ERG rearrangements and *PTEN* loss in Prostate Cancer

Alba Font Tello

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Als meus avis,

Neus i Lluís.

Estenc la mà

Estenc la mà i no hi ets.
Però el misteri d'aquesta teva absència se'm revela
més dòcilment i tot del que pensava.

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i en mi mateix hi hauràs deixat l'empremta
de la vida que visc, no solitari
sinó amb el món i tu per companyia,
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2. Concurrent *TMPRSS2-ERG* and *SLC45A3-ERG* rearrangements plus *PTEN* loss are not found in low grade prostate cancer and define an aggressive tumor subset. Hernández S*****, **Font-Tello A***, Juanpere N, de Muga S, Lorenzo M, Salido M, Fumadó L, Serrano L, Cecchini L, Serrano S, Lloreta J. Prostate. 2016 Jun;76(9):854-65. doi: 10.1002/pros.23176.

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ABSTRACT

Prostate cancer (PrCa) is a highly heterogeneous disease and its prognosis, diagnosis and management are still controversial. *ERG* rearrangements and *PTEN* loss are frequent and concomitant events in a subset of PrCa.

The aim of this thesis is to analyze the potential use of *TMPRSS2-ERG* and *SLC45A3-ERG* rearrangements, along with ERG and *PTEN* expression in the stratification and prognosis of PrCa patients.

TMPRSS2-ERG and *ERG* mRNA overexpression levels are related to a more aggressive phenotype and could be useful PrCa progression markers. Single *TMPRSS2-ERG* is associated with low grade PrCa and subsequent development of *SLC45A3-ERG* results in higher *ERG* expression. The triple hit (*TMPRSS2-ERG*, *SLC45A3-ERG* and *PTEN* loss) is not found in low grade nor low stage tumor, it is associated with Gleason pattern 4 and T3-4 stage and it defines a group of tumors that should be excluded from watchful waiting and are candidates for intense therapy.

Key words: *TMPRSS2-ERG*, *SLC45A3-ERG*, *PTEN*, prognosis, prostate cancer.

El càncer de pròstata (CaPr) és una malaltia altament heterogènia i el seu pronòstic, diagnòstic i gestió són polèmiques. Els reordenaments d'*ERG* i la pèrdua de *PTEN* són esdeveniments freqüents i concomitants en un subconjunt de CaPr.

L'objectiu d'aquesta tesi és analitzar l'ús potencial dels reordenaments *TMPRSS2-ERG* i *SLC45A3-ERG*, juntament amb l'expressió d'ERG i *PTEN* en l'estratificació i pronòstic dels pacients amb CaPr.

Nivells de sobreexpressió de *TMPRSS2-ERG* i *ERG* estan relacionats amb un fenotip més agressiu i podrien ser útils marcadors de progressió del CaPr. *TMPRSS2-ERG* s'associa amb CaPr de baix grau i el posterior desenvolupament de *SLC45A3-ERG* causa una major expressió d'*ERG*. El "triple hit" (*TMPRSS2-ERG*, *SLC45A3-ERG* i pèrdua de *PTEN*) no es troba en tumors de baix grau i estadi, s'associa amb patró 4 de Gleason i estadis T3-4, i defineix un grup de tumors que són candidats a teràpia intensa i s'han d'excloure d'espera en observació.

Paraules clau: *TMPRSS2-ERG*, *SLC45A3-ERG*, *PTEN*, pronòstic, càncer de pròstata.

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INTRODUCTION

1. Anatomy and Histology of the Prostate

1.1. Prostate Anatomy

The prostate is a fibromuscular and glandular organ of the male reproductive system. This exocrine gland is about the size of a walnut in young adults, but its size can increase with age. It is a pyramidal-shaped organ that consists of an apex, which is the narrowest caudal part; a wide base, situated cranially; and an anterior, a posterior and two lateral surfaces.

Anatomically, the prostate is continuous with the bladder neck, anterior to the rectum, it is surrounding the prostatic urethra, and the levator ani muscles lie around its lateral surfaces **[Figure 1]**. The ejaculatory ducts perforate the proximal posterior part of the prostate to open to the prostatic urethra at the level of the verumontanum (mid-prostate). The seminal vesicles are located postero-superior to the prostate, and together they contribute to the production of the seminal fluid, a slightly alkaline fluid that facilitates sperm transit and survival, as well as fertilization. The prostate also plays a pivotal role in male ejaculation.[1–4]

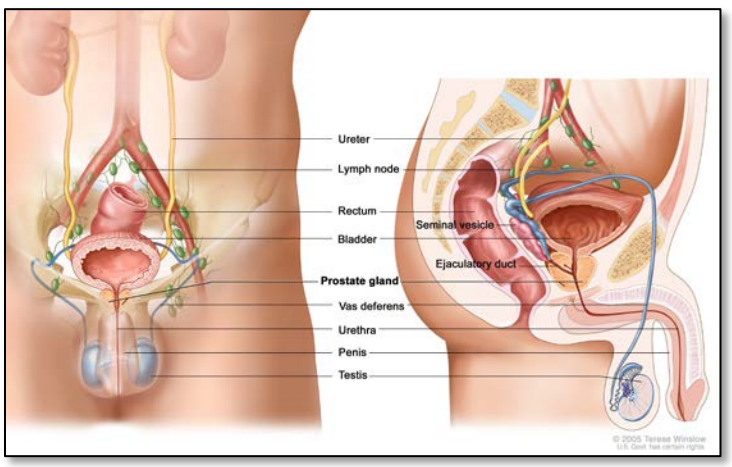

Figure 1. Anatomical relations of the prostate.
From the website of the National Cancer Institute
(https://www.cancer.gov)

The current model of zonal anatomy of the prostate was first described by McNeal[5]. The prostate is an organ conformed by three glandular zones, which are surrounded by layers of fibromuscular stroma, classically referred to as prostatic capsule. These three areas have distinctive anatomical and histological features as well as different propensity for disease **[Figure 2]**. The fibro-muscular zone (FZ) is a stromal barrier that forms the antero-medial surface of the prostate, extending from the apex to the base, and acting as a shield for the glandular structures and the urethra. The glandular zones are described taking the urethra as the primary anatomic reference, and are called central (CZ), transition (TZ) and peripheral (PZ) zones. The CZ extends from the verumontanum to the base of the prostate and its ducts arborize proximally around the ejaculatory ducts. It constitutes 20% of the glandular prostate, its lateral borders are fused to the PZ and it is relatively resistant to adenocarcinomas and other diseases. Instead, the PZ is the most common site for the development of prostatic adenocarcinomas, with around 70% of prostate cancers arising from this structure. It is located postero-laterally, around the CZ and the distal prostatic urethra, and represents the major glandular component of the prostate (70%). The ducts of the PZ are distributed distally from the verumontanum to the prostate apex. The TZ extends bilaterally from the mid-gland to the base, in contact with both CZ and PZ, and accounting for 10% of the glandular component of the prostate. Whereas a relatively low number of prostate cancers (around 20%) arise from this area, it is the most common site for benign prostatic hyperplasia (BPH). Finally, the periurethral zone lies

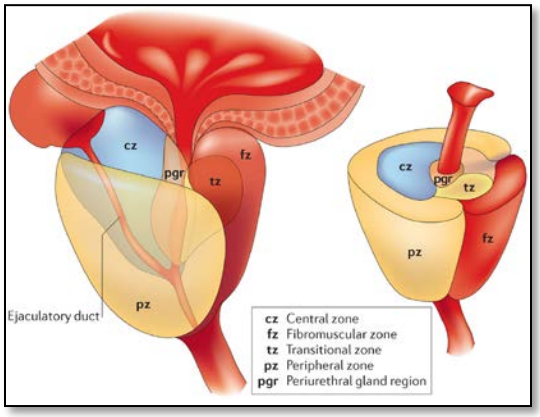

Figure 2. Zonal anatomy of the prostate gland.

From Verze P. *et al*, Nat. Rev. Urol. 2016[8]

around the proximal urethra and it is also commonly affected by BPH. [5–8]

1.2. Prostate Histology

The glandular prostate consists of tubular-acinar epithelial glands and supporting fibro-muscular stroma, which are closely interconnected through different signaling pathways to ensure the normal development and homeostasis of the prostate.[8] **[Figure 3]**

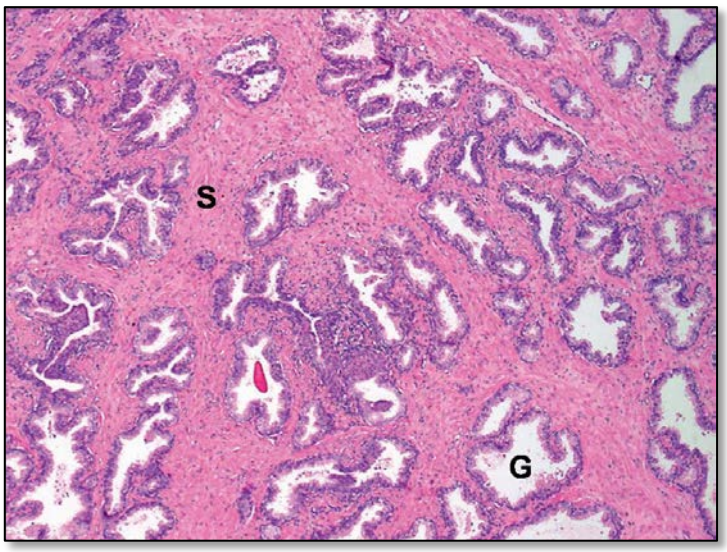

Figure 3. Hematoxylin-eosin (H&E) staining of the prostate gland, 10x.
Glands (G) surrounded by fibro-muscular stroma (S).
From Weather's Functional Histology, 2013[10]

The fibro-muscular stroma accounts for a great part of the prostate and helps maintaining the gland homeostasis, providing an adequate microenvironment for the epithelial compartment. It is composed of collagenous fibrous tissue, androgen receptor(AR)-positive smooth muscle cells, fibroblasts, immune cells, nerve fibers and vasculature; and it surrounds the individual glands.[9] The glands consist of pseudo-stratified columnar epithelium formed by columnar, basal and neuroendocrine cells: [10,11] **[Figure 4]**

- Columnar cells contain apical secretory granules and round large basal nuclei. These are the most abundant epithelial cells of the

prostate gland and are located along the glandular lumen forming a continuous layer. These are AR-positive cells and express cytokeratins 8 and 18.

- The basal layer of cells is below the luminal epithelium, in contact with the basal membrane, separating the epithelium from the stroma. Abounding cuboidal epithelial cells that maintain the glandular epithelium, contribute to its regeneration, and are located between columnar cells. Characteristic expression markers are p63 and cytokeratins 5 and 14, whereas AR expression levels are low or undetectable.
- Neuroendocrine cells are scattered irregularly along the basal layer, express endocrine markers like chromogranin A and synaptophysin, and are AR-negative.

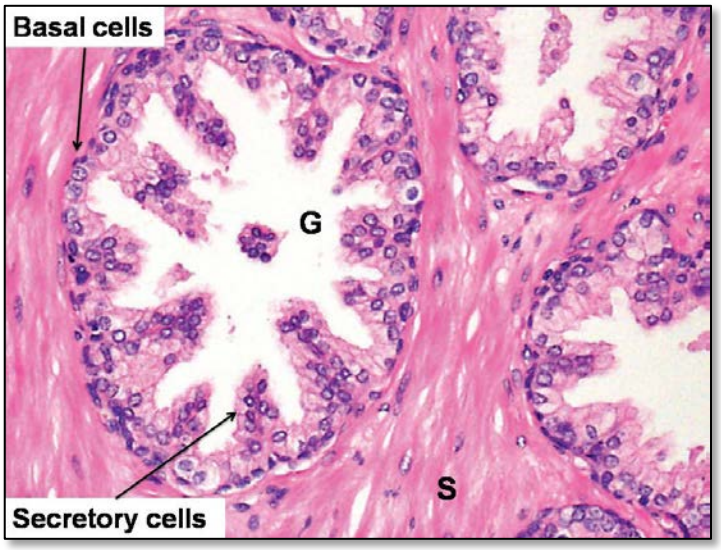

Figure 4. Hematoxylin-eosin (H&E) staining of the prostate gland, 40x.
Glands (G) surrounded by fibro-muscular stroma (S).
From Weather's Functional Histology, 2013[10]

Functionally, the glandular acini are responsible for the production of prostatic fluid, which is released and stored into the luminal space until ejaculation, when the smooth muscle contractions lead to its expulsion into the urethra at the level of the verumontanum.[8,12]

The histology of the prostate is distinct on each of the anatomical zones. On the one hand, the CZ is characterized by large ducts and acini embedded in compact muscular stroma, which arborize from the verumontanum toward the prostate base, surrounding the ejaculatory ducts. On the other hand, the PZ consists of simple columnar epithelium, formed by small rounded acini that empty into long and narrow ducts. In this zone, the stroma is loose and intertwined with muscle bundles. The PZ ducts run from the verumontanum to the prostate apex. Whereas there is a notorious difference between the CZ and PZ; the histology of the TZ is similar to the PZ. The ducts of the TZ are distributed around the pre-prostatic sphincter, towards the bladder neck; and are inlaid in a compact stroma with interlacing fibers of smooth muscle.[13]

2. Prostate Cancer

2.1. Epidemiology

Prostate cancer (PrCa) is the most frequent malignant tumor diagnosed in men (aside from non-melanoma skin cancers) and the second cause of death by cancer. During 2017, it is estimated that there will be over 160,000 new diagnosed cases and more than 26,000 deaths by PrCa in the US.

The widespread implementation of the prostate-specific antigen (PSA) blood-test during the late 1980s lead to an increase of PrCa incidence, but this trend has been decreasing significantly since the mid-1990s. Also, the development of better detection methods and improved treatments have led to a decrease on the mortality rates.[14]

Similarly in Spain, PrCa is the tumor with highest incidence among men (21.67%), one of the main causes of death by cancer (8.62%), and the neoplasm with the highest 5-year prevalence (31.4%).[15]

2.2. Etiology

Despite major efforts on the research of PrCa risk factors, few have been well established. The difficulties on finding clear risk factors might rely on the fact that PrCa is a highly heterogeneous disease. Age, ethnicity, family history and some genetic variants are well known risk factors; whereas other factors like diet, specific nutrients or foods, or obesity among others, have also been extensively studied but their association with PrCa incidence is still poorly understood.

PrCa incidence varies along with age, being more common in older men. It is rare among men younger than 40, but its incidence rapidly increases after the age of 50, and the group most frequently diagnosed comprises men between 65 and 74 years old.[16] Furthermore, prostate cancer diagnosed in older men tends to be more aggressive, with higher Gleason Scores (GS) and tumor stages.[17]

Another important risk factor for the development of PrCa is family history; indeed PrCa has the highest familial risks and heritability of all major cancers.[18] It is estimated that 5-10% of PrCa cases may be hereditary, probably related to inherited genetic factors. For instance, mutations in *breast cancer 2* gene (*BRCA2)* and *homeobox B13* gene (*HOXB13)* are well known alterations conferring higher cancer risk, but their prevalence is low. Nonetheless, shared environmental factors among family members may also play an important role.[19]

Although the mechanisms are still unclear, ethnicity is a well-established PrCa risk factor. Men of African-American and Afro-Caribbean ancestry have higher risk of developing this disease whereas men of Asian origins have the lowest risk.[16,20] Similarly, geography is also an important risk factor; and geographic areas such as USA and Northern Europe are the highest risk areas whereas the risk in South-East Asia is really low.[21]

Besides, environmental factors seem to have an important role as PrCa risk factors as well, and their effect is likely dependent on other factors, such as ethnicity, genetic susceptibility or geography.[22] Notably, risk of mortality driven by PrCa is also much higher in men of African-American origin, elderly men and patients with advanced PrCa.[16]

The complex interplay among these and other factors could explain the diverse incidence and severity of PrCa.

2.3. Prostate cancer diagnosis

2.3.1. Screening

There is a lot of controversy around PrCa screening, and many ongoing studies are assessing the consequences of current PSA-screening tendencies. Since its implementation, PSA screening has been used as an early detection method, as well as a follow-up method during treatment. However, its use as a mass-screening method has always been a controversial practice.

PSA is a serine protease encoded by the Kallikrein Related Serine Peptidase 3 (KLK3), a member of the 15 *Kallikrein* (*KLK*) genes that cluster on human chromosome 19, and it was first associated with its role on semen liquefaction and sperm motility. KLKs constitute the largest contiguous cluster of peptidases in humans, share similarities in their structure and functionality, and have a wide range of tissue-specificities. These peptidases are synthesized as pre-proenzymes, then processed to proenzymes and finally become active when their pro-domain is cleaved causing a conformational change in their substrate binding pocket and conferring them with proteolytic activity.[23,24]

Human kallikrein 2 (hk2) and PSA (respectively encoded by *KLK2* and *KLK3*) are essential for human reproduction and male fertility, they are produced by the columnar secretory cells of the glands and their expression

profiles are restricted to the prostate epithelia. Since they are androgen responsive, AR activity can be indirectly studied when assessing hk2 and PSA levels. Also, although they are mostly found in the semen, a small portion can be found in the blood; and serum PSA levels tend to increase in PrCa.[24,25]

PSA screening test, along with digital rectal examination (DRE), aim to detect prostate cancer at early stages, reducing disease-specific morbidity and mortality. However, PSA screening method leads to overdiagnosis and consequently overtreatment, as it has poor specificity and sensitivity, and it is still unclear whether it provides more benefits than harms.

As reviewed by Fleshner *et al* [26], two large randomized control trials – the European Randomized Study of Screening for Prostate Cancer (ERSPC)[27] and the US Prostate, Lung, Colorectal and Ovarian (PLCO) trial[28]– have been assessing the efficacy and impact of PSA screening. The results point towards many disadvantages of the screening, as it leads to many unnecessary biopsies and many false-positive results that translate to overtreatment of the patients, with all its consequences and side-effects; and little benefits. Many recommendations and guidelines regarding PSA screening highlight the importance of shared decision making and agree on the fact that screening is not beneficial for men with a life expectancy shorter than 10 years. Otherwise, there is not a clear agreement in terms of the age group included, the frequency of screening or the PSA threshold.

For instance, in 2008 the United States Preventive Services Task Force (USPSTF) recommended a D grade for PSA screening in men over 75 years old and in 2012 they extended it to all men, recommending against its use as it does not seem that the harms outweigh the benefits. Since then, PSA screening, prostate biopsies and PrCa incidence have decreased, but concomitantly, there has been a trend on patients being diagnosed with higher grade and stage tumors. This could be an indication that many patients with intermediate risk PrCa are now being underscreened and in turn underdiagnosed, and consequently they would not beneficiate of early

detection and potential curability. Nonetheless, the USPSTF is reviewing this topic, taking into consideration subpopulations and risk factors when considering screening.

On the other hand, the European Association of Urology recommendations include PSA screening on men with higher risk of PrCa (for example men over 50, or over 45 years old for African American men, or men with PrCa family history) and discourage its use on men with a life expectancy shorter than 15 years. Also, it is recommended not to include any men for PSA screening without counseling them about its risks and benefits.[29]

New biological markers[30,31] like *TMPRSS2-ERG* rearrangement, PCA3 or KLKs, among others, can provide a better sensitivity and specificity when combined with PSA test. At the same time, the use of nomograms and risk calculators could be beneficial. More studies are needed to have a clear understanding and determine the most appropriate screening method for PrCa.

2.3.2. Signs and symptoms

PrCa is a complex disease that usually does not cause any signs or symptoms during early stages. Even more, as in most cases the cancer grows really slowly, some patients may never find out they had PrCa and will die from unrelated causes. Nonetheless, some advanced cases can be the root of symptoms such as problems urinating, blood in the urine or semen, erectile dysfunction, pain in the lower back, the hips and/or the upper thighs, or enlargement of the prostate. It is important to determine whether these symptoms are caused by PrCa, as they can be symptoms of other diseases like for example BPH or prostatitis. In metastatic cases, patients can develop other symptoms; most commonly from bone metastasis (PrCa has a high tropism towards bone), causing pain or fractures.[32]

2.3.3. Clinical diagnosis

The diagnosis of PrCa is generally made on the basis of several features, mainly assessed by PSA blood test and/or DRE. Unfortunately, although PSA blood test and DRE are complementary, they are still suboptimal methods. Blood counts, biochemical profiles, and imaging techniques like transrectal ultrasonography (TRUS) or magnetic resonance imaging (MRI) also have an important role on the diagnosis of PrCa. Still, the definitive diagnosis relies on the histopathological identification of prostatic adenocarcinoma, either from samples of prostate biopsy cores, transurethral resection of the prostate (TURP) or prostatectomy for BPH.

- PSA blood test

  The small fraction of PSA that is released into the bloodstream can be measured as a continuous parameter. It is important to mention that PSA is a specific marker for prostatic tissue, but it is not a cancer specific marker. PSA levels may be temporary or chronically affected not only by PrCa but also by other prostate diseases such as BPH, prostatitis or trauma, among others. Typically, levels under 4 ng/mL are considered basal normal levels of PSA, and high PSA is considered in men with levels over 10 ng/mL. Although some patients with PrCa have lower levels, PSA values may reflect the risk of both cancer and higher GS in some other patients. Nonetheless, PSA should be used along with other diagnostic methods to determine the need for performing a biopsy.

- Digital Rectal Examination

  DRE is the physical examination of the prostate via the rectum for any abnormalities regarding size, shape, texture, and most important hardness; can be useful to distinguish between PrCa and other conditions like BPