteína de 34KDA producida casi exclusivamente por las células epiteliales de la glándula prostática, la cual circula unida a alfa-1-antiquimiotripsina y la alfa-2-macroglobulina, y su deber es la de dividir la semenogelina I y II en polipéptidos de menor tamaño, evitando así formación del coágulo seminal[8–12]. En condiciones normales una pequeña cantidad, menos de 4 ng/ml, es liberada al torrente sanguíneo, pero en un proceso neoplásico estos niveles se elevan[8]. Por tal motivo se considera realizar biopsia de próstata a aquellos hombres con un nivel de PSA sérico mayor a 4 ng/ml[8]. Sin embargo, el PSA también se ha encontrado elevado en otras enfermedades como cáncer de mama, carcinoma de células renales, cáncer de ovario y neoplasia suprarrenal[13]. De igual forma, en hiperplasia prostática benigna (HPB), prostatitis, cistitis, traumatismo perineal y cirugía del tracto urinario reciente podría encontrarse elevada[8,14].

El PSA puede transitar en el suero libremente (fPSA) o acompañado de inhibidores de proteasa (cPSA) con el fin de evitar proteólisis. Al sumar el fPSA y PSA acompañado de inhibidores de la proteasa se obtiene como resultado el PSA sérico total (tPSA), gran parte de este, alrededor del 70-90% puede estar ligado a alfa-1-antiquimiotripsina, en menor proporción a la alfa-2-macroglobulina, alfa-1 antitripsina o a un inhibidor de la proteína C[12]. Consecuentemente, cerca de un 10-30% del PSA total (tPSA) circula libremente (fPSA), esta forma libre del PSA se caracteriza por asumir 3 formas moleculares específicas[15]. Una de ellas se encuentra predominantemente en la zona de transición de la próstata en pacientes con HPB y se denomina BPSA[16], la segunda representación se llama PSA intacto (iPSA) y por último se tiene el proPSA, hallado en su mayoría en la zona periférica de la glándula prostática, el cual se asocia a CP[17].

Según la *American Cancer Society*, la sensibilidad del PSA para valores de referencia de 4 ng/ml y 3 ng/ml para el diagnóstico de cáncer es del 21% y 32% respectivamente. Una especificidad de 91% para valores de corte de 4 ng/ml y de 85% para valores de 3 ng/ml de PSA[18].

En EE. UU. se llevó a cabo el estudio (PLCO) para evaluar la incidencia de cáncer de ovario, colorrectal, pulmonar y de próstata. En el caso de CP se evaluaron hombres entre los 55-74 años, a quienes se le realizó tamización anual con PSA durante 13 años, y como resultado se obtuvo que realizar tamización con PSA no lleva a disminución de la incidencia de CP (RR: 1,09, IC 95%: 0,87-1,36)[19]. Otro gran estudio fue el *The European Randomized Study of Screening for Prostate Cancer* (ERSPC), donde se realizó tamización con PSA durante 11 años a hombres de ciertos países europeos, evaluando la mortalidad por CP; los resultados indicaron una reducción relativa en las tasas de mortalidad de un 21% (RR: 0,79, IC 95%: 0,68 a 0,91)[20].

Un metaanálisis de Cochrane realizado en 2011 resumió los resultados de 5 experimentos poblacionales, con un total de 341.351 participantes, y mostró que realizar tamización con PSA es efectivo para la detección de cáncer de próstata (RR: 1,35: IC 95%: 1,06-1,72), sin embargo esta prueba no disminuyó la mortalidad (RR: 0,95; IC 95%: 0,85-1,07)[21]. Otro metaanálisis del año 2010 obtuvo resultados similares; tenía como objetivo mostrar evidencia de los beneficios de la tamización con el PSA, para lo cual se tomaron resultados de 6 experimentos con un total de 387.286 participantes. Los resultados mostraron que la tamización con PSA se relaciona con una probabilidad aumentada de diagnosticar cáncer de próstata (RR: 1,46, IC 95%: 1,21-1,77), pero al igual que en el estudio anterior no se observa disminución en la mortalidad por CP (RR: 0,88, IC 95%: 0,71-1,09)[22], de manera tal que en la actualidad no se recomienda realizar tamización poblacional de CP.

Lo anterior nos sugiere la importancia de determinar otros biomarcadores más específicos, que lleven a una disminución de biopsias innecesarias, al igual que a una detección precoz del CP, y así mejorar la supervivencia de los pacientes.

¿Qué hay más reciente?

Gen de cáncer de próstata 3

El *Long non-coding RNA prostate cancer associated 3 gene test* (PCA3) se ha visto implicado en un número importante de investigaciones que reflejan el fundamento para el estudio del CP[23]. Este se encuentra implicado en la supervivencia de la célula cancerígena; este efecto lo logra en parte modulando la señalización al receptor de andrógenos y ejerce su función principal en el núcleo y en la fracción microsómica de la célula[24].

Tomar este test requiere que el proceso se divida en 2 grandes partes; primero debe realizarse un masaje prostático y luego se obtiene la muestra de orina, esto facilitará la aparición del biomarcador en la orina[24].

Los estudios han mostrado resultados promisorios, pero controvertidos a la vez por distintas causas. Son promisorios porque se ha encontrado que la medición de este biomarcador puede diferenciar entre cáncer y prostatitis crónica/HPB[1]. En parte el motivo para que esto sea posible se relaciona con la alta expresión de la molécula en tejido cancerígeno de 60 a 100 veces más que en tejido inflamado, pero sin neoplasia, y cabe resaltar también que esto se observó en el 95% de los pacientes[23]. Christos et al.[1] mencionan que este biomarcador es útil para diferenciar entre HBP del cáncer localizado, sin embargo, sugieren que el uso de la molécula para tamización no debe ser única, sino más bien debería ser un panel de nuevos biomarcadores que provean mayor precisión diagnóstica[23]. Se menciona además el uso de la beta 2 microglobulina (B2M) y mucina intestinal (MUC3) asociados al PCA3[1]. Se conoció en este estudio que los valores tenidos en cuenta para decir si es relevante el aumento de PCA3 son: 195 DU (con este valor de referencia se mejora la precisión diagnóstica hasta en un 77%)[1].

Si bien las ventajas del uso de PCA3 para el diagnóstico de CP han sido dilucidadas y se ha apoyado la idea de

usarlo en programas terapéuticos como un apoyo[23], hay una contraparte que discute la validez clínica de los hallazgos encontrados en los diferentes estudios. Esta controversia se basa en el hecho de que existan pocos estudios que provean un mayor conocimiento del biomarcador, y a los que existen actualmente se les critica que hayan usado muestras poblacionales pequeñas que hacen menos fidedigno el resultado final. Se necesitan más estudios que incluyan una proporción mayor de pacientes y a los cuales se les haga un seguimiento en el tiempo más prolongado[23]. Producto de lo mencionado anteriormente se ha encontrado que hay un fallo para demostrar que las asociaciones entre PCA3 y cáncer de próstata tienen alguna relevancia en el pronóstico del paciente[24]. De acuerdo a este estudio, usando de esta manera los biomarcadores, se incrementa el valor predictivo de la prueba y es posible diferenciar entre condiciones inflamatorias benignas de aquellas que requieren un seguimiento especial, como lo sería la neoplasia[24].

β2-microglobulina

La β2-microglobulina (B2M) es un polipéptido de bajo peso molecular (11.815 D), sintetizado por células nucleadas y que forma complejos con el complejo mayor de histocompatibilidad I[25] (por lo cual se asume que desempeña un rol en la respuesta inmune). Fue identificado inicialmente en la orina de pacientes con enfermedad tubular renal[26] y posteriormente en pacientes con cáncer de mama[27] y cáncer gástrico[28].

Se ha reportado que la B2M estimula el crecimiento e incrementa la expresión de genes que codifican para la osteocalcina y la sialoproteína ósea en las células cancerígenas del CP mediante la activación de la cascada enzimática de la proteín quinasa A dependiente de AMP cíclico. De este modo, cuando hay una sobreexpresión de B2M en células cancerígenas prostáticas, se genera un aumento en la codificación de genes que en última instancia serán los responsables de generar metástasis, tales como la osteocalcina, sialoproteína ósea, ciclina A, ciclina D1 y factor de crecimiento endotelial vascular. Estas vías génicas sugieren que la señalización mediada por B2M podría ser un target terapéutico para el tratamiento de CP con metástasis ósea (fig. 1)[29–31]. Igualmente, otra vía molecular en la que se ha visto involucrada la B2M es en la interacción con la homeostasis del hierro. Cuando la B2M interactúa con la proteína hemocromatosis (HFE) (que actúa como su receptor), se produce una modulación en la homeostasis del hierro y una posterior transdiferenciación epitelio-mesenquimal (EMT), que termina promoviendo el crecimiento, la invasión y la metástasis hacia huesos y tejidos blandos (fig. 2)[32].

En estudios realizados por Zhang et al. se demostró que el marcador B2M sérico podría ser usado como un marcador diagnóstico de CP y útil en la diferenciación entre procesos malignos e HPB al encontrar que los niveles en suero de B2M se notaban significativamente elevados en pacientes con CA de próstata, a diferencia de los niveles del mismo marcador en pacientes con HPB o de los pacientes del grupo control. Sin embargo, no se encontró una diferencia marcada entre los niveles del B2M entre los pacientes con HPB y los del grupo control.

Así mismo, pacientes con niveles de PSA mayores ($\geq$ 20 mg/ml) tienen niveles de B2M mayores y viceversa[33]. Por lo tanto, los niveles de B2 M se asocian con mayor agresividad clínica[33].

La B2M puede, por lo tanto, ser un biomarcador eficiente para el diagnóstico clínico, seguimiento y pronóstico del CP y a su vez un blanco terapéutico posiblemente eficaz para revertir la EMT y, de esta manera, evitar la progresión metastásica[34].

Examen 4K (panel 4-calicreínas séricas)

El 4K *score test* (por su descripción en inglés), como su nombre indica, combina los valores plasmáticos de 4 calicreínas usadas como marcadores prostáticos: PSA total (tPSA), PSA libre (fPSA), PSA intacto (iPSA) y la calicreína-2 (hK2)[35]. En su mayoría el PSA en plasma se encuentra unido a inhibidores de proteasas y una pequeña cantidad se encuentra libremente (fPSA), este PSA libre asume 3 formas moleculares: iPSA, pro-PSA y BPSA. Un menor valor de fPSA en proporción con tPSA se relaciona más con CP, mientras que un mayor valor se asocia con enfermedad benigna. La calicreína-2 se ha encontrado aumentada en pacientes con CP de alto grado. Adicionalmente, en el 4K test se toman datos como la edad de la persona, hallazgos al tacto rectal (nódulos) y antecedente de biopsia previa[35].

En diferentes estudios: Gothenburg ERSPC[36], ProtecT[37], Rotterdam ERSPC[38], Braun et al.[39] y Parekh et al.[40], entre otros, se ha concluido que el uso del 4K test reduce prácticamente a la mitad la práctica de biopsias en aquellos pacientes con PSA en zona gris (4-10 ng/ml), ya que permite identificar a aquellos individuos susceptibles a desarrollar cáncer clínicamente relevante de aquellos con enfermedad no maligna.

En conclusión, el panel 4K permite individualizar la predicción de CP aun si previamente no se le ha realizado al paciente la prueba de tamización o una biopsia. Este panel disminuye alrededor de 41-71% de biopsias practicadas innecesariamente[35]. Sin embargo, existen limitaciones en cuanto a la disponibilidad del panel en el escenario clínico cotidiano. Actualmente solo se encuentra disponible en los Estados Unidos y el costo para los pacientes oscila alrededor de 1.185 dólares americanos[35].

Mucinas

Las mucinas son proteínas glucosiladas de elevado peso molecular, presentes en la mayoría de tejidos epiteliales, incluidos el tracto urinario y el aparato reproductor[41].

En la actualidad se conocen un total de 21 genes codificantes de mucinas, estos han sido divididos en 2 grupos: MUC1, MUC3 y MUC4 codificantes para mucinas de membrana, siendo estas necesarias en la interacción célula-célula, mientras que los genes MUC2, MUC5AC, MUC5B y MUC6 codifican para mucinas secretoras de moco[42].

La función de las mucinas en general es la de proteger e hidratar el epitelio. Sin embargo, en procesos neoplásicos se ha visto afectada su función[43].

MUC1 ha sido la más estudiada en el desarrollo de diferentes cánceres, entre ellos CP, donde se ha visto implicada en la angiogénesis, proliferación y transducción de señales tumorales, así como también en la invasión y metástasis del tumor[43], ya que aminora la respuesta inmune y promueve la propagación tumoral a otros tejidos, al perderse las interacciones célula-célula y célula-matriz extracelular[41].

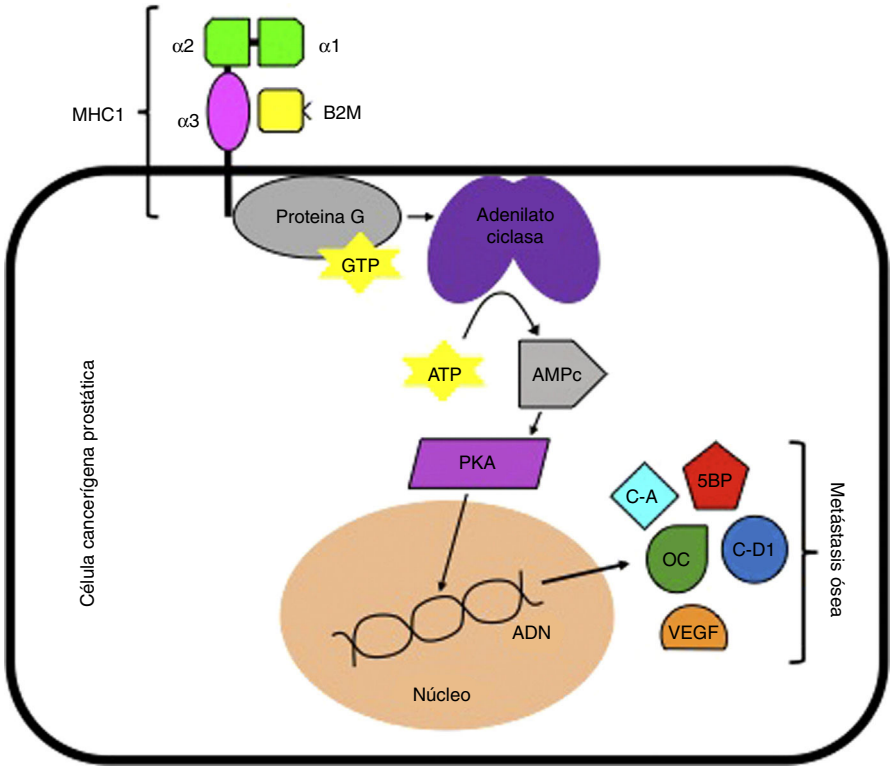

Figura 1 Señalización mediada por B2M. ADN: ácido desoxirribonucléico; AMPc: adenosín monofosfato c; ATP: adenosín trifosfato; B2M: beta 2 microglobulina; C-A: ciclina A; C-D1: ciclina D1; GTP: guanosín trifosfato; MHC I: complejo mayor de histocompatibilidad 1; OC: osteocalcina; PKA: proteína quinasa A; SBP: sialoproteína ósea; VEGF: factor de crecimiento endotelial vascular.

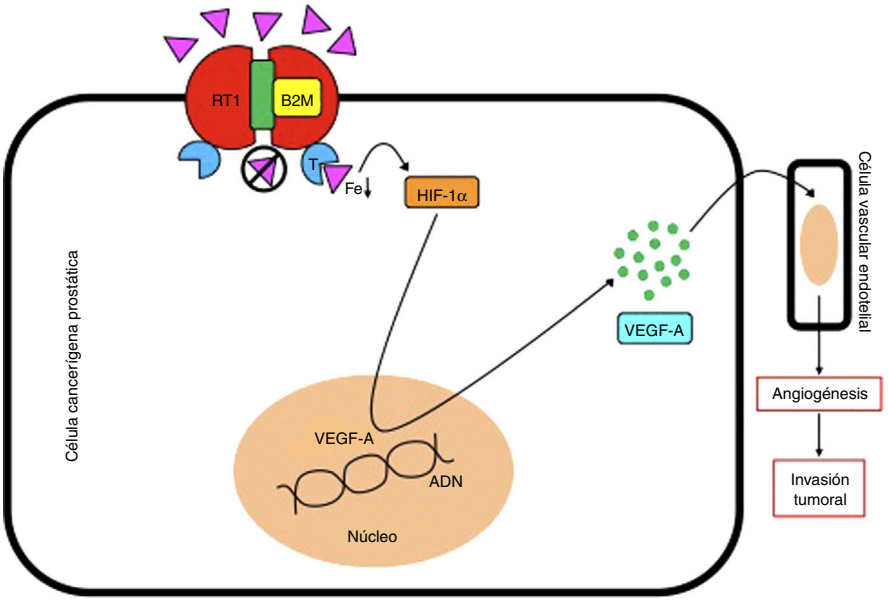

Figura 2 La beta 2 microglobulina (B2M) regula directamente los niveles de hierro (Fe) celular al formar complejos con la proteína hemocromatosis (HFE), que bloquean el receptor de transferrina 1 (RT1) y previenen la entrada de hierro a la célula, y como consecuencia una disminución de su concentración intracelular. Este fenómeno promueve la expresión del factor inductor de hipoxia 1α (HIF-1α), que actuará sobre el núcleo celular para generar la síntesis del factor de crecimiento vascular endotelial A (VEGF-A), que inducirá el proceso de angiogénesis en las células vasculares endoteliales, y como consecuencia un crecimiento, invasión y metástasis tumoral.
ADN: ácido desoxirribonucléico; T: transferrina.

En tejido prostático maligno, mediante microarreglos tisulares, se ha reportado una sobreproducción de MUC1, junto con una distribución aberrante y alteraciones en la glucosilación de esta mucina[41,43]. Adicionalmente, en estudios realizados por Cozzi et al., se demostró la sobreexpresión de MUC1 en el 58% de las muestras de tejido prostático neoplásico y en el 90% de los nodos linfáticos metastásicos, mientras que en tejido libre de cáncer no se evidenció lo anteriormente mencionado[42].

Otros biomarcadores

Recientemente ha habido un interés sobre proteínas involucradas con la autofagia mitocondrial, pues estas pueden estar asociadas a carcinogénesis[44]. Dichas proteínas pueden ser vistas como nuevos biomarcadores diagnósticos y pronósticos en el cáncer de próstata. Sin embargo, no son los únicos biomarcadores nuevos sobre los cuales se ha centrado la atención, pues se ha visto que al realizar un perfilamiento genético del cáncer, y también al observar la presencia de células madre en el tejido neoplásico, se pueden obtener nuevos marcadores de la enfermedad. A continuación se mencionan 4 de ellos:

Leucine-rich pentatricopeptide repeat motif-containing protein

La proteína leucine-rich pentatricopeptide repeat motif-containing (LRPPRC) estabiliza el oncogén BCL-2 y bloquea la autofagia, alterando así los mecanismos reguladores de muerte celular[44]. En la comparación de la inmunohistoquímica de la expresión de LRPPRC que realizan Mancini et al. entre tejido neoplásico y HBP se encuentra que esta proteína efectivamente se expresó más en el tejido con cáncer[44]. Este hallazgo confirma que la LRPPRC puede ser vista como un nuevo biomarcador diagnóstico y la mitocondria como un objetivo terapéutico[44].

Perfilamiento genético del cáncer de próstata: ADN circulante del tumor

El análisis de fragmentos de ADN circulante del tumor supone la posibilidad de poder encontrar dichos pedazos de ADN libres en fluidos corporales y en el plasma de los pacientes con tumores sólidos[44]. La ventaja de estas secuencias es que contienen la información genética completa del tumo,; lo que permitiría analizarlo para determinar el pronóstico y la respuesta al tratamiento[44]. Además de los beneficios mencionados anteriormente, el ctDNA permite realizar análisis no invasivo, pues no sería necesario obtener una biopsia como tal[44]. Finalmente, el ctDNA permite identificar mutaciones *de novo*, lo que en último término impacta el curso del tratamiento.

Células madre

Estudios recientes han demostrado la presencia de células madre en la próstata y su posible rol en la progresión de la enfermedad y la respuesta al tratamiento[44]. Lo que se ha observado es que a partir de estas células de originan algunas neoplasias, y su presencia podría constituir un factor predictor pronóstico[44]. Algunos biomarcadores asociados a células madre podrían en un futuro ser usados como ayuda en el enfoque a la terapia personalizada del CP[44].

Alfa metilacil coenzima A racemasa

La alfa metilacil CoA racemasa, o también llamada P504S, es una enzima peroxisomal y mitocondrial necesaria para la producción de ácidos biliares y la beta-oxidación de los ácidos grasos de cadena ramificada[45]. En cáncer de próstata es usada como marcador molecular, puesto que mediante microarreglos de ADN se ha detectado una expresión elevada de alfa metilacil CoA racemasa en el tejido prostático afectado, comparado con tejido prostático benigno[46,47].

Como se puede notar, existen diferentes biomarcadores que en la actualidad se encuentran en investigación para determinar su rendimiento diagnóstico en pacientes con CP. Cabe anotar que los descritos no son todos los biomarcadores en desarrollo en la actualidad, y aún es muy temprano para tomar estas herramientas, pues faltan estudios con mayor tamaño de muestra, validez y confiabilidad, que nos permitan trabajar con certeza sobre nuestra población masculina, sin embargo es claro que el presente y futuro debe estar enfocado en la investigación de éste tipo de biomarcadores en cáncer de próstata, así como en otros tipos de tumores.

Conflicto de intereses

No existe conflicto de intereses.

Bibliografía

1. Kontos CK, Adamopoulos PG, Scorilas A. Cancer prognostic and predictive biomarkers in prostate cancer. 2015;7159:1-10.
2. Ouyang H, Zhou Y, Zhang L, Shen G. Diagnostic value of microRNAs for urologic cancers: A systematic review and meta-analysis. Medicine (Baltimore). 2015;94:e1272.
3. Survival rates for prostate cancer [Internet]. American Cancer Society. [cosultado 5 May 2016].Disponible en: http://www.cancer.org/cancer/prostatecancer/detailedguide/prostate-cancer-survival-rates
4. Center MM, Jemal A, Lortet-Tieulent J, Ward E, Ferlay J, Brawley O, et al. International variation in prostate cancer incidence and mortality rates. Eur Urol. 2012;61:1079–92.
5. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBOCAN 2012 v1.0, Cancer incidence and mortality worldwide: IARC CancerBase No. 11 [Internet]. Int Agency Res Cancer [Internet]. 2013. Disponible en: http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx
6. Luis J, Matiz P, Julián N, Reyes A, Paula M, Becerra S. Evolución de la mortalidad por cáncer de próstata en Colombia: estudio ecológico. Urol Colom. 2014;23:3-10.
7. Hoffman RM. Screening for prostate cancer. N Engl J Med. 2011;365:1–7.
8. Saini S. PSA and beyond: Alternative prostate cancer biomarkers. Cell Oncol [Internet]. 2016;39:97–106.
9. Potenziani, J. PSA (antígeno prostático específico) Enfoque 2012. Vitae. 2012;51:1-21.
10. García HA, Carbonell J. Urología en pocas palabras. Primera Edición. Cali: Editorial Universidad del Valle; 2011, p. 210-221.
11. Ortiz Sanchez JM. La eficiencia del índice de PSA libre en el diagnóstico de cáncer de próstata [Internet]. 2014. [consultado 1 Jun 2016]. Disponible en: http://cybertesis.unmsm.edu.pe/bitstream/cybertesis/3829/1/Ortiz_sj.pdf
12. Salman J, Schoots I, Carlsson S, Jenster G, Roobol M. Prostate specific antigen as a tumor marker in prostate cancer: Biochemical and clinical aspects. Adv Cancer Biomarkers. 2015;9:3–114.

13. Chadha KC, Miller A, Nair BB, Schwartz SA, Trump DL, Underwood W. New serum biomarkers for prostate cancer diagnosis. Clin cancer Investig J. 2015;3:72–9.
14. Ilic D, Neuberger MM, Djulbegovic M, Dahm P. Screening for prostate cancer. Cochrane database Syst Rev. 2013;1:CD004720.
15. Balk SP. Biology of prostate-specific antigen. J Clin Oncol. 2003;21:383–91.
16. Mikolajczyk SD, Millar LS, Wang TJ, Rittenhouse HG, Wolfert RL, Marks LS, et al. BPSA, a specific molecular form of free prostate-specific antigen, is found predominantly in the transition zone of patients with nodular benign prostatic hyperplasia. Urology. 2000;55:41–5.
17. Mikolajczyk SD, Millar LS, Wang TJ, Rittenhouse HG, Marks LS, Song W, et al. A precursor form of prostate-specific antigen is more highly elevated in prostate cancer compared with benign transition zone prostate tissue. Cancer Res. 2000;60:756–9.
18. Wolf AM, Wender RC, Etzioni RB, Thompson IM, Amico Vd A, Volk RJ, et al. American Cancer Society guideline for the early detection of prostate cáncer. Update 2010. Cancer J. 2010;60:70–98.
19. Andriole GL, Crawford ED, Grubb RL, Buys SS, Chia D, Church TR, et al. Prostate cancer screening in the randomized prostate, lung, colorectal, and ovarian cancer screening trial: Mortality results after 13 years of follow-up. J Natl Cancer Inst. 2012;104:125–32.
20. Schroder F, Hugosson J, Roobol M, Tammela T, Ciatto S. Prostate-cancer mortality at 11 years of follow-up. N Engl J Med. 2012;366:981–90.
21. Ilic D, Connor DO, Green S, Wilt TJ. Screening for prostate cancer: An updated Cochrane systematic review. BJU Int. 2011;107:882–91.
22. Djulbegovic M, Beyth RJ, Neuberger MM, Stoffs TL, Vieweg J, Djulbegovic B. Screening for prostate cancer: Systematic review and meta-analysis of randomised controlled trials. BMJ. 2010;341:c4543, 1-9.
23. Wei W, Leng J, Shao H, Wang W. High PCA3 scores in urine correlate with poor-prognosis factors in prostate cancer patients. Int J Clin Exp Med. 2015;8:16606-16612.
24. Jedinak A, Curatolo A, Zurakowski D, Dillon S, Bhasin MK, Libermann TA, et al. Novel non-invasive biomarkers that distinguish between benign prostate hyperplasia and prostate cancer. BMC Cancer. 2015;15:259.
25. Pedersen LO, Hansen AS, Olsen AC, Gerwien J, Nissen MH, Buus S. The interaction between beta 2-microglobulin and purified class-I major histocompatibility (MHC) antigen. Scand J Immunol. 1994;39:64–72.
26. Berggard I, Bearn AG. Isolation and properties of a low molecular weight B2-globulin occurring in human biological fluids. J Biol Chem. 1968;243:4095–103.
27. Klein T, Levin I, Niska A, Joren R, Gal R, Schater J. Correlation between tumour and serumP2 m. Eur J Immunogenet. 1996;1:417–23.
28. Auer I, Watzel C, Greulich M. The plasma concentration of b2-microglobulin in the diagnosis of malignancy of the gastrointestinal tract. Med Klin. 1979;74:1581–3.
29. Huang W, Wu D, Xie Z, Pohl J, Hsieh C, Weitzmann MN, et al. β 2-microglobulin is a signaling and growth-promoting factor for human prostate cancer bone metastasis. Cancer Res. 2006;66:9108–16.
30. Rowley DR, Dang TD, Mcbride L, Gerdes MJ, Lu B. β-2 microglobulin is mitogenic to pc-3 prostatic carcinoma cells and antagonistic to transforming growth factor β 1 action microglobulin is mitogenic to pc-3 prostatic carcinoma cells and antagonistic to transforming growth factor flu action1. Cancer Res. 1995;55:781–6.
31. Yang J, Qian J, Wezeman M, Wang S, Lin P, Wang M, et al. Targeting B2-microglobulin for induction of tumor apoptosis in human hematological malignancies. Cancer Cell. 2006;10:295–307.
32. Josson S, Nomura T, Lin JT, Huang WC, Wu D. B2-microglobulin induces epitelial to mesenchymal transition and confers cancer lethality and bone metastasis in human cancer cells. Cancer Res. 2011;71:2600–10.
33. Zhang YX, Wang L, Ji PY, Zhao GG, Zhong GP, Wang ZP. Correlation of serum β2-microglobulin levels with prostate-specific antigen, gleason score, clinical stage, tumor metastasis and therapy efficacy inprostate cancer. Arch Med Res. 2013;44:259–65.
34. Huang WC, Zhau H. B2-microglobulin signaling blockade inhibited androgen receptor axis and caused apoptosis in human prostate cancer cells. Clin Cancer Res. 2008;14:5341–7.
35. Mcdonald ML, Parsons JK. 4-Kallikrein test and kallikrein marker s in prostate ca ncer screening. Urol Clin NA. 2016;43:39–46.
36. Vickers AJ, Cronin AM, Aus G, Puihl C-G, Becker C, Pettersson K, et al. Impact of recent screening on predicting the outcome of prostate cancer biopsy in men with elevated PSA: Data from the European randomized study of prostate cancer screening in Gothenburg, Sweden. Cancer. 2010; 116:2612-2620.
37. Bryant RJ, Sjoberg DD, Vickers AJ, Robinson MC, Kumar R, Marsden L, et al. Predicting high-grade cancer at ten-core prostate biopsy using four kallikrein markers measured in blood in the ProtecT Study. J Natl Cancer Inst. 2015;107:1-6.
38. Vickers AJ, Cronin AM, Roobol MJ, Savage CJ, Pettersson K, Scardino PT, et al. A four-kallikrein panel predicts prostate cancer in men with recent screening: data from the European randomized Study of Prostate Cancer Screening. Rotterdam. 2010;16:3232–9.
39. Braun K, Sjoberg DD, Vickers AJ, Lilja H, Bjartell AS, Catto J. A four-kallikrein panel predicts high-grade cancer on biopsy: Independent validation in a community cohort. Eur Urol. 2015:1–7.
40. Parekh DJ, Punnen S, Sjoberg DD, Asroff SW, Bailen JL, Cochran JS, et al. A multi-institutional prospective trial in the USA confirms that the 4K score accurately identifies men with high-grade prostate cancer. Eur Urol. 2015;68:464–70.
41. O'Connor JC, Julian J, Lim SD, Carson DD. MUC1 expression in human prostate cancer cell lines and primary tumors. Prostate Cancer Prostatic Dis. 2005;8:36–44.
42. Cozzi PJ, Wang J, Delprado W, Perkins AC, Allen BJ, Russell J, et al. Clin Exp Metastasis. 2006;22:565–73.
43. Li Y, Cozzi PJ. MUC1 is a promising therapeutic target for prostate cancer therapy. Curr Cancer Drug Targets. 2007;7:259–71.
44. Mancini M, Zazzara M, Zattoni F. Stem cells, biomarkers and genetic profiling: approaching future challenges in urology. Riv Urol V 83. 2016:4–13.
45. Ananthanarayanan V, Deaton RJ, Yang XJ, Pins MR, Gann PH. Alpha-methylacyl-CoA racemase (AMACR) expression in normal prostatic glands and high-grade prostatic intraepithelial neoplasia (HGPIN): Association with diagnosis of prostate cancer. Prostate. 2005;63:341–6.
46. Alinezhad S, Väänänen R-M, Ochoa NT, Vertosick EA, Bjartell A, Boström PJ, et al. Global expression of AMACR transcripts predicts risk for prostate cancer–a systematic comparison of AMACR protein and mRNA expression in cancerous and noncancerous prostate. BMC Urol. 2016;16:10.
47. Jiang Z, Woda BA, Wu CL, Yang XJ. Discovery and clinical application of a novel prostate cancer marker: Alpha-methylacyl CoA racemase (P504S). Am J Clin Pathol. 2004;122:275–89.

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Frequency of allelic variants of the TMPRSS2 gene in a prostate cancer-free Southwestern Colombian population

Prevalencia de variantes alélicas del gen TMPRSS2 en una población del suroeste de Colombia libre de cáncer de próstata

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Abstract

OBJECTIVE: To describe the frequency of the TMPRSS2 gene and its variants in a prostate cancer-free Southwestern Colombian population.

MATERIALS AND METHODS: An observational study was conducted that included cancer-free persons, regardless of age, from Southwestern Colombia. Blood samples were drawn from the patients for DNA extraction. Blood drops were collected and dried on filters and immersed in phosphate buffer, utilizing the DNeasy kit. The preparation process was carried out using the TruSeq Exome Library Prep® kit and the resulting libraries were normalized with the TruSeq Rapid Exome® kit. The commercial kits were provided by Illumina®. We sequenced the full exome and identified the variants associated with the TMPRSS2 gene. Descriptive statistics were employed for the data analysis.

RESULTS: The study population was made up of 162 persons from whom 7,315,466 sequence data were obtained. The TMPRSS2 gene was found in 414 data (4.3%). The most common SNP was rs140530035 (32.1%) and the most relevant SNP sequenced was rs12329760 (10.6%).

CONCLUSION: TMPRSS2 was not frequent in the population studied. The most important polymorphism associated with the TMPRSS2 gene was rs12329760.

KEYWORDS: Gene; Prostate cancer; TMPRSS2; Polymorphism.

Resumen

OBJETIVO: Estimar la prevalencia del gen TMPRSS2, y sus variantes, en pacientes libres de cáncer de próstata de una población del suroeste de Colombia.

MATERIALES Y MÉTODOS: Estudio observacional, al que se incluyeron pacientes libres de cáncer de próstata, sin importar su edad, residentes de una población del sudoeste de Colombia. Se recolectaron muestras de sangre para extraer el ADN mediante filtros, inmersos en una solución tampón de fosfato, para evaluarse en el equipo comercial DNeasy. Para la lectura de resultados se utilizó el manual TruSeq Exome Library Prep® y se normalizaron con TruSeq Rapid Exome®, proporcionados por Illumina®. Se obtuvo la secuenciación del exoma completo y se identificaron las variantes asociadas con el gen TMPRSS2. Para el análisis de los datos se implementó estadística descriptiva.

RESULTADOS: Se registraron 162 pacientes, de quienes se obtuvieron 7,315,466 datos de secuenciación. El gen TMPRSS2 se encontró en 414 datos (4.3%). El SNP más común fue rs140530035 (32.1%) y el secuenciador más relevante rs12329760 (10.6%).

CONCLUSIÓN: la identificación del gen TMPRSS2 no es frecuente en pacientes libre de cáncer de próstata del suroeste de Colombia. El polimorfismo rs12329760 tuvo mayor relación con el gen TMPRSS2.

PALABRAS CLAVE: Gen TMPRSS2; cáncer de próstata; polimorfismo.

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INTRODUCTION

One of the most prevalent neoplastic pathologies associated with male sex is prostate cancer. The estimated prevalence is 1.1 million people worldwide (1–3) and it is impacted by ethnicity and geographic location (4). Populations of African descent are the most affected, showing an 11% increase in prevalence in recent years(5). The Southwest region of Colombia is inhabited by populations of Latin American and African descent in approximately the same proportion but with different rates of disease incidence(6).

Variants of certain genes have been associated with a higher frequency of prostate cancer (BRCA1-2, ATM, NBN, TMPRSS2, among others) (7). Serine proteases, such as the TMPRSS2 gene, are recognized through their mechanisms of action in inflammatory and immune processes. That gene is located on chromosome 21q22.3 and is expressed at the apex of the secretory epithelium of the glands. Fusion with members of the ETS family is the most frequent chromosomal re-arrangement found in 50% of prostate cancers, mainly produced by the microdeletion of a portion of the TMPRSS2 gene (8). The TMPRSS2 gene and the fusion gene (TMPRSS2:ERG) have been associated with the severity and prognosis of prostate cancer, although the actual pathophysiologic process or the variant associated with that condition are not very well known (9). The fusion gene has been widely studied and at present has been postulated as one of the most important biomarkers for diagnostic and prognostic purposes in the prostate cancer population (10). There are reports in the literature on the single nucleotide polymorphisms (SNPs) most frequently related to those clinical scenarios.

The present study is important because there are no similar descriptive studies characterizing the presence of the TMPRSS2 gene and its variants in a population from Southwestern Colombia. Our study focuses on describing the frequency of the allelic variants of the TMPRSS2 gene in that population.

MATERIALS AND METHODS

A descriptive, observational study was conducted on persons, regardless of age, from Southwestern Colombia (Nariño, Cauca, Putumayo, and Valle), within the time frame of 2014 to 2016.

Sample size

According to the expected frequency for hereditary prostate cancer (≈ 15%), alpha 5%, and an expected error of 5%, the calculated sample size was 162 people and convenience sampling was carried out.

Complete exome sequencing was performed, which enabled the sequencing of all protein-coding regions (exome) in the genome, thus identifying the variants that could alter the sequence of a protein. It was carried out as follows:

DNA extraction

Blood was drawn from each patient for DNA extraction. All drops of blood were collected and dried on filter paper. The filter paper was then immersed in a phosphate buffer utilizing the DNeasy kit from the QIAGEN® company (Hilden, Germany-Operational). Each extraction was quantified, and its quality was verified, to continue the sequencing processing.

Sequencing protocol

DNA aliquots from each sample underwent a preparation process with the TruSeq Exome Library Prep®. The resulting libraries were then normalized for sequencing using the TruSeq Rapid Exome®. The kits were provided by Illumina® from San Diego, California, USA. The normalized

fragments with their corresponding adaptors for sequencing were charged in a HiSeq2500 machine.

We sequenced the full exome and identified the related variants, specifically the SNPs for the TMPRSS2 gene that is associated with prostate cancer (PCa).

The present project was conducted following all ethical international standards. Descriptive statistics were performed in R and the results are shown in frequency tables for each gene and its associated variants. Finally, we looked for the variants in the following public databases: Exome Aggregation Consortium (ExAC), PharmGKB(11), Clinvar(12), Ensemble, and dbSNP(13), searching for a pattern through which we could use the variants we found as markers.

RESULTS

One hundred sixty-two patients were included in the study, providing 7,315,466 sequence data, and the TMPRSS2 gene was found in 414 data (4.3%). Missense variants were identified in 23% of the data, although the most frequent variants were synonymous variants and introns. Only one stop variant was found in those data (**Table 1**).

In addition, the most common variants for the TMPRSS2 gene were: rs140530035 (32.12%), rs17854725 (19.8%), and rs2298659 (13.5%) (**Table 2**).

Table 1. Associated Variants.

Variant	Absolute Frequency	Percentage (%)
5UTR	3	0.72
Intron	144	34.78
Missense	98	23.67
Stop	1	0.24
Synonymous	168	40.58

Table 2. Variants identified for TMPRSS2 gene

Variants	Absolute Frequency	Percentage (%)
No identifier available	36	8.70
rs12329760	44	10.63
rs140530035	133	32.13
rs143049780	1	0.24
rs148125094	1	0.24
rs149527323	1	0.24
rs17854725	82	19.81
rs181414852	1	0.24
rs2298659	56	13.53
rs3787950	15	3.62
rs61735789	2	0.48
rs61735790	1	0.24
rs61735792	1	0.24
rs61735793	1	0.24
rs61735794	3	0.72
rs61735795	1	0.24
rs75603675	35	8.45

DISCUSSION

Transmembrane protease serine 2, also called TMPRSS2, is a protease composed of 492 amino acids expressed on the cell surface of multiple organs and they are theorized to be strategically located to regulate cell-cell interactions. The TMPRSS2 gene has been shown to be positively regulated by androgenic hormones in neoplastic tissue, possibly modulating the inflammatory response of prostate cells through the activation of PAR-2 (14,15).

Prostate cancer is one of the most frequent cancers in males and the TMPRSS2 gene has historically been associated with that malignant tumor. Numerous authors have conducted studies over the past decades in an attempt to link the presence of the TMPRSS2 gene with the frequency of cancer and its prognosis (16).

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Although there are studies that have found that the TMPRSS2 gene does not represent a worse prognosis for prostate cancer (17), an important fusion of that gene with the ERG gene was described, with an increasing relation to the diagnosis and aggressiveness of prostate cancer (present in 50% of high-risk prostate cancers) (18,19).

We found a low frequency of the allelic variant associated with the TMPRSS2:ERG fusion gene in our cancer-free population from Southwestern Colombia. The rs12329760 variant, albeit not the most frequent SNP found in the present study, is reported in the literature to have a non-negligible allele frequency (AF) in populations from East Asia and Northern Europe (0.38 and 0.37, respectively), with a major homozygote ratio (> 7%). Frequency in the Hispanic population is 0.155, with a low number of homozygotes (20). It should be noted that Southwestern Colombia has a large population of African descent, in which a higher frequency of said allelic variant (0.29) has been identified. That is an important fact to keep in mind when identifying new biomarkers for prostate cancer.

The most sequenced polymorphism in the present study was rs140530035. It is a very common intron in the world population and the allele frequency of that variant reaches 0.9 (21). Comparing populations, inhabitants of northern Europe (Finland) have an AF of 0.99, whereas it is only 0.66 in the so-called Latino population, according to Lek et al. 2016 (21).

The rs17854725 and rs2298659 polymorphisms are synonymous variants that are rare in the Latin American population, according to the literature, with an AF of 0.15 or less, and they have no known pathologic associations. Likewise, the rs75603675 polymorphism is not known to be associated with any pathology (21).

Strengths and limitations

The present study is the first to describe the relation of the TMPRSS2 gene and its allelic variants to a Southwestern Colombian cancer-free population. An advantage of the project was the quality of the study's samples, analyses, and data. Several variants associated with the TMPRSS2 gene were identified. That is very important information for the performance of future longitudinal studies in cancer-free patients to determine the risk for that disease.

A limitation of the present study was the fact that we did not find any information associated with the presence of a pathologic relationship to prostate cancer.

CONCLUSIONS

The TMPRSS2 gene was not frequent in the cancer-free Southwestern Colombian population studied. Nonetheless, the most common variants for the TMPRSS2 gene were: rs140530035 (32.12%), rs17854725 (19.8%), and rs2298659 (13.5%).

REFERENCES

1. Bray F, et al. Global estimates of cancer prevalence for 27 sites in the adult population in 2008 GLOBOCAN. Int J Cancer 2013;132(5):1133-45. DOI: 10.1002/ijc.27711
2. Valencia O, et al. Incidence and Prevalence of Cancer in Colombia: The Methodology Used Matters. J Glob Oncol 2018;(4):1-7. DOI: 10.1200/JGO.17.00008
3. Bashir MN. Epidemiology of Prostate Cancer. Asian Pacific J Cancer Prev 2015;16(13):5137-41.
4. Rebbeck TR. Prostate cancer genetics: variation by race, ethnicity, and geography. Semin Radiat Oncol 2016;27(1):3-10. DOI: 10.1016/j.semradonc.2016.08.002
5. Parkin DM, et al. Fifty years of cancer incidence: CI5 I-IX. Int J Cancer 2010;127:2918-27. DOI: 10.1002/ijc.25517
6. Poveda-Matiz JL, et al. Evolución de la mortalidad por cáncer de próstata en Colombia: estudio ecológico. Urol Colomb 2014;23(1):3-10. Dirección URL: <http://www.elsevier.es/es-revista-urologia-colombiana-398-articulo-evolucion-mortalidad-por-cancer-prostata-X0120789X14284174>.

7. Antczak A, et al. The variant allele of the rs188140481 polymorphism confers a moderate increase in the risk of prostate cancer in Polish men. Eur J Cancer Prev 2015;24(2):122-7. DOI: 10.1097/CEJ.0000000000000079
8. Antalis T, et al. Membrane-anchored serine proteases in health and disease. Prog Mol Biol Transl Sci 2011;99:1-50. DOI: 10.1016/B978-0-12-385504-6.00001-4
9. Fernández-Serra A, et al. Molecular characterization and clinical impact of TMPRSS2-ERG rearrangement on prostate cancer: comparison between FISH and RT-PCR. Biomed Res Int 2013;2013:465179. DOI: 10.1155/2013/465179
10. Wang J, et al. Expression of variant TMPRSS2/ERG fusion messenger RNAs is associated with aggressive prostate cancer. Cancer Res 2006;66(17):8347-51. DOI: 10.1158/0008-5472.CAN-06-1966
11. Whirl-Carrillo M, et al. Pharmacogenomics knowledge for personalized medicine. Clin Pharmacol Ther. 2012;92(4):414-7. DOI: 10.1038/clpt.2012.96
12. Landrum M, et al. ClinVar: Improving access to variant interpretations and supporting evidence. Nucleic Acids Res 2018;46(D1):D1062-D1067. DOI: 10.1093/nar/gkx1153
13. Sherry S, et al. dbSNP: The NCBI database of genetic variation. Nucleic Acids Res 2001;29(1):308-11.
14. Blee AM, et al. TMPRSS2-ERG controls luminal epithelial lineage and antiandrogen sensitivity in PTEN and TP53 -mutated prostate cancer. Clin Cancer Res 2018;24(18):4551-4565. DOI: 10.1158/1078-0432.CCR-18-0653
15. Knuuttila M, et al. Intratumoral androgen levels are linked to TMPRSS2-ERG fusion in prostate cancer. Endocr Relat Cancer 2018;25(9):807-19. DOI: 10.1530/ERC-18-0148
16. Fernández-Serra A, et al. Cáncer de próstata: la revolución de los genes de fusión. Actas Urol Esp 2011;35(7):420-8. Dirección URL: <http://www.elsevier.es/es-revista-actas-urologicas-espanolas-292-articulo-cancer-prostata-revolucion-los-genes-S0210480611000702>.
17. Tomlins SA, et al. Role of the TMPRSS2-ERG gene fusion in prostate cancer. Neoplasia. Neoplasia 2008;10(2):177-88. Dirección URL: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2244693/>.
18. Bismar TA, et al. PTEN genomic deletion is an early event associated with ERG gene rearrangements in prostate cancer. BJU Int 2011;107(3):477–85. DOI: 10.1111/j.1464-410X.2010.09470.x
19. Hsu F-C, et al. Comparison of two methods for estimating absolute risk of prostate cancer based on single nucleotide polymorphisms and family history. Cancer Epidemiol Biomarkers Prev 2010;19(4):1083-8. DOI: 10.1158/1055-9965.EPI-09-1176
20. Karczewski KJ, et al. The ExAC browser: displaying reference data information from over 60 000 exomes. Nucleic Acids Res 2017;45(D1):D840-5. DOI: 10.1093/nar/gkw971
21. Lek M, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature 2016;536(7616):285-91. DOI: 10.1038/nature19057

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REVIEW PAPER

UROLOGICAL ONCOLOGY

Molecular alterations associated with prostate cancer

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Introduction The amount of information and knowledge about pathways and genetic alterations regarding prostate cancer, including the tools available for its study has been recently increasing. Additionally, a variety of molecular signaling pathways control cell proliferation, however, this incompletely understood process is disturbed in cancer cells.

Materials and methods A literature review was made using the MEDLINE, Embase and LILACS databases searching for the following keywords: prostate neoplasms, prostate cancer, molecular medicine, genomics, pathways, and cell cycle.

Results Different biological mechanisms have been associated with the development of prostate cancer, such as alterations in tumor suppressor genes, oncogenes (TP53, RB1, among others) and CDKIs; DNA methylation; chromosomal alterations and rearrangements; changes in PTEN and PI3K / mTOR; global defects in apoptosis; alterations in the androgen receptor (AR); and epigenetic mechanisms.

Conclusions Good clinical practice and a practical approach have to be based on basic knowledge, thus, in this article, the main genetic alterations, mutations and pathways involved in prostate cancer development were reviewed.

Key Words: prostatic neoplasms ‹› molecular medicine ‹› genetics ‹› review

INTRODUCTION

Recently, the quantity and quality of tools available for the genetic study of cancer and the whole genome have increased, with even greater detail available for the exome alone. Many molecular signaling pathways provide negative or positive regulatory signals that control cell proliferation in a way that attempts to preserve cell number and homeostasis, but this process is completely altered in cancer cells [1, 2].

Normal cells must acquire at least eight attributes to transition from a normal cell to a cancer cell. These attributes include the following: 1) genetic instability and mutation; 2) autonomous growth; 3) insensitivity to internal and external anti-proliferative signals; 4) resistance to apoptosis and other forms of induced cell death; 5) unlimited cell division potential; 6) ability to form new blood vessels (angiogenesis); 7) local invasive behavior that enables the distinction of benign and malignant neoplasms; 8) evasion of the immune system.

Additionally, cancer cells require energy for autonomous growth and unlimited replication. Tumor-associated inflammatory mediators also cause preneoplastic cells to progress to invasive cancer cells; finally, cancer cells gain the ability to metastasize, that is, to migrate and colonize organs or tissues [1, 3, 4]. The purpose of this article was to review and describe the main biomolecular mechanisms associated with prostate cancer. Therefore, the somatic genetic alterations that are involved in the pathogenesis of prostate carcinoma progression are shown in Figure 1 [5, 6].

MATERIAL AND METHODS

We performed a systematic literature search in Medline via Ovid, Scopus (including Embase) and LILACS from their inception to nowadays with the following keywords: prostate neoplasms, prostate cancer, molecular medicine, genomics, pathways, and cell cycle. We included reviews, systematic reviews, basic science studies and analytical studies, which tried to explain the molecular disturbances associated with prostate cancer. According to the heterogeneity expected, we synthesized information based on the molecular mechanism. Information about the most promising biomarkers associated with prostate cancer can be found elsewhere [7].

Tumor suppressor genes and oncogenes

Suppressor genes negatively regulate cell growth, and therefore, play an important role in the normal cell cycle, DNA repair and cell signaling. The loss of the function of both alleles of a suppressor gene leads to carcinogenesis; thus, different pathways can result in cancer, such as 1. Homozygous gene deletion, 2. Loss of one allele and mutational inactivation of the second, 3. Mutations in both alleles, or 4. Loss of one allele and epigenetic inactivation of the second allele (e.g., DNA methylation) [1]. The two best characterized suppressor genes thus far are the retinoblastoma gene (RB1) and the TP53 gene, which are described later.

Oncogenes are positively associated with cell proliferation and are the mutated form of normal genes (proto-oncogenes). Two such oncogenes are MYC and MET. MYC is responsible for the regulation of cell proliferation. This amplified gene is frequently present in prostate cancer (PCa), and its expression in prostatic cells has been associated with immortalization [8]. In contrast, MET has been reported in renal cell carcinoma (RCC), primarily in the hereditary type [9].

The mechanisms by which a proto-oncogene can become an activated oncogene are as follows: 1) proto-oncogene mutation, 2) gene amplification and 3) chromosomal rearrangement. An example of the third mechanism is the translocation that leads to the fusion of the TMPRSS2 gene with the ERG oncogene in a large proportion of PCa cases [10]. Figure 2 schematically represents the cell cycle and describes how a cell in G0 is allowed to proliferate based on a signal, is duplicated in S phase, the phase in which DNA is synthesized, and subsequently segregates its genomic complement, which results in two daughter cells in a process called M phase (mitosis). These two processes are separated by two critical gaps termed Gap 1 and Gap 2. The entire cycle lasts approximately 24 hours, and each phase depends on the previous one. In addition, some mechanisms function to verify the integrity of the DNA. If any alterations are found, the cell attempts to repair the damage, but if repair is not possible, the cell enters an active process termed apoptosis, which will be described later. The loss of the ability to respond to DNA damage leads to genetic instability, increases the mutation rate and mutates genes associated with cancer, thereby contributing to carcinogenesis and progression of the disease [11, 12].

Retinoblastoma protein (RB1)

RB1 is important for controlling the R-point, which is a decisive point in late G1 phase during which the cell is committed to undergo division. Thus, if this

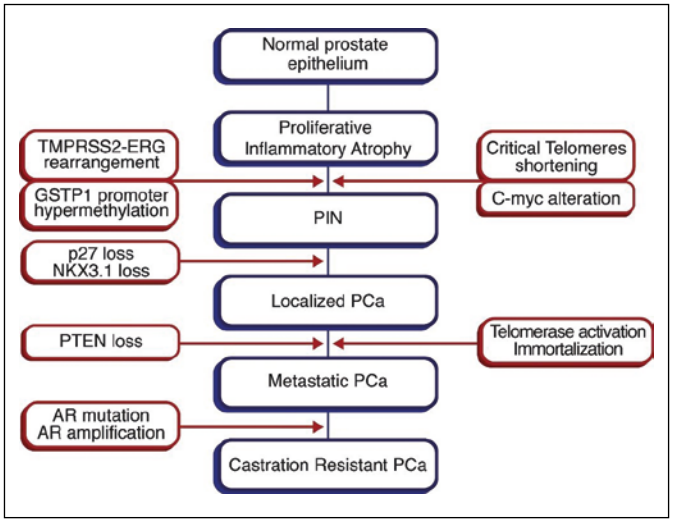

Figure 1. *Natural history of prostate cancer and the molecular alterations involved.*

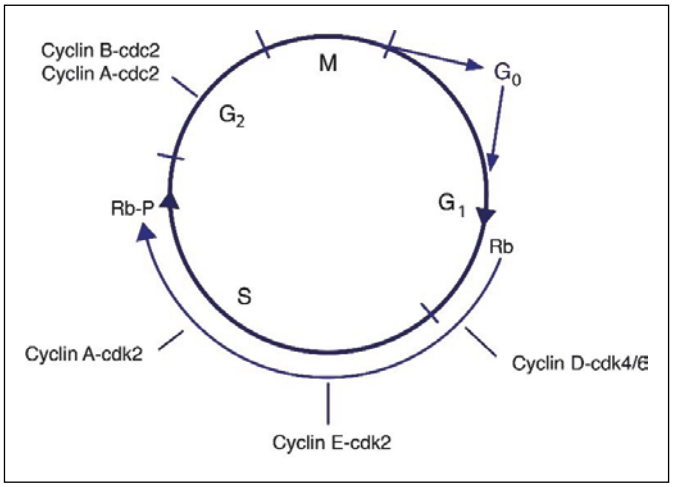

Figure 2. *Cell cycle scheme.*

control is lost, the cell continues to proliferate. All of the above events are due to inactivation of the RB1 pathway, which is mutated in at least 30% of bladder and prostate tumors, although RB1 mutations have not been strongly associated with these cancer types. It has also rarely been associated with renal carcinoma [13, 14].

Cyclin-dependent kinase inhibitors

The temporal sequence of events during the cell cycle is dependent on cyclins and cyclin-dependent kinases (CDKs). CDKs phosphorylate protein substrates that are involved in the execution of specific activities in each phase. In contrast, cyclin-dependent kinase inhibitors (CDKIs) bind directly to CDKs and suspend their activity and their ability to phosphorylate other proteins [15]. CDKIs belong to one of two classes: the Cip / Kip Family, which includes the CDKN1A (p21), CDKN1B (p27) and CDKN1C (p57) proteins, and the INK4 (inhibits CDK4) family, which includes the INK4B (p15), INK4A (p16), INK4C (p18) and INK4D (p19) proteins. The p16 protein binds to CDKs 4 and 6 and inhibits their interaction with cyclin D1; normally, active CDK4 and 6 mediate the passage of the cell through G1 phase via the phosphorylation of RB1 [1, 16]. The latter has also been associated with bladder cancer (by deletion of INK4A) and with renal cancer, and p16 inactivation has been shown to occur by hypermethylation of the DNA (epigenetic mechanism) [17]. In prostate cancer, hypermethylation of INK4A is typically seen in 60% of cases, although INK4B is rarely inactivated [18].
Decreased CDKN1B has been correlated with decreased overall survival and disease-free survival after radical prostatectomy. In addition to positive CDKN1B in prostate biopsies, it has been associated with increased biochemical recurrence, and in mice, absence is associated with prostatic hyperplasia [19, 20].

Tumor suppressor TP53

TP53 is a suppressor gene that plays an important role in response to cellular damage. It signals a halt to the cycle or leads to damage repair pathways (Figure 3), but if repair is not possible, the cell will undergo apoptosis. This suppressor is often mutated in genitourinary cancers. Additionally, Figure 4 shows the possible causes of alterations in TP53 and its responses in the cell cycle.
TP53-induced apoptosis is mediated by Bcl-2 through an intrinsic pathway, and alterations in the regulation of this pathway have direct relevance in the etiology of cancer. This pathway is associated with the activation of transcriptional genes and the inhibition of other genes that block the cascade. On the one hand, TP-53 is dependent on the activation of the Apaf-1/caspase-9 pathway, but on the other hand, Bax (Bcl-2 family) is not essential for TP53-dependent apoptosis.
In addition, different tumor suppressive pathways are associated with TP53, and some examples are the response to DNA damage, cell senescence and apoptosis, and thus, it is logical to consider that TP53 is frequently mutated in cancer [1, 21].

Methylation of DNA

The covalent modification of the C5 position of the cytosine by a methyl group is mediated by DNA methyltransferase and results in the for-

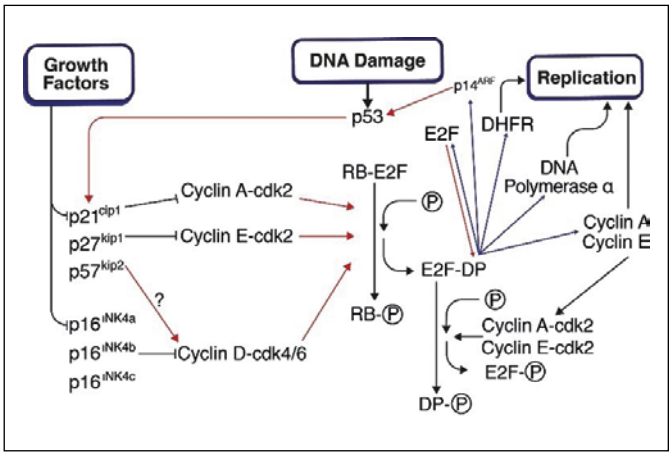

Figure 3. *TP53-dependent repair mechanisms.*

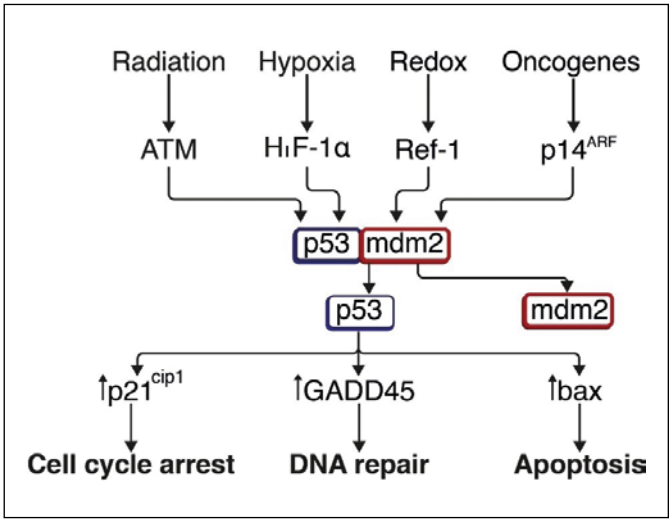

Figure 4. *Causes of alterations in TP53 and its response in the cell cycle.*

mation of 5-methylcytosine, which is an epigenetic modification that occurs in vertebrates and is part of normal and essential embryological development. As vertebrates have evolved, CpG dinucleotides have become depleted within the genome, but in some areas, this depletion is not seen; these areas are termed CpG islands and represent approximately 1% of the genome. The epigenetic properties of methylation can affect the genetic activity without alterations in the DNA sequence and represent an alternative means to inactivate the gene apart from a mutation or deletion. The three major pathways that DNA methylation uses to result in genetic alterations include the following: 1) inherent mutational effects of 5-methylcytosine, 2) epigenetic effects of the promoter on transcription and 3) activation and potential induction of a gene due to instability of the chromosome by DNA hypomethylation [1].

DNA methylation and prostate cancer

Glutathione S transferases are a family of enzymes that are responsible for the detoxification of a large group of xenobiotics that catalyze the nucleophilic attack of reduced glutathione in potentially harmful electrophilic compounds. The aberrant methylation of the CpG island at the glutathione S transferase pi (GSTP1) locus is the most frequent somatic alteration reported in PCa [22]. This methylation has been detected in up to 90% of PCa and in 70% of prostatic intraepithelial neoplasia (PIN), however it might be present in normal or hyperplastic tissue [22]. Due to oxidative stress, this aberrant methylation leads to the overexpression of GSTP1 in prostatic epithelial columnar cells. These findings are also associated with worse clinical outcomes [23].

The gene for the ras association domain of the familial protein 1 isoform A (RASSF1A) is located on chromosome 3p21. This is a suppressor gene that is methylated in 60–70% of prostate carcinomas, and it has been observed that this alteration is more frequent in high-grade tumors than in less aggressive tumors [24, 25]. The methylation patterns are not consistent since they can consist of either hypo- or hypermethylation, and in addition, the methylation is conserved in all metastases, suggesting an alteration that follows clonal selection [26, 27].

DNA damage and repair

Cancer is fundamentally a genetic disease. Alterations in different genes will lead to the activation of different pathways associated with cancer, leading to changes in tumor suppressor genes and oncogenes through epigenetic, mutational and copy number distortions.

To counteract these elements, the cell employs different defense mechanisms including the use of free radicals such as alpha tocopherol, vitamin C, carotenoids, bilirubin and urate and protective enzymes such as superoxide dismutase, glutathione peroxidase and glutathione transferase. In addition to the previously described methylation of GSTP1, associations have been found between the polymorphisms of this gene and the risk of biochemical recurrence in patients with PCa [28].

The cell additionally employs a series of mechanisms termed the DNA damage response (DDR), which involves a number of genes. DDR relies on the replication machinery, as well as on specific mechanisms such as repairs of base cleavage, nucleotide cleavage, double helix rupture and imbalance [1].

Chromosomal abnormalities

Deletions of chromosomal segments are frequently found, although gains and amplifications are seen more frequently in cases of advanced disease [12]. These changes have been demonstrated through cytogenetic techniques such as genomic hybridization, fluorescence in situ hybridization or detection of microsatellites. Cytogenetic methods detect numerical changes, while molecular analytic methods identify recombinations that do not lead to changes in copy number [12].

The most frequently altered autosomes are 8, 13, 7, 10, 16, 6 and 17, likely in this order. In addition, gains or amplification of parts of the X chromosome and losses of the Y are also observed. A decreased copy number and loss of heterozygosity of chromosome 8p are also consistent in previous studies (observed in approximately 50% of cases). Specific alterations are observed in each of the chromosomes, but special attention must be paid to the functional impact each alteration may have on the tumor phenotype and the indication or expression of the tumor suppressor genes or oncogenes in the affected regions [12].

Recurrent genetic rearrangements in PCa

Recurrent gene fusions have been identified, primarily between the androgen-regulated gene TMPRSS2 and ERG, which is a member of the ETS (E26 transforming sequence) family. This fusion occurs in 90% of all fusions that involve ETS genes in prostate cancer [29]. The other fusions occur as a result of more complex types of translocations [10, 30]. In 60% of cases, the fusion occurs due to a deletion of the

sequence that separates the two genes (3 Mb).
These rearrangements can be readily identified through reverse transcription polymerase chain reaction (RT-PCR) or by multicolor fluorescence in situ hybridization (FISH).
At present, several clinical studies have evaluated these markers in urine and blood, while other studies have evaluated the expression of the ERG protein using a simpler method (immunostaining) [31, 32, 33].
The TMPRSS2-ERG fusion status is considered a possible diagnostic marker, although its prognostic significance is still unclear, which is a fundamental part of patient follow-up [34, 35].
The TMPRSS2 gene can be merged with other members of the ETS family including ETV1, ETV4 and ETV5 [36, 37].
Studies have been performed with different technologies including next generation RNA sequencing; in these studies, it was found that some fusions are single events or events that occur in only one patient, which might imply that we actually know very little about PCa [38].

PTEN and PI3K/mTOR

The PI3K/mTOR (mammalian target of rapamycin) pathway plays an important role in cell growth, proliferation and oncogenesis in PCa [39, 40, 41]. PTEN is a negative regulator of this pathway. In retrospective studies, it has been shown how the loss of PTEN and consequently, the activation of the mTOR pathway lead to a poor prognosis in PCa [5]. PTEN deletions have been found in up to 20% of patients with PCa and have been associated with earlier biochemical relapse, metastasis, resistance to castration, presence of ERG gene fusions and the accumulation of nuclear TP53 [5].

Association with telomeres

A potential association between telomere length and prostate cancer has been found. Initially, this association was found only in studies with small sample sizes, but subsequently, some studies with larger sample sizes were performed in which associations were observed between a short telomere length and decreased overall survival and increased biochemical recurrence. These findings have been consistent even when adjusted for age, Gleason score and lymph node involvement. It has been proposed that cancer that develops from these areas can lead to greater genotype and phenotype heterogeneity, as well as to an increase in aggressiveness [42]. Some studies have even suggested that the risk of death is increased up to 14 times in patients with short telomeres compared with patients with long telomeres [43].

Apoptosis

Apoptosis is an orderly, energy-requiring process in which the cellular content is degraded and condensed into an apoptotic body that is finally digested by neighboring cells or macrophages [44]. The positive or negative signals of the apoptotic process finally converge in a family of proteases termed cysteine proteases with aspartic acid specificity. Caspases total at least 13, and some of them are initiators (caspase – 8, caspase – 9, caspase – 10), whereas others are executioners (caspase – 3, caspase – 6 and caspase – 7). Caspases are derived from procaspases, which are larger inactive forms that require proteolytic cleavage in order to become active. They are frequently activated by other caspases (initiator) to generate an activating cascade of executioner caspases. The latter type attacks different anti-apoptotic intracellular proteins such as Bcl-2 and Bcl-XL. They not only destroy their anti-apoptotic functions but also release carboxyl-terminal fragments to remove the cell [45]. They also degrade DNA repair and replication proteins such as DNA-PKcs and replication factor C, leading to nuclear dysregulation.
Nuclear proteins such as laminin, NuMa and SAF-A are fragmented and undergo nuclear dissolution and nuclear condensation (milestones in the cell that lead to apoptosis). Proteolysis of cytoskeletal proteins such as keratin and actin also occurs, which leads to the destruction of the integrity of the internal structure. A final step is the breakdown of cell-to-cell interaction proteins such as beta-catenin and focal adhesion kinase, which precipitates the phenotypic and irreversible changes associated with apoptosis [45].

Global defects in apoptosis

In both PCa and PIN, a high level of apoptosis is seen, although compared with other malignancies, PCa has low apoptotic activity along with increased replication. As PCa progresses, it is unclear whether androgen-resistant cells have an increased or decreased apoptosis rate because both have been found in patients with castration-resistant prostate cancer [46]. In contrast, an advanced infiltrative tumor whose DNA is mutated and that is fast-growing may have a high rate of apoptosis despite the protective mechanisms that the cell has acquired.
Apoptosis can be initiated by two pathways: the intrinsic and the extrinsic pathways (Figure 5). The intrinsic pathway monitors conditions within

the cell and responds to various forms of stress. Pro-apoptotic signals can originate from damaged and unrepaired DNA or from the lack of signals from the cell surface (cell-cell or cell-matrix contacts, including hormones or diminished growth factors).
Mitochondria and the Bcl-2 family are major components of the intrinsic pathway. The Bcl-2 family contains 12 pro-apoptotic proteins including Bax, Bak, Bok, Bik, Bas, Bid and Bim. It also contains six pro-survival proteins including Bcl-2, Bcl-XL, Bcl-W and Mcl1 [47].
Each protein in the family responds to different stimuli; however, their primary function is to increase the permeability of the mitochondrial membrane [48]. Subsequently, cytochrome c is released from the intermembrane space into the cytoplasm, where it binds to Apaf-1 proteins and forms the apoptosome complex. Caspase-9 is activated, which then activates the entire cascade described above.
In addition, other activations occur such as ones that involve Bid, which is regulated by initiating caspases in the cytosol. Caspase-8 allows the dimerization of Bid with Bax or Bcl-2. This active form of Bax inhibits Bcl-2, which leads to apoptosis. This process can be blocked by anti-apoptotic proteins and by the IAP proteins that inhibit specific caspases [1].
The extrinsic pathway mediates apoptosis after receiving external signals from surface receptors called 'death receptors', such as tumor necrosis factor receptor 1 (TNFR1) and Fas receptor. The death receptor domain is located in its intracellular region and allows binding to adapter proteins that also contain a death domain (RIP, TRADD, FADD). Additionally, these proteins have an effector domain that binds to the caspase recruitment domain (CARD) of the initiating caspase [49]. Subsequently, the initiator caspase is cleaved and is able to activate the cascade. The most well-known death receptor is CD95 or Fas, but this receptor does not appear to have a direct effect on the etiology of cancer. In contrast, IGF-1 can activate the PI3K / AKT anti-apoptotic pathway and stimulate the expression of Bcl-like proteins along with Bax suppression. In addition, the expression of IGF binding proteins may also be altered in PCa [12, 50, 51].

Androgens and prostate cancer

Most treatments for PCa are based on androgenic suppression, but they are rarely curative. To cure the disease, different mechanisms should be considered and are described as follows (Figure 6): 1) some carcinomas do not express androgen receptor (AR) in some cases because the gene is silenced by a hypermethylated promoter; 2) several peptide growth

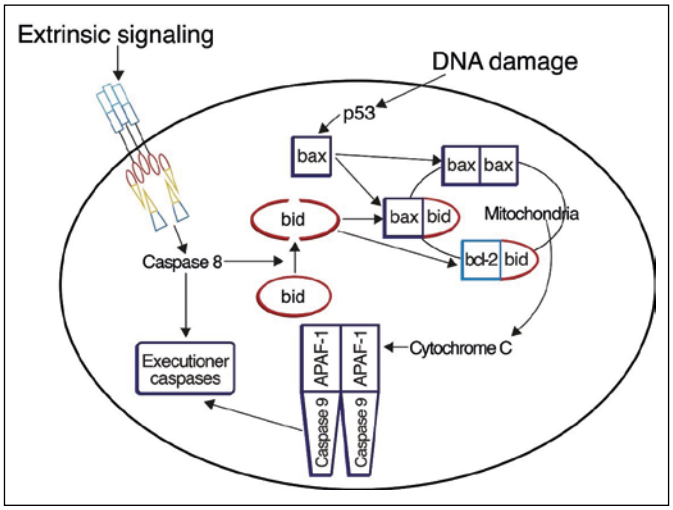

Figure 5. *Intrinsic and extrinsic pathways of apoptosis.*

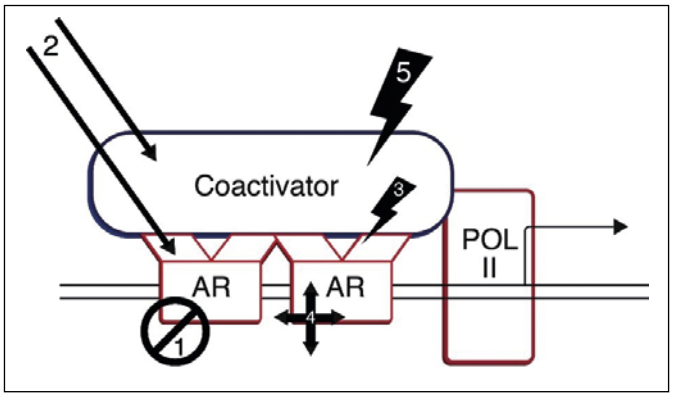

Figure 6. *Mechanisms of altered androgen signaling in prostate carcinoma: 1. The androgen receptor (AR) is absent in some carcinomas. 2. Growth factors activate the AR or a coactivator in a ligand-independent fashion. 3. AR mutations alter ligand specificity and affinity. 4. AR expression is increased by gene amplification. 5. Coactivators are differentially expressed or mutated.*

factors and cytokines such as fibroblast growth factor 7 (FGF7), epidermal growth factor (EGF) and interleukin-6 (IL-6) can activate the AR synergistically with or independently of a steroid ligand; 3) in some carcinomas, somatic mutations in the AR alter its receptor specificity and cause it to respond to estrogen, progesterone, dehydroepiandrosterone or synthetic anti-androgens; 4) amplification of the AR gene can occur in up to 30% of cases, even in the presence of depletion, which leads to increased sensitivity to a minimal androgen level; 5) different co-activating proteins have been identified as mediators of the effects of AR on chromatin structure, as well as transcriptional initiators and their interactions with other signaling pathways. All of the circumstances

described above could lead to androgen-independent tumor growth [12].

Expression profiles

With the advent of the analysis of gene expression patterns by cDNA or oligonucleotide microarrays, research related to the diagnosis, prognosis and new therapeutic markers has become increasingly important. For example, it has been found that hepsin is not a good marker since its down-regulation increases tumor heterogeneity in prostate carcinomas [52]. Another marker is the P504S protein, which is identical to Alpha-Methyl-Acyl-CoA Racemase (AMACR); the latter is a peroxisomal enzyme that is involved in the metabolism of branched-chain amino acids, which might be useful for the differentiation of hyperplasia and atrophy from cancer [53, 54].

Epigenetics / Environmental factors

Lifestyle and dietary habits have been found to be triggers of the oncogenic cascade in PCa. For example, dietary carcinogens, estrogens and oxidants act as a trigger for chronic inflammatory changes within the prostatic tissue and thus act as a promoter of PCa [5, 55, 56]. It has been suggested that the intake of red meat (formation of heterocyclic aromatic amines and polycyclic aromatic hydrocarbon, which have carcinogenic properties) or animal fat is a risk factor for PCa. However, when prevention studies on both of these micronutrients and other elements of the diet were performed, the suppression of those elements was not found to prevent prostate cancer [57]. Additionally, sexually transmitted diseases as part of a system that triggers chronic inflammation in epithelial cells have been associated with the development of PCa (Figure 7) [58, 59, 60].

Given the change from persistent oxidative stress, a survival response is generated by glutathione S transferase, cyclooxygenase-2 and other mediators. In general, the epigenetic silencing of multiple genes occurs, including the silencing of a fundamental gene, GSTP1, which is found throughout all stages of prostate cancer progression [5].

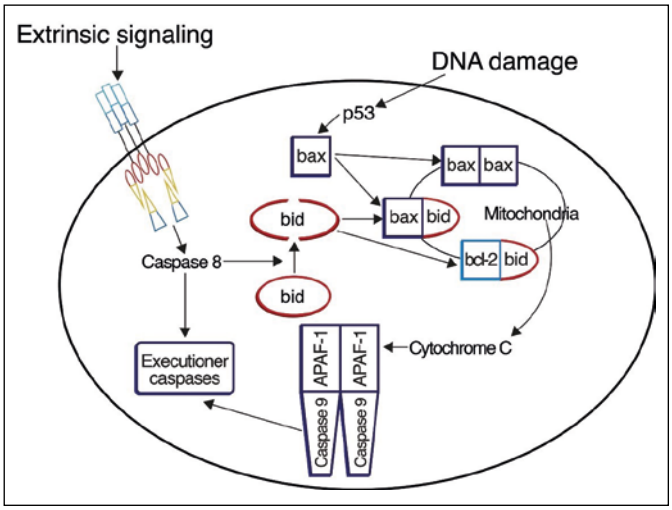

Figure 7. *Epigenetic factors associated with prostate cancer.*

CONCLUSIONS

The natural history of prostate cancer involves numerous genetic and molecular alterations that cause the normal prostatic epithelial cell to become cancerous and resistant to castration. Different biological mechanisms have been associated with the development of prostate cancer, such as alterations in tumor suppressor genes, oncogenes (TP53, RB1, among others) and CDKIs; DNA methylation; chromosomal alterations and rearrangements; changes in PTEN and PI3K / mTOR; global defects in apoptosis; alterations in the AR; and epigenetic mechanisms. These are not the only mechanisms, but they have been found to be associated with prostate cancer at a higher frequency than others. Similarly, the development of prostate cancer does not have a unique etiology, but rather, it is predominantly multifactorial and can be explained by the different mechanisms described here.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

References

1. Gonzalgo M, Stefanos K, Meeker A. Part IV. Molecular and cellular biology. In: Wein A, Kavoussi L, Partin A, Peters C, eds. Campbell Walsh Urology. Eleventh. Philadelphia: Elsevier Inc. 2016; p. 459.
2. Feitelson MA, Arzumanyan A, Kulathinal RJ, et al. Sustained proliferation in cancer: Mechanisms and novel therapeutic targets. Semin Cancer Biol. 2015; 35: S25-54.
3. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. Cell. 2011; 144: 646-674.
4. Luo J, Solimini NL, Elledge SJ. Principles of cancer therapy: oncogene and non-oncogene addiction. Cell. 2009; 136: 823-837.
5. Netto GJ. Molecular Updates in Prostate Cancer. Surg Pathol Clin. 2015; 8: 561-580.
6. Netto GJ, Cheng L. Emerging critical role of molecular testing in diagnostic

genitourinary pathology. Arch Pathol Lab Med. 2012; 136: 372-390.

7. Esquivel Parra L, Caicedo Bolaños A, Guaitarilla J, García-Perdomo H. Una mirada general a los biomarcadores para la tamización y el diagnóstico temprano del cáncer de Próstata. Urol Colomb. 2017; 26: 110-116.

8. Gil J, Kerai P, Lleonart M, et al. Immortalization of primary human prostate epithelial cells by c-Myc. Cancer Res. 2005; 65: 2179-2185.

9. Schmidt L, Duh FM, Chen F, Kishida T, et al. Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. Nat Genet. 1997; 16: 68-73.

10. Tomlins SA, Rhodes DR, Perner S, et al. Recurrent Fusion of TMPRSS2 and ETS Transcription Factor Genes in Prostate Cancer. Science. 2005; 310: 644-648.

11. Bartek J, Lukas J, Bartkova J. Perspective: defects in cell cycle control and cancer. J Pathol. 1999; 187: 95-99.

12. Schulz W, Burchardt M, Cronauer M. Molecular biology of prostate cancer. Mol Hum Reprod. 2003; 9: 437-448.

13. Kubota Y, Fujinami K, Uemura H, et al. Retinoblastoma gene mutations in primary human prostate cancer. Prostate. 1995; 27: 314-320.

14. Logothetis CJ, Xu HJ, Ro JY, et al. Altered expression of retinoblastoma protein and known prognostic variables in locally advanced bladder cancer. J Natl Cancer Inst. 1992; 84: 1256-1261.

15. Sherr CJ, Roberts JM. Inhibitors of mammalian G1 cyclin-dependent kinases. Genes Dev. 1995; 9: 1149-1163.

16. Serrano M, Hannon GJ, Beach D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature. 1993; 366: 704-707.

17. Merlo A, Herman J, Mao L, et al. 5′ CpG island methylation is associated with transcriptional silencing of the tumour suppressor p16/CDKN2/ MTS1 in human cancers. Nat Med. 1995; 1: 686-692.

18. Herman JG, Merlo A, Mao L, et al. Inactivation of the CDKN2 / p16 / MTS1 Gene Is Frequently Associated with Aberrant DNA Methylation in All Common Human Cancers DNA Methylation in All Common Human Cancers. Cancer Res. 1995; 55: 4525-4530.

19. Freedland SJ, DeGregorio F, Sacoolidge JC, et al. Preoperative p27 status is an independent predictor of prostate specific antigen failure following radical prostatectomy. J Urol. 2003; 169: 1325-1330.

20. Cordon-Cardo C, Koff A, Drobnjak M, et al. Distinct altered patterns of p27KIP1 gene expression in benign prostatic hyperplasia and prostatic carcinoma. J Natl Cancer Inst. 1998; 90: 1284-1291.

21. Oda E, Ohki R, Murasawa H, et al. Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. Science. 2000; 288: 1053-1058.

22. Jerónimo C, Usadel H, Henrique R, et al. Quantitation of GSTP1 methylation in non-neoplastic prostatic tissue and organ-confined prostate adenocarcinoma. J Natl Cancer Inst. 2001; 93: 1747-1752.

23. Maruyama R, Toyooka S, Toyooka KO, et al. Aberrant promoter methylation profile of prostate cancers and its relationship to clinicopathological features. Clin Cancer Res. 2002; 8: 514-519.

24. Liu L, Yoon J-H, Dammann R, Pfeifer GP. Frequent hypermethylation of the RASSF1A gene in prostate cancer. Oncogene. 2002; 21: 6835-6840.

25. Kuzmin I, Gillespie JW, Protopopov A, et al. The RASSF1A tumor suppressor gene is inactivated in prostate tumors and suppresses growth of prostate carcinoma cells. Cancer Res. 2002; 62: 3498-3502.

26. Yegnasubramanian S, Wu Z, Haffner MC, et al. Chromosome-wide mapping of DNA methylation patterns in normal and malignant prostate cells reveals pervasive methylation of gene-associated and conserved intergenic sequences. BMC Genomics. 2011; 12: 313.

27. Aryee MJ, Liu W, Engelmann JC, et al. DNA methylation alterations exhibit intraindividual stability and interindividual heterogeneity in prostate cancer metastases. Sci Transl Med. 2013; 5: 169.

28. Nock NL, Bock C, Neslund-Dudas C, et al. Polymorphisms in glutathione S-transferase genes increase risk of prostate cancer biochemical recurrence differentially by ethnicity and disease severity. Cancer Causes Control. 2009; 20: 1915-1926.

29. Kumar-Sinha C, Tomlins SA, Chinnaiyan AM. Recurrent gene fusions in prostate cancer. Nat Rev Cancer. 2008; 8: 497-511.

30. Perner S, Mosquera J-M, Demichelis F, et al. TMPRSS2-ERG fusion prostate cancer: an early molecular event associated with invasion. Am J Surg Pathol. 2007; 31: 882-888.

31. Falzarano SM, Zhou M, Carver P, et al. ERG gene rearrangement status in prostate cancer detected by immunohistochemistry. Virchows Arch. 2011; 459: 441-447.

32. Park K, Tomlins SA, Mudaliar KM, et al. Antibody-based detection of ERG rearrangement-positive prostate cancer. Neoplasia. 2010; 12: 590-598.

33. Chaux A, Albadine R, Toubaji A, et al. Immunohistochemistry for ERG expression as a surrogate for TMPRSS2-ERG fusion detection in prostatic adenocarcinomas. Am J Surg Pathol. 2011; 35: 1014-1020.

34. Fine SW, Gopalan A, Leversha MA, et al. TMPRSS2-ERG gene fusion is associated with low Gleason scores and not with high-grade morphological features. Mod Pathol. 2010; 23: 1325-1333.

35. Leyten GHJM, Hessels D, Jannink SA, et al. Prospective multicentre evaluation of PCA3 and TMPRSS2-ERG gene fusions as diagnostic and prognostic urinary biomarkers for prostate cancer. Eur Urol. 2014; 65: 534-542.

36. Helgeson BE, Tomlins SA, Shah N, et al. Characterization of TMPRSS2:ETV5 and SLC45A3:ETV5 gene fusions in prostate cancer. Cancer Res. 2008; 68: 73-80.

37. Tomlins SA, Mehra R, Rhodes DR, et al. TMPRSS2:ETV4 gene fusions define a third molecular subtype of prostate cancer. Cancer Res. 2006; 66: 3396-4000.

38. Pflueger D, Terry S, Sboner A, et al. Discovery of non-ETS gene fusions in human prostate cancer using next-generation RNA sequencing. Genome Res. 2011; 21: 56-67.

39. Sarker D, Reid AHM, Yap TA, de Bono JS. Targeting the PI3K/AKT pathway for the treatment of prostate cancer. Clin cancer Res. 2009; 15: 4799-4805.

40. Bismar TA, Yoshimoto M, Vollmer RT, Duan Q, Firszt M, Corcos J, et al. PTEN genomic deletion is an early event

associated with ERG gene rearrangements in prostate cancer. BJU Int. 2011; 107: 477-485.

41. Han B, Mehra R, Lonigro RJ, et al. Fluorescence in situ hybridization study shows association of PTEN deletion with ERG rearrangement during prostate cancer progression. Mod Pathol. 2009; 22: 1083-1093.

42. Fordyce CA, Heaphy CM, Joste NE, Smith AY, Hunt WC, Griffith JK. Association between cancer-free survival and telomere DNA content in prostate tumors. J Urol. 2005; 173: 610-614.

43. Heaphy CM, Yoon GS, Peskoe SB, et al. Prostate cancer cell telomere length variability and stromal cell telomere length as prognostic markers for metastasis and death. Cancer Discov. 2013; 3: 1130-1141.

44. Fadok VA, Savill JS, Haslett C, et al. Different populations of macrophages use either the vitronectin receptor or the phosphatidylserine receptor to recognize and remove apoptotic cells. J Immunol. 1992; 149: 4029-4035.

45. Wolf BB, Green DR. Suicidal tendencies: apoptotic cell death by caspase family proteinases. J Biol Chem. 1999; 274: 20049-20052.

46. Koivisto P, Visakorpi T, Rantala I, Isola J. Increased cell proliferation activity and decreased cell death are associated with the emergence of hormone-refractory recurrent prostate cancer. J Pathol. 1997; 183: 51-56.

47. Adams JM, Cory S. The Bcl-2 protein family: arbiters of cell survival. Science. 1998; 281: 1322-1326.

48. Kroemer G, Reed JC. Mitochondrial control of cell death. Nat Med. 2000; 6: 513-519.

49. Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. Science. 1998; 281: 1305-1308.

50. Grimberg A, Cohen P. Role of insulin-like growth factors and their binding proteins in growth control and carcinogenesis. J Cell Physiol. 2000; 183: 1-9.

51. Chan JM, Stampfer MJ, Ma J, Gann P, et al. Insulin-like growth factor-I (IGF-I) and IGF binding protein-3 as predictors of advanced-stage prostate cancer. J Natl Cancer Inst. 2002; 94: 1099-1106.

52. Srikantan V, Valladares M, Rhim JS, Moul JW, Srivastava S. HEPSIN inhibits cell growth/invasion in prostate cancer cells. Cancer Res. 2002; 62: 6812-6816.

53. Jiang Z, Woda BA, Rock KL, et al. P504S: a new molecular marker for the detection of prostate carcinoma. Am J Surg Pathol. 2001; 25: 1397-1404.

54. Rubin MA, Zhou M, Dhanasekaran SM, et al. Alpha- Methylacyl coenzyme A racemase as a tissue biomarker for prostate cancer. JAMA. 2002; 287: 1662-1670.

55. Sfanos KS, Hempel HA, De Marzo AM. The role of inflammation in prostate cancer. Adv Exp Med Biol. 2014; 816: 153-181.

56. De Marzo AM, Platz EA, Sutcliffe S, et al. Inflammation in prostate carcinogenesis. Nat Rev Cancer. 2007; 7: 256-269.

57. Jurado-Penagos A, García Perdomo HA. Efectividad de los micronutrientes en la prevención del cáncer de próstata. Rev Fac Med. 2014; 62: 65-72.

58. Elkahwaji JE. The role of inflammatory mediators in the development of prostatic hyperplasia and prostate cancer. Res Rep Urol. 2012; 5: 1-10.

59. Sutcliffe S, Neace C, Magnuson NS, Reeves R, Alderete JF. Trichomonosis, a common curable STI, and prostate carcinogenesis--a proposed molecular mechanism. PLoS Pathog. 2012; 8: e1002801.

60. Sutcliffe S, Platz EA. Inflammation and prostate cancer: a focus on infections. Curr Urol Rep. 2008; 9: 243-249. ■

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Una mirada global y actualizada del cáncer de próstata

An updated and global review on prostate cancer

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| Resumen |

Introducción. El cáncer de próstata es una patología importante en la salud pública y tiene alto impacto mundial. El conocimiento y manejo de esta enfermedad debe ser del dominio de todo médico general y especialista que tenga a cargo pacientes que la padezcan.

Objetivo. Obtener una visión actualizada de la epidemiología, los factores de riesgo, la clasificación, el diagnóstico y el tratamiento del cáncer de próstata.

Materiales y métodos. Se realizó una búsqueda en las bases de datos Embase y MEDLINE desde enero del 2000 hasta marzo del 2017 mediante la cual se hizo un recorrido a través de las condiciones de riesgo, tamizaje, diagnóstico, nuevos biomarcadores y tratamiento del cáncer de próstata.

Resultados. Factores genéticos y medioambientales son foco de estudio en la actualidad. La sospecha diagnóstica del cáncer de próstata sigue siendo con el antígeno específico prostático y el tacto rectal y su diagnóstico se debe hacer con la biopsia de próstata. Se han hecho cambios importantes en cuanto a la clasificación y tratamiento de los pacientes con esta enfermedad.

Conclusión. Existe mucha investigación en curso y por venir sobre la prevención, el diagnóstico y el tratamiento de esta condición tan importante, relevante y pertinente para los hombres alrededor del mundo.

Palabras clave: Próstata; Neoplasias de la Próstata; Biomarcadores; Revisión (DeCS).

| Abstract |

Introduction: Prostate cancer is a major public health concern with a high impact worldwide. Knowledge and management of this disease should be mastered by general practitioners and specialists who treat patients with this pathology.

Objective: To obtain an updated and detailed review of the epidemiology, risk factors, classification, diagnosis and treatment of prostate cancer.

Materials and methods: A search was carried out in the Embase and MEDLINE databases from January 2000 to March 2017, comprising risk factors, screening, diagnosis, new biomarkers and treatment of prostate cancer.

Results: Genetic and environmental factors are currently the focus of studies. Prostate-specific antigen and digital rectal examination are still used to suspect prostate cancer, while diagnosis is achieved with a prostate biopsy. Important changes have been made regarding the classification and treatment of patients with this disease.

Conclusion: Significant changes have been made in the area. Several ongoing and upcoming researches on prevention, diagnosis and treatment of this condition are available, which are relevant for men around the world.

Keywords: Prostate; Prostatic Neoplasms; Biomarkers; Review (MeSH).

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Introducción

Dada su frecuencia en las poblaciones, el cáncer de próstata (CAP) es una patología de importancia en la salud pública a nivel nacional e internacional (1). Debido a las dificultades del sistema de salud, la poca disponibilidad de especialistas y la alta prevalencia, el conocimiento de esta condición debe ser del dominio de todo médico general y no se debe quedar en esferas de la medicina especializada como la urología y la oncología. No obstante, el tratamiento, de manera integral, debe ser dado por centros de excelencia en cáncer (2).

El objetivo de esta investigación fue obtener una visión actualizada a través de una revisión detallada y al día de la epidemiología, los factores de riesgo, la clasificación, el diagnóstico y el tratamiento del CAP.

Materiales y métodos

Se realizó una búsqueda en las bases de datos Embase y MEDLINE (a través de OVID) desde enero de 2000 hasta marzo de 2017. Las palabras clave utilizadas fueron: "prostate" OR "prostate, neoplasm" AND "diagnosis" OR "treatment". Además, se realizó una exhaustiva evaluación manual de la bibliografía proporcionada en los artículos encontrados.

Resultados y discusión

Epidemiología

El CAP es la neoplasia con mayor frecuencia en hombres alrededor del mundo y representa la segunda causa de muerte por cáncer en esta población en EE. UU. (3). Esta patología presenta una incidencia de 131.5 por cada 100 000 habitantes (3) con una distribución según raza de 123 por cada 100 000 habitantes en la raza blanca y 208 por cada 100 000 habitantes en la raza negra (4). Se estima que 1 de cada 7 hombres serán diagnosticados a lo largo de su vida con CAP y que 1 de cada 38 hombres morirán como consecuencia de este (5).

El estudio GLOBCAN notificó que en países del norte de Europa (Dinamarca, Noruega y Suecia) se ha incrementado el diagnóstico de CAP 8.2% por año; sin embargo, se presenta una mortalidad en descenso desde el 2000 de 3.1% por año (6). En EE. UU. y Canadá se han encontrado datos similares, con una incidencia estable de 4.3% y una disminución en la mortalidad de 3.1%; no obstante, en países en vía de desarrollo la mortalidad ha ido en aumento (6,7). En cuanto a la epidemiología nacional, Colombia tiene una de las incidencias más bajas de CAP en Latinoamérica y una proporción de 28% entre incidencia y mortalidad, un valor muy cercano al promedio mundial de 28.6% e inferior al de países como Ecuador (40.41%), Cuba (46.65%) y Perú (37.74%) (6); del igual forma, la mortalidad ha disminuido en los últimos 4 años (7) y las regiones con el mayor número de pacientes con CAP reportados son Bogotá D.C., Valle y Antioquia (las regiones más pobladas y con mayor cantidad de urólogos) (5,7).

Factores de riesgo

Raza

Los pacientes de raza negra presentan mayor prevalencia de CAP (8); además, en esta población se presenta a edades más tempranas y con mayor volumen tumoral, mayor antígeno prostático y peor pronóstico (9,10). Algunos autores relacionan estos resultados con las inequidades sociales y dificultades de acceso a servicios de salud a las que se expone esta población (11); sin embargo, existe mucha más evidencia que sustenta la raza como factor riesgo para CAP. Por otro lado, se han encontrado tasas mucho menores en asiáticos, lo cual se ha relacionado con la dieta, los estilos de vida y los factores ambientales (12).

Historia familiar

Cerca del 10-15% de los hombres con CAP tienen, por lo menos, un familiar con antecedente de esta patología (8,13). Se estima que contar con un familiar de primer grado de consanguinidad con CAP incrementa el RR 2 a 4 veces y es 5 veces mayor si son dos los familiares con dicho diagnóstico.

Inflamación

La inflamación crónica se considera un factor de riesgo dado que conlleva a hiperproliferación celular; esto, a su vez, genera una alteración en los niveles de antioxidantes, en la reparación del DNA y en la apoptosis. Además, se ha encontrado que el antecedente de una infección de transmisión sexual tiene un OR=1.5 (14) y haber tenido o tener prostatitis tiene un OR=1.57 (15). A pesar de ser una de las hipótesis más fuertes, aún no es claro el mecanismo que llevaría a la inflamación a producir el CAP o si es una causa suficiente para su desarrollo.

Estrés oxidativo

Algunos estudios han sugerido que las especies reactivas de oxígeno (ROS), como el superóxido o el peróxido, crean un ambiente de mutagénesis propicio para el inicio del CAP (16,17). Este elemento podría estar asociado con la hipótesis de la inflamación crónica.

Andrógenos

Existe evidencia de que un aumento en la concentración de los niveles de testosterona incrementa la incidencia de CAP, aunque no se ha establecido una relación dosis-respuesta ni una concentración a partir de la cual se incremente el riesgo; además, no se ha encontrado mayor riesgo de CAP en pacientes con hipogonadismo tratados con terapia de reemplazo de testosterona (18).

Estrógenos

Se ha evidenciado que los estrógenos pueden predisponer e incluso causar CAP. En este aspecto es necesario recalcar que el 17β-estradiol ya se ha clasificado como carcinógeno, sobre todo en cáncer de mama y endometrial. Se cree que el efecto de los estrógenos en el CAP es causado por mutaciones directas a través de la regulación por efectos epigenéticos o por alteración endocrina propiamente dicha (19).

Dieta

Diversos estudios han sugerido que una dieta baja en grasas y calcio y con aumento en el consumo de vitamina E y licopenos, así como el ejercicio regular, podrían comportarse como factores protectores para el desarrollo de CAP. Por otro lado, la ingesta elevada de grasas saturadas de origen animal y las carnes rojas han sido descritas como factores de riesgo; sin embargo, los hallazgos no son consistentes entre los diferentes estudios, por ejemplo, en el estudio SELECT no se demostró el factor protector del uso de la vitamina E ni el selenio (20).

Aumento de niveles del factor de crecimiento similar a la insulina (IGF-1)

El factor de crecimiento similar a la insulina es un factor mitogénico y antiapoptótico. Altos niveles implican más riesgo de CAP (21); sin embargo, otros estudios no lo encuentran como factor de riesgo.

Genética

Se han encontrado alteraciones en genes supresores como el p53 y el PTEN, los cuales se relacionan con aumento de la incidencia y progresión y agresividad del CAP. Entre otros genes alterados se ha encontrado: oncogén RAS, EIF3S3, BCL2 (anti-apoptosis), EGFR, FGFR2c, ERBB2, BRCA 2, MET, además de algunas mutaciones en el cromosoma 1 (riesgo CAP familiar) y 8 (cáncer esporádico). Asimismo, se han evidenciado polimorfismos genéticos en algunas enzimas como: 5 alfa reductasa, mayor en raza negra; receptor de vitamina D (VDR), el cual ha sido reconocido como un factor protector, aunque en pacientes de raza negra se disminuye e incrementa el riesgo de CAP; receptor androgénico (AR), el cual aumenta el riesgo de CAP familiar, y telomerasa, un factor para cáncer esporádico.

Muchas de las investigaciones han centrado su atención en el gen BRCA2 (Breast Cancer susceptibility protein type 2), el cual presenta un patrón de herencia autosómica dominante con una dominancia incompleta. Este gen codifica para una proteína del mismo nombre, cuya función es actuar como centro reclutando proteínas reguladoras para reparar las rupturas de doble cadena por recombinación homóloga; además, facilita la reparación de cadenas simples al promover la formación del complejo RAD51-ssDNA (cadena simple de DNA) (22).

A lo largo de la historia, el gen BRCA2 se ha relacionado con el cáncer de mama; sin embargo, hallazgos recientes indican que este puede jugar un papel importante en el CAP. No se ha logrado identificar con certeza el mecanismo por el cual sus mutaciones predisponen al desarrollo del CAP, aunque por su función se deduce que alteraciones y mutaciones de este predisponen a una menor reparación de los daños del genoma, lo cual podría resultar en alteraciones del ciclo celular y, por consiguiente, en una mayor proliferación celular.

Por lo general, los pacientes con mutaciones del gen BRCA2 presentan mayor incidencia de CAP (23), estadios más avanzados (T3-T4), fenotipos más agresivos y menor sobrevida a pesar de recibir un tratamiento local con intento curativo similar (24).

Obesidad

Algunos autores sugieren que la obesidad juega un papel en el desarrollo del CAP, pues se cree que la resistencia a la insulina producida por la obesidad lleva a una elevación de esta hormona, la cual, por su capacidad anabólica, podría generar desarrollo de cáncer o su progresión (25). Se cree que los obesos tienen menos probabilidad de tener el antígeno específico prostático (PSA) elevado y por consiguiente menos probabilidad de realización de biopsia y de diagnosticar CAP; esto, junto a las asociaciones con los niveles circulantes de hormonas metabólicas y sexuales, lleva a que se sugiera a la obesidad como un factor de riesgo para CAP agresivo (26).

Alcohol

La relación de la ingesta de alcohol con el CAP es controvertida. Rota *et al.* (27), en un metaanálisis con 52 899 casos de cáncer (50 estudios de casos y controles y 22 cohortes), no encontraron evidencia material entre la ingesta de alcohol y CAP, incluso no se hallaron diferencias estadísticas en el grupo de alta ingesta (≥4 bebidas alcohólicas al día) (27).

Cigarrillo

Es conocida la capacidad cancerígena del tabaco, así como el mecanismo por el cual se genera el daño genético. En el CAP no se ha descrito un aumento en la incidencia, sin embargo sí se ha encontrado que puede generar mayores tasas de muerte que, aunque son modestas, podrían tener impacto a nivel de salud pública por tratarse de un factor de riesgo modificable (28).

Historia natural

En autopsias se ha encontrado una prevalencia a nivel histológico de 30-40% de CAP en hombres >50 años, de 5% o menos en <30 años y de 60-70% en >79 años (29). Se calcula que el 1.5% de estos se hacen detectables por clínica cada año. El CAP es de carácter progresivo y su agresividad biológica está directamente relacionada con el grado de diferenciación celular (escala de Gleason), el TNM, el valor de PSA, entre otros factores.

Clasificación histopatológica

Para la clasificación de histopatología es utilizado el sistema Gleason, el cual expone el grado de diferenciación celular encontrado en el estroma prostático. Esta clasificación está compuesta por dos valores: el grado encontrado más frecuentemente y el siguiente, así se obtiene un valor final (por ejemplo: 4+5=9), el puntaje va de 2 a 10. En el caso en que los dos valores se encuentren en las mismas proporciones, se coloca el más indiferenciado primero.

La nueva clasificación de Gleason realizada por el Colegio Americano de Patología relaciona la puntuación con el pronóstico que tiene cada grupo (Tabla 1) (30).

Tabla 1. Clasificación de Gleason del Colegio Americano de Patología.

Grado	Puntuación	Características
1	≤6	Sólo glándulas bien diferenciadas
2	3+4=7	Glándulas predominantemente bien diferenciadas con menor componente de glándulas mal diferenciadas, fusionadas o cribiformes
3	4+3=7	Glándulas predominantemente mal diferenciadas, fusionadas o cribiformes con menor componente de las glándulas diferenciadas
4	8 puede 4+4; 3+5; 5+3	Solo glándulas mal diferenciadas, fusionadas o cribiformes; predominantemente glándulas bien diferenciadas y menor componente que carece de glándulas; predominante carencia de glándulas y menor componente de las glándulas bien diferenciadas
5	9 o 10	Carece de formación de glándulas (o con necrosis) con o sin glándulas mal diferencias, fusionada o glándulas cribiformes

Fuente: Elaboración con base en Epstein *et al.* (30).

Clasificación clínica y patológica

La clasificación clínica y patológica se realiza con base en la clasificación TNM 2016 (Tabla 2) (31). Esta consta de tres elementos: la T, relacionada con el compromiso tumoral en la glándula y fuera de ella; la N, asociada con el compromiso nodular local, y la M,

relacionada con la extensión o difusión a distancia por metástasis del tumor.

Tabla 2. Clasificación TNM para cáncer de próstata.

Tumor primario, (T) clínico	TX	El tumor primario no puede ser evaluado
	T0	No hay evidencia de tumor primario
	T1	El tumor primario no es clínicamente aparente (no visible, no palpable)
	T1a	Tumor incidental en 5% o menos del tejido prostático resecado
	T1b	Tumor incidental en más del 5% del tejido prostático resecado
	T1c	Tumor identificado por biopsia con aguja (por elevación del APE)
	T2	Tumor primario confinado a la próstata
	T2a	Tumor compromete <50% de un lóbulo
	T2b	Tumor compromete >50% de un lóbulo
	T2c	Tumor compromete ambos lóbulos
	T3	El tumor se extiende más allá de la cápsula prostática (invasión al ápex prostático o a la capsula prostática es clasificado como T2).
	T3a	Extensión extracapsular unilateral o bilateral
	T3b	El tumor compromete vesículas seminales
	T4	Tumor fijo o que invade estructuras adyacentes diferentes de las vesículas seminales: cuello vesical, esfínter externo, recto, elevadores del ano o pared pélvica
	El tumor detectado por biopsia en uno o ambos lóbulos prostáticos, que no es palpable o visible por imagenología, se clasifica como T1c.	
	Márgenes positivos deben ser indicados como R1 (enfermedad microscópica residual)	
Ganglios linfáticos regionales (N)	Nx	Metástasis regionales no evaluables
	N0	No hay metástasis regionales
	N1	Metástasis en uno o varios ganglios regionales
Metástasis a distancia (M)	Mx	Metástasis a distancia no evaluables
	M0	No hay metástasis a distancia
	M1	Metástasis a distancia
	M1a	A ganglios linfáticos no regionales
	M1b	A hueso
	M1c	A otro sitio
	Cuando hay más de un sitio de metástasis se clasifica como M1c	

Fuente: Elaboración con base en (31).

Estadificación

La estadificación del CAP se muestra en la Tabla 3. Para determinar este estadio hay que tener en cuenta que si el PSA o el valor de Gleason no están disponibles, la clasificación debe tener en cuenta el T o el valor de PSA o el Gleason que esté disponible.

Tabla 3. Estadificación del cáncer de próstata.

Estadio	T	N	M	PSA	Gleason
I	T1a-c	N0	M0	<10	≤6
	T2a	N0	M0	<10	≤6
	T1-T2a	N0	M0	X	X
IIa	T1a-c	N0	M0	<20	7
	T1a-c	N0	M0	≥10 - <20	≤6
	T2a	N0	M0	<20	≤7
	T2b	N0	M0	<20	≤7
	T2b	N0	M0	X	X
IIb	T2c	N0	M0	Cualquier	Cualquier
	T1-2	N0	M0	≥20	Cualquier
	T1-2	N0	M0	Cualquier	≥8
III	T3a-b	N0	M0	Cualquier	Cualquier
IV	T4	N0	M0	Cualquier	Cualquier
	Cualquier	N1	M0	Cualquier	Cualquier
	Cualquier	Cualquier	M1	Cualquier	Cualquier

T: tumor; N: nódulo; M: metástasis; PSA: antígeno específico prostático.
Fuente: Elaboración con base en (31).

Clasificación de riesgo para carcinoma localizado

La clasificación de riesgo para carcinoma localizado utilizada por tradición para el CAP es la de D'amico (Tabla 4) (32). Sin embargo, durante los últimos años se han generado diferentes cambios de acuerdo al pronóstico tan heterogéneo que puede presentarse con diferentes factores, por lo cual se han realizado modificaciones y una nueva clasificación sugerida por la National Comprehensive Cancer Network (NCCN) (Tabla 5) (33).

Tabla 4. Clasificación de D'Amico para carcinoma localizado.

Riesgo	PSA	Gleason	TNM
Bajo	<= 10	<= 6	<= T2a
Intermedio	10 a 20	7	T2b
Alto	>20	>= 8	>= T2c

PSA: antígeno específico prostático; TNM: tumor, nódulo, metástasis.
Fuente: Elaboración con base en D'Amico *et al.* (32).

Tabla 5. Nueva clasificación para carcinoma localizado sugerida por la National Comprehensive Cancer Network.

Riesgo	PSA	Gleason	TNM	Otros
Muy bajo	<10	≤6	T1c	<3 cores de la biopsia positivos, todos con <50% del core comprometido; Densidad de PSA <15 ng/mL/gr
Bajo	<10	≤6	T1-T2a	
Intermedio	10 a 20	7	T2b-T2c	
Alto	>20	8 a 10	T3a	

PSA: antígeno específico prostático; TNM: tumor, nódulo, metástasis.
Fuente: Elaboración con base en (33).

Diagnóstico

En la actualidad, el PSA y el tacto rectal constituyen los métodos diagnósticos más usados en la clínica para detectar el CAP; sin embargo, estos tienen bajo rendimiento diagnóstico, tanto a nivel individual como en conjunto (5).

Antígeno específico prostático

El PSA, también llamado calicreína III, es una glicoproteína de 34kDA, la cual es casi exclusiva de las células epiteliales prostáticas y circula unida a la alfa-1-antiquimiotripsina y la alfa-2-macroglobulina; su función es dividir la semenogelina I y II en polipéptidos de menor tamaño, evitando así formación del coágulo seminal (34-38).

El PSA es encontrado en el fluido prostático en concentraciones de 1 000 000 ng/mL; en condiciones normales una pequeña cantidad —<4 ng/mL— es liberada al torrente sanguíneo, pero en un proceso neoplásico estos niveles se elevan (34). Por tal motivo se considera realizar biopsia de próstata a aquellos hombres con un nivel de PSA sérico >4ng/mL (34). Sin embargo, este valor también se ha encontrado elevado en otras patologías como cáncer de mama, carcinoma de células renales, cáncer de ovario, neoplasia suprarrenal (39) y patologías urológicas como hiperplasia prostática benigna (HPB), prostatitis, cistitis, instrumentación y cirugía del tracto urinario reciente (34,40). Cabe aclarar que el tacto rectal no incrementa los valores de PSA.

Según la American Cancer Society, la sensibilidad del PSA para valores de referencia de 4 ng/mL y 3 ng/mL para el diagnóstico de cáncer es de 21% y 32%, respectivamente. La especificidad es de 91% para valores de corte de 4 ng/mL y de 85% para valores de 3 ng/mL (41).

En el estudio PLCO, en lo concerniente a CAP, se evaluaron hombres entre los 55 y los 74 años, a quienes se les realizó tamizaje anual con PSA durante 13 años. Como resultado se obtuvo que realizar tamizaje con PSA no lleva a disminución de la incidencia de CAP (RR: 1.09, IC95%: 0.87-1.36) (42).

Otro gran estudio fue el ERSPC, donde se realizó tamizaje con PSA durante 11 años a hombres de ciertos países europeos, evaluando la mortalidad por CAP. Los resultados indicaron una reducción relativa del 21% en las tasas de mortalidad (RR: 0.79, IC95%: 0.68-0.91) (43).

Un metaanálisis de Cochrane realizado en el 2011 resumió los resultados de cinco experimentos poblacionales con un total de 341 351 participantes y mostró que realizar tamizaje con PSA es efectivo para la detección de CAP (RR: 1.35, IC95%: 1.06-1.72); sin embargo, esta prueba no disminuyó la mortalidad (RR: 0.95, IC95%: 0.85-1.07) (44), de manera tal que en la actualidad no se recomienda realizar tamizaje poblacional de CAP.

No existe evidencia suficiente para determinar la mejor medida de tamización en salud pública, por ahora se sugiere tamizaje de oportunidad en hombres entre 50-70 años (de acuerdo a la expectativa de vida de la población) que ingresen a la consulta del urólogo y pacientes con factores de riesgo (raza negra y familiares con CAP).

Es de vital importancia hacer claridad en que al decidir el inicio de la búsqueda del diagnóstico del CAP debe realizarse el tacto rectal en conjunto con PSA.

Otros biomarcadores en el diagnóstico del cáncer de próstata

La falencia del PSA ha llevado a la necesidad de identificar nuevos biomarcadores con mayor sensibilidad y especificidad que permitan alcanzar un diagnóstico temprano del CAP (5). El PSA, al encontrarse elevado tanto en condiciones benignas (hiperplasia prostática benigna) como malignas (45), ha generado que se soliciten biopsias costosas e innecesarias a pacientes que no lo requerían desde un comienzo (45). Como consecuencia, se han explorado otras técnicas y moléculas para hacer un diagnóstico más específico, tales como el PCA3, la microglobulina, las mucinas, entre otras (5). Algunos de estas técnicas fueron incluidas en una revisión detallada publicada con anterioridad por Esquivel-Parra *et al.* (5), pero se sugiere revisar para profundizar en el tema.

Otras herramientas que intentan disminuir el número de biopsias innecesarias, pero que aún no han tenido éxito y se utilizan para pacientes con valores de PSA entre 4 y 10 ng/mL, son:

Relación PSA libre/PSA total: con relación a éste ítem, la relación eleva la especificidad para el diagnóstico del CAP en aquellos casos con los niveles mencionados ante presencia de duda en cuanto a la indicación para la toma de biopsia.

El PSA puede transitar en el suero libremente (fPSA) o acompañado de inhibidores de proteasa (cPSA) con el fin de evitar proteólisis. Al sumar el fPSA y cPSA se obtiene el PSA sérico total (tPSA), gran parte de este (70-90%) puede estar ligado a alfa-1-antiquimiotripsina y en menor proporción a la alfa-2-macroglobulina, alfa-1 antitripsina o a un inhibidor de la proteína C (38). En consecuencia, cerca del 10-30% del tPSA circula libremente (fPSA) (5).

Con índices <0.07, la probabilidad de CAP es casi del 90%. Aún no se encuentra definido un valor límite, sin embargo se recomienda el uso de 0.20 para decidir entre biopsia o seguimiento; los valores por encima de este sugieren un diagnóstico y tratamiento adecuado para hiperplasia prostática benigna. Por otro lado, se sugiere la realización de biopsia en valores menores a este por la probabilidad elevada de CAP.

Densidad de PSA: este ítem se calcula con base en el valor de PSA total dividido por el volumen de la próstata en cm^3 (determinado por ecografía). Se sugiere realizar biopsia ante valores >0.15 ng/mL/cm^3, ya que estos sugieren adenocarcinoma.

Velocidad de PSA: con relación a este ítem, se encuentra que un valor >0.75 ng/mL/año, sugiere la presencia de cáncer. Además, para aquellos pacientes que han sido sometidos a prostatectomía por patología benigna, sugieren un incremento >0.4 ng/mL/año.

Aunque se cuenta con todas estas herramientas para intentar elevar la especificidad del PSA, hasta el momento no han demostrado disminuir el número de biopsias innecesarias y en la actualidad se considera la toma de biopsia prostática en todo paciente con PSA >4 ng/mL; sin embargo, algunos autores sugieren tomar un valor único de PSA como <2.5 ng/mL (puede ser aplicado para pacientes <50 años dado que faltan estudios para confirmar su valor) para definir la necesidad de biopsia prostática.

Biopsia transrectal de próstata guiada por ecografía

Es el estándar de oro para el diagnóstico del CAP en la actualidad. Las muestras son tomadas en la periferia prostática, que es el sitio en el que más se presenta carcinoma. Por lo general, en la primera biopsia se deben tomar mínimo seis cilindros por cada lóbulo; en caso de contar con biopsia negativa con persistencia de PSA elevado, es necesario proceder a la realización de una biopsia por saturación (>10-12 muestras/lóbulo). Existen estudios que demuestran que la biopsia de próstata requiere adecuada analgesia/anestesia y el uso de profilaxis antibiótica.

La ecografía transrectal de próstata solo se encuentra indicada si va acompañada de biopsia, no debe realizarse en otra condición. Las

indicaciones para biopsia de próstata son PSA >4ng/mL y presencia de alteraciones en la superficie prostática (nódulo o próstata pétrea) predominantemente, aunque hay variantes que no son el objeto de esta revisión.

Medicina nuclear

La gammagrafía ósea se debe realizar obligatoriamente de manera inicial en pacientes con PSA >10 ng/mL o en aquellos clasificados como de riesgo intermedio y alto. La probabilidad de que los pacientes con niveles de PSA <10 ng/mL tengan una gammagrafía positiva se encuentra por debajo del 1%; sin embargo, un PSA >49 tiene un LR+ (likelihood ratio positivo) >6, es decir, hay 6 veces más probabilidades de encontrar una gammagrafía positiva en pacientes con CAP que en pacientes sin CAP (46).

Tomografía computarizada (TC)

La TC abdominal, al igual que la resonancia magnética (RM), evalúa de forma indirecta la invasión nodal al medir el diámetro de los ganglios linfáticos. Sin embargo, su sensibilidad es baja y la invasión microscópica no puede ser detectada. La sensibilidad es <40%. Si se utiliza un umbral de 10mm, la mediana de la sensibilidad, la especificidad, el valor predictivo negativo y el valor predictivo positivo estimado son de 7%, 100%, 85% y 100%, respectivamente.

Aunque la biopsia por aspiración con aguja fina puede ser un buen complemento en casos con imágenes positivas, la dificultad de alcanzar los ganglios por su posición hace que esta no sea muy sensible para la estadificación y tenga una tasa de falsos negativos de 40%. Para la TC, al igual que la RM, la detección de invasión ganglionar microscópica es <1% en pacientes con puntaje Gleason <8, PSA <20 ng/mL o enfermedad localizada. Su uso debe ser reservado para pacientes de alto riesgo.

Aunque la TC de hueso tiene baja especificidad, se prefiere su uso sobre otras técnicas que evalúan metástasis óseas. Se recomienda su realización en pacientes sintomáticos, sin importar los niveles de PSA, la clasificación de Gleason o el estadio clínico (47).

Resonancia magnética de próstata/pelvis

La imagen ponderada en T2 es la más útil para la estadificación local en la RM. A 1.5T (Tesla), la RM tiene baja sensibilidad para detectar extensión extraprostática de carcinoma (22-82%) o invasión de vesículas seminales (0-71%), pero mayor especificidad (61-100% y 62-100%, respectivamente). La exactitud global de la RM para distinguir las etapas T1/T2 de la T3 es 50-85%; estos resultados se deben a que la RM no puede detectar la extensión extraprostática microscópica, de tal manera que su sensibilidad aumenta con el radio de extensión dentro de la grasa periprostática (47).

El uso de la sonda endorrectal mejora la precisión del estadio a 1.5T, además se ha demostrado una mejor precisión en el uso combinado de sondas endorrectales y externas frente al uso solo de las externas. La alta intensidad de campo permite una alta resolución T2-WI y los resultados a 3T parecen mejores que a 1.5T. Aunque la experiencia del lector sigue siendo de suma importancia, la precisión de la RM a 3T varía entre 67% y 93%, dependiendo de la experiencia del personal. La predicción de la etapa patológica por parte de la RM puede mejorar cuando se combina con datos clínicos. Dada la baja sensibilidad para la extensión extraprostática focal (microscópica), la RM de próstata multiparamétrica no se recomienda para estadificación local en pacientes de bajo riesgo. Sin embargo, puede ser útil en la planificación del tratamiento en seleccionados pacientes de bajo riesgo (48).

Tomografía por emisión de positrones/tomografía computarizada

La tomografía por emisión de positrones de 11C- o 18F-colina (PET/TC) tiene una buena especificidad para las metástasis ganglionares, pero la sensibilidad es de 10-73%. En un metaanálisis de 609 pacientes, la sensibilidad y la especificidad de la PET/TC para las metástasis a ganglios pélvicos fue de 62% (IC95%: 51-66) y 92% (IC95%: 89-94), respectivamente (49); dada su baja sensibilidad, esta no se recomienda para la estadificación inicial en metástasis ganglionar, por lo que en la actualidad se están desarrollando estudios con psmaPET-TC (antígeno de membrana específico de próstata-PET/TC).

La PET/TC de 18F-colina muestra una sensibilidad superior a la TC ósea convencional a la hora de evaluar metástasis en hueso. No está claro si la PET/TC de 11C-colina es más sensible que la exploración ósea convencional, pero tiene mayor especificidad con menos lesiones indeterminadas; sin embargo, la relación costo-efectividad de estas intervenciones todavía no se ha evaluado. Por lo tanto, se prefiere la TC ósea sobre la base de la disponibilidad y el costo.

Tratamiento

El tratamiento de elección depende del estadio del tumor en el momento del diagnóstico. Se pueden emplear seis modalidades:

Quirúrgica
Radioterapia externa conformacional
Braquiterapia
Hormonoterapia
Vigilancia activa
Observación.

Carcinoma de próstata localizado <cT2c, Nx, M0

El abordaje del carcinoma de próstata localizado depende del nivel del riesgo, determinado con anterioridad por D´amico y las guías del NCCN, realizándose de la siguiente forma:

Riesgo muy bajo: puede realizarse cualquier modalidad del tratamiento. Se han encontrado buenos resultados con la observación y la vigilancia activa para evitar el sobretratamiento; sin embargo, en pacientes con expectativa de vida >10 años se podría considerar el tratamiento quirúrgico.

Riesgo bajo: al igual que en el grupo anterior, puede realizarse cualquier modalidad de tratamiento. La expectativa de vida también cobra un papel importante, ya que en aquellos pacientes con pronóstico <10 años es probable que no se justifique el tratamiento quirúrgico. En aquellos que presenten una expectativa de vida >10 años, y se consideren candidatos a tratamiento quirúrgico, la prostatectomía radical es el tratamiento de elección, sin necesidad de realizar linfadenectomía, la cual ha demostrado mayor efectividad en comparación con la radioterapia (50).

Riesgo intermedio: la prostatectomía radical retropúbica con linfadenectomía pélvica bilateral es el manejo de elección en estos pacientes con expectativa de vida >10 años; este, como todo procedimiento quirúrgico, puede tener complicaciones como sangrado, infección, incontinencia urinaria y disfunción eréctil, sin embargo las de mayor importancia para el paciente son las dos últimas. Cabe aclarar que la frecuencia de estas dos ha disminuido dada la mejoría de la técnica quirúrgica y la preservación de las bandeletas neurovasculares junto con el esfínter externo. Otra

modalidad de tratamiento pata estos pacientes es la radioterapia externa, que puede ser 3D conformacional, con intensidad modulada (74- 80 Gy) o braquiterapia de baja tasa (preferible para pacientes de bajo riesgo). Todas tienen complicaciones relacionadas, similares a la cirugía, como incontinencia, disfunción eréctil, proctitis o cistitis. *Riesgo alto:* en este grupo de pacientes el tratamiento se realiza de la misma forma que en el paciente de riesgo intermedio.

Carcinoma de próstata localmente avanzado cT3-4, Nx, M0

Una de las opciones terapéuticas para carcinoma de próstata localmente avanzado es la prostatectomía radical retropúbica con linfadenectomía pélvica bilateral en pacientes jóvenes muy seleccionados, con estadio clínico T3, gleason <8 y PSA <20 ng/mL, esto dado que hasta 25% pueden estar sobreestadificados. Otra opción es la radioterapia externa combinada con terapia hormonal (análogos LHRH neoadyuvante, concurrente y adyuvante (por 1-3 años)).

Carcinoma de próstata avanzado cualquier T, N1, M1

El tratamiento de elección en los pacientes con CAP avanzado es la hormonoterapia (quirúrgica o médica). Los medicamentos que se utilizan en la actualidad para la orquiectomía médica son los análogos LHRH (acetato de leuprolide, acetato de goserelina y acetato de triptorelina) y los antagonistas del receptor de la hormona liberadora de gonadotropina (GnRH)(Degarelix), cuyo uso lo debe llevar a cabo personal especializado. A estos medicamentos se les puede adicionar otros emergentes como la abiraterona (inhibidor de la síntesis de testosterona), la enzalutamida (inhibidor del receptor de andrógenos) y el radio-223 cuando el paciente es denominado resistente a la castración (51). Los criterios para ser definido como resistente a la castración son (51) nivel de testosterona <50 ng/dl o 1.7 nmol/l más

Progresión bioquímica: tres elevaciones consecutivas del PSA al menos con una semana de diferencia y con resultado en dos incrementos del 50% por encima del nadir y un PSA >2, o
Progresión radiológica: aparición de dos o más lesiones nuevas en una gammagrafía ósea o una lesión de tejidos blandos usando los criterios RECIST (Response Evaluation Criteria in Solid Tumors).

Los antiandrógenos se utilizan como coadyuvantes en el tratamiento y existen de dos tipos: esteroideos y no esteroideos. Dentro de estos últimos se encuentran la flutamida, la bicalutamida, entre otros, sin embargo sus indicaciones, dosis y seguimiento deben ser realizados por el especialista (51).

En aquellos pacientes con CAP metastásico y resistente a la castración se pueden hacer clasificaciones de acuerdo con el estado funcional y con la presencia de síntomas o metástasis viscerales. Para aquellos con deterioro del estado funcional, el tratamiento aún está en investigación dado el pobre pronóstico de los pacientes. Por el contrario, para aquellos con buen estado funcional, se cuenta con diferentes tratamientos como los antiandrógenos de nueva generación y la quimioterapia. Los pacientes asintomáticos o levemente sintomáticos podrían recibir abiraterona, enzalutamida y radio 223, mientras que los sintomáticos y que tienen metástasis viscerales son candidatos a quimioterapia (docetaxel o cabazitaxel, como segunda línea), cuyo manejo es dado por el especialista en oncología o en juntas interdisciplinarias (51).

Conclusiones

La presente investigación presenta una visión actualizada en diferentes aspectos del CAP. Se trata de una patología muy frecuente, con una incidencia que varía entre mantenerse estable o aumentar y una prevalencia que, con claridad, está en aumento, quizá debido a la mejoría para diagnóstico y tratamiento. Con respecto a los factores de riesgo, se tienen los clásicamente reconocidos, como raza, edad y factores genéticos; los que todavía no se ha podido encontrar el rol que juegan, como la alimentación, y algunos que antes se creía eran factores protectores y ahora apuntan a jugar un papel carcinógeno, como los estrógenos.

Es importante realizar una adecuada clasificación y estadificación clínica, anatómica y patológica del CAP, ya que de dicho proceso depende la aproximación y utilización de técnicas de imágenes y radiología, además del tratamiento oportuno, individualizado e indicado según la medicina basada en la evidencia.

La caracterización genética y microbiológica del CAP es hacía donde apuntan y apuntarán la mayoría de esfuerzos e investigaciones, ya que se requieren pruebas con mayor sensibilidad y especificidad que lleven a un diagnóstico más temprano y certero y, de ser posible, faciliten terapias más específicas y menos mórbidas.

Se realizó un recorrido a través de las condiciones de riesgo, tamizaje, diagnóstico, nuevos biomarcadores y tratamiento del CAP. Varios elementos han cambiado en los últimos años, principalmente acerca de la comprensión de la fisiología del cáncer, los factores asociados, la búsqueda de nuevos biomarcadores en cada una de las etapas del cáncer y varios elementos relacionados con el tratamiento; sin embargo, hay mucha investigación en curso acerca de la prevención, el diagnóstico y el tratamiento de esta condición tan importante, relevante y pertinente para los hombres alrededor del mundo.

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Referencias

1. **Restrepo JA, Bravo LE, García-Perdomo HA, García LS, Collazos P, Carbonell J.** Incidencia, mortalidad y supervivencia al cáncer de próstata en Cali, Colombia, 1962-2011. *salud publica de méxico.* 2014;56(5):440-7.
2. **García-Perdomo H.** Cancer Centers of Excellence in Colombia : A fundamental way to work together. *Urol Colomb.* 2017;26(3):157-8. http://doi.org/cm95.
3. **Siegel RL, Miller KD, Jemal A.** Cancer statistics, 2016. *CA Cancer J Clin.* 2016;66(1):7-30. http://doi.org/cm96.
4. American Cancer Society. Cancer Facts & Figures for African Americans 2016-2018. Atlanta: Cancer Society; 2016.
5. **Esquivel-Parra LM, Caicedo-Bolaños AM, Guaitarilla-Soto JM, García-Perdomo HA.** Una mirada general a los biomarcadores para la tamización y el diagnóstico temprano del cáncer de próstata. *Urol Colomb.* 2017;26(2):110-6. http://doi.org/cm97.
6. **Pveda-Matiz JL, Arenas-Reyes NJ, Sáenz-Becerra MP, Daza-Almendrales FP.** Evolución de la mortalidad por cáncer de próstata en Colombia: estudio ecológico. *Urol Colomb.* 2014;23(1):3-10. http://doi.org/f3gmns.

7. **Center MM, Jemal A, Lortet-Tieulent J, Ward E, Ferlay J, Brawley O, *et al.*** International variation in prostate cancer incidence and mortality rates. *Eur Urol.* 2012;61(6):1079-92. http://doi.org/f2kw49.

8. **Hayes RB, Liff JM, Pottern LM, Greenberg RS, Schoenberg JB, Schwartz AG, *et al.*** Prostate cancer risk in U.S. blacks and whites with a family history of cancer. *Int J Cancer.* 1995;60(3):361-64. http://doi.org/ds5rtn.

9. **Hoffman RM, Gilliland FD, Eley JW, Harlan LC, Stephenson RA, Stanford JL, *et al.*** Racial and ethnic differences in advanced-stage prostate cancer: the Prostate Cancer Outcomes Study. *J Natl Cancer Inst.* 2001;93(5):388-95.

10. **Taksler GB, Keating NL, Cutler DM.** Explaining racial differences in prostate cancer mortality. *Cancer.* 2012;118(17):4280-9. http://doi.org/fz4bg2.

11. **Graham-Steed T, Uchio E, Wells CK, Aslan M, Ko J, Concato J.** 'Race' and prostate cancer mortality in equal-access healthcare systems. *Am J Med.* 2013;126(12):1084-8. http://doi.org/f2w2hb.

12. **Sim HG, Cheng CW.** Changing demography of prostate cancer in Asia. *Eur J Cancer.* 2005;41(6):834-45. http://doi.org/bdfnh6

13. **Whittemore AS, Wu AH, Kolonel LN, John EM, Gallagher RP, Howe GR, *et al.*** Family history and prostate cancer risk in black, white, and asian men in the United States and Canada. *Am J Epidemiol.* 1995;141(8):732-40. http://doi.org/cm98.

14. **Caini S, Gandini S, Dudas M, Bremer V, Severi E, Gherasim A.** Sexually transmitted infections and prostate cancer risk: a systematic review and meta-analysis. *Cancer Epidemiol.* 2014;38(4):329-38. http://doi.org/f6gjv8.

15. **Dennis LK, Lynch CF, Torner JC.** Epidemiologic association between prostatitis and prostate cancer. *Urology.* 2002;60(1):78-83. http://doi.org/fkj4sw.

16. **Oh B, Figtree G, Costa D, Eade T, Hruby G, Lim S, *et al.*** Oxidative stress in prostate cancer patients: a systematic review of case control studies. *Prostate Int.* 2016;4(3):71-87. http://doi.org/cm99.

17. **Khandrika L, Kumar B, Koul S, Maroni P, Koul HK.** Oxidative stress in prostate cancer. *Cancer Lett.* 2009;282(2):125-36. http://doi.org/c3hf8q.

18. **Michaud JE, Billups KL, Partin AW.** Testosterone and prostate cancer: an evidence-based review of pathogenesis and oncologic risk. *Ther Adv Urol.* 2015;7(6):378-87. http://doi.org/cncj.

19. **Nelles JL, Hu WY, Prins GS.** Estrogen action and prostate cancer. *Expert Rev Endocrinol Metab.* 2011;6(3):437-51. http://doi.org/fb648s.

20. **Jurado-Penagos A, García-Perdomo HA.** Efectividad de los micronutrientes en la prevención del cáncer de próstata. *Rev. Fac. Med.* 2014;62(Suppl 1):S65-72. http://doi.org/cnck.

21. **Gennigens C, Menetrier-Caux C, Droz JP.** Insulin-Like Growth Factor (IGF) family and prostate cancer. *Crit Rev Oncol Hematol.* 2006;58(2):124-45. http://doi.org/bsrtpj.

22. **Fradet-Turcotte A, Sitz J, Grapton D, Orthwein A.** BRCA2 functions: from DNA repair to replication fork stabilization. *Endocr Relat Cancer.* 2016;23(10):T1-17. http://doi.org/f9bt3g.

23. **Gulati R, Cheng HH, Lange PH, Nelson PS, Etzioni R.** Screening men at increased risk for prostate cancer diagnosis: Model estimates of benefits and harms. *Cancer Epidemiol Biomarkers Prev.* 2017;26(2):222-7. http://doi.org/cncm.

24. **Song WH, Kim SH, Joung JY, Park WS, Seo HK, Chung J, *et al.*** Prostate Cancer in a Patient with a Family History of BRCA Mutation: a Case Report and Literature Review. *J Korean Med Sci.* 2017;32(2):377-81. http://doi.org/f9ndt6.

25. **Kaaks R, Stattin P.** Obesity, endogenous hormone metabolism, and prostate cancer risk: a conundrum of "highs" and "lows". *Cancer Prev Res (Phila).* 2010;3(3):259-62. http://doi.org/cz4p86.

26. **Perdana NR, Mochtar CA, Umbas R, Hamid AR.** The Risk Factors of Prostate Cancer and Its Prevention: A Literature Review. *Acta Med Indones.* 2016;48(3):228-38.

27. **Rota M, Scotti L, Turati F, Tramacere I, Islami F, Bellocco R, *et al.*** Alcohol consumption and prostate cancer risk: A meta-analysis of the dose-risk relation. *Eur J Cancer Prev.* 2012;21(4):350-9. http://doi.org/fpbwkh.

28. **Islami F, Moreira DM, Boffetta P, Freedland SJ.** A systematic review and meta-analysis of tobacco use and prostate cancer mortality and incidence in prospective cohort studies. *Eur Urol.* 2014;66(6):1054-64. http://doi.org/f2vzkr.

29. **Bell KJ, Del Mar C, Wright G, Dickinson J, Glasziou P.** Prevalence of incidental prostate cancer: A systematic review of autopsy studies. *Int J Cancer.* 2015;137(7):1749-57. http://doi.org/cncn.

30. **Epstein JI, Egevad L, Amin MB, Delahunt B, Srigley JR, Humphrey PA, *et al.*** The 2014 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma: Definition of Grading Patterns and Proposal for a New Grading System. *Am J Surg Pathol.* 2016;40(2):244-52. http://doi.org/f3vjqr.

31. **Amin MB, Edge S, Greene F, Byrd DR, Bokland RK, Washington RK, *et al.*, editors.** AJCC Cancer Staging Manual. 8th ed. Springer International Publishing; 2016.

32. **D'Amico AV, Whittington R, Malkowicz SB, Schultz D, Blank K, Broderick GA, *et al.*** Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinically localized prostate cancer. *JAMA.* 1998;280(11):969-74. http://doi.org/cvj2jv

33. National Comprehensive Cancer Network Foundation. NCCN Guidelines for patients: Prostate Cancer. Washingto D.C.: NCCN Foudatio; 2016.

34. **Saini S.** PSA and beyond: alternative prostate cancer biomarkers. *Cell Oncol.* 2016;39(2):97-106. http://doi.org/f8g7cg.

35. **Potenziani-Bigelli JC.** PSA (Antígeno Prostático Específico) Enfoque 2012. VITAE Academia Biomédica Digital. 2012;(51):1-21.

36. **Garcia HA, Carbonell J.** Urología en pocas palabras. Un enfoque práctico para el médico general. Santiago de Cali: Editorial Universidad del Valle; 2011.

37. **Ortiz Sanchez JM.** La eficiencia del índice de PSA libre en el diagnostico de Cáncer de Próstata [tesis doctoral]. Lima: Facultad de Medicina, Universidad Nacional Mayor de San Marcos; 2014.

38. **Salman JW, Schoots IG, Carlsson SV, Jenster G, Roobol MJ.** Prostate Specific Antigen as a Tumor Marker in Prostate Cancer: Biochemical and Clinical Aspects. *Adv Exp Med Biol.* 2015;867:93-114. http://doi.org/f8bzzn.

39. **Chadha KC, Miller A, Nair BB, Schwartz SA, Trump DL, Underwood W.** New serum biomarkers for prostate cancer diagnosis. *Clin cancer Investig J.* 2015;3(1):72-9. http://doi.org/cncq.

40. **Ilic D, Neuberger MM, Djulbegovic M, Dahm P.** Screening for prostate cancer. Cochrane *Database Syst Rev.* 2013;1(1):CD004720. http://doi.org/bmpz.

41. **Wolf AM, Wender RC, Etzioni RB, Thompson IM, Amico A Vd, Volk RJ, *et al.*** American cancer society guideline for the early detection of prostate cancer: update 2010. *CA Cancer J Clin.* 2010;60(2):70-98. http://doi.org/bsxhtz.

42. **Andriole GL, Crawford ED, Grubb RL, Buys SS, Chia D, Church TR, *et al.*** Prostate cancer screening in the randomized prostate, lung, colorectal, and ovarian cancer screening trial: Mortality results after 13 years of follow-up. *J Natl Cancer Inst.* 2012;104(2):125-32. http://doi.org/fxx25h.

43. **Schröder FH, Hugosson J, Roobol MJ, Tammela TL, Ciatto S, Nelen V, *et al.*** Prostate-Cancer Mortality at 11 Years of Follow-up. *N Engl J Med.* 2012;366(11):981-90. http://doi.org/f2zx8f.

44. **Ilic D, O'Connor D, Green S, Wilt TJ.** Screening for prostate cancer : an updated Cochrane systematic review. BJU Int. 2011;107(6):882-91. http://doi.org/b4sx4t.

45. **Kontos CK, Adamopoulos PG, Scorilas A.** cancer Prognostic and predictive biomarkers in prostate cancer. *Expert Rev Mol Diagn.* 2015;15(2):1567-76. http://doi.org/cncr.

46. García P HA, Varela R. Validez diagnóstica del antígeno prostático específico para la presencia de metástasis en pacientes con cáncer de próstata. *Urol Colomb.* 2010;19(3):13-8.

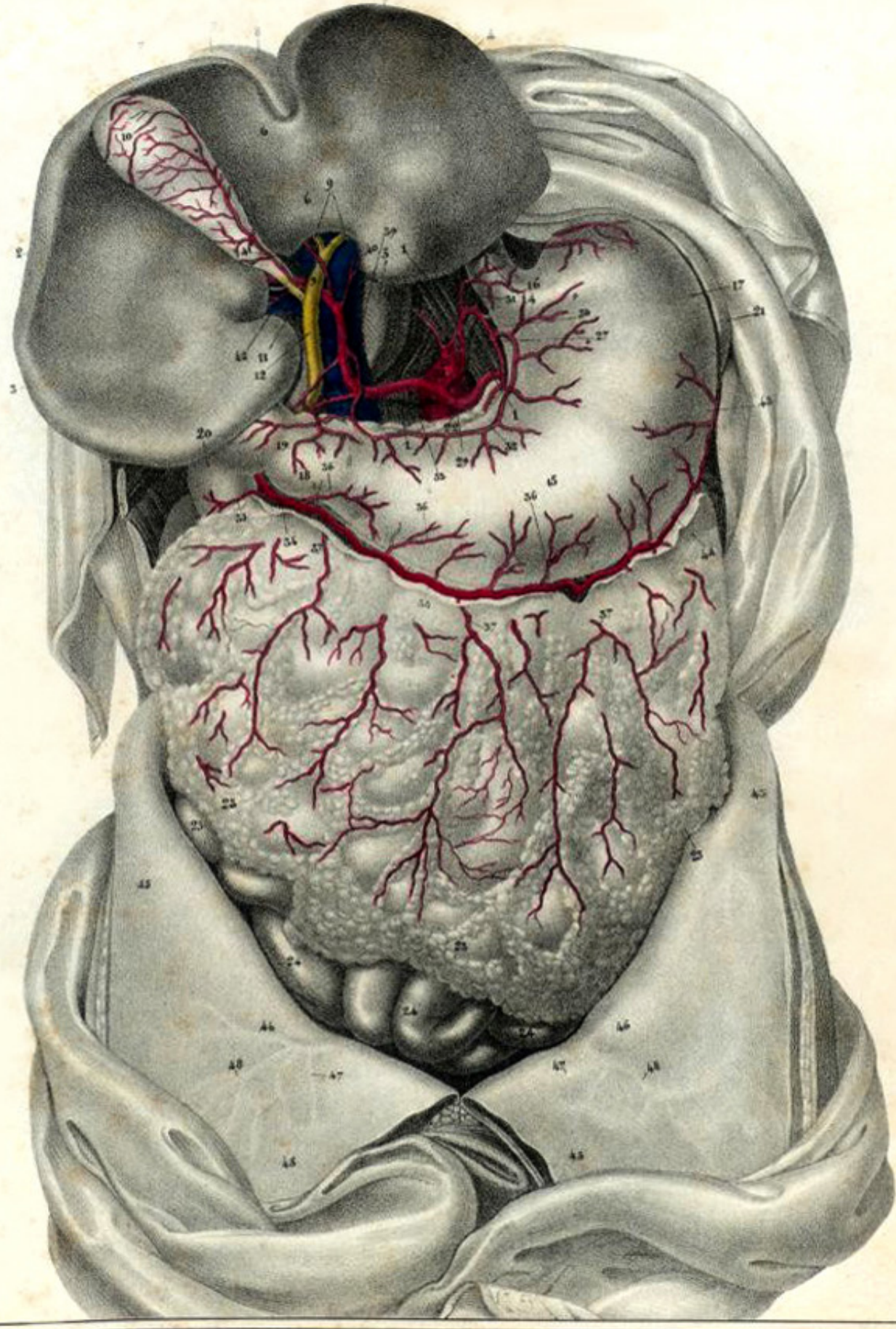
47. Mottet N, Bellmunt J, Briers E, van den Bergh RCN, Bolla M, van Casteren NJ, *et al.* Guidelines on Prostate Cancer. European Association of Urology; 2015.

48. Albert JM, Swanson DA, Pugh TJ, Zhang M, Bruno TL, Kudchadker RJ, *et al.* Magnetic resonance imaging-based treatment planning for prostate brachytherapy. *Brachytherapy.* 2013;12(1):30-7. http://doi.org/f4k25z.

49. von Eyben FE, Kairemo K. Meta-analysis of (11)C-choline and (18) F-choline PET/CT for management of patients with prostate cancer. *Nucl Med Commun.* 2014;35(3):221-30. http://doi.org/f5smr6.

50. Wallis CJD, Saskin R, Choo R, Herschorn S, Kodama RT, Satkunasivam R, *et al.* Surgery Versus Radiotherapy for Clinically-localized Prostate Cancer: A Systematic Review and Meta-analysis. *Eur Urol.* 2016;70(1):21-30. http://doi.org/f8p4dx.

51. Mottet N, Bellmunt J, Briers E, Bolla M, Bourke L, Cornford P, *et al.* Guidelines on Prostate Cancer. European Association of Urology; 2017.



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Lith. de Frey.

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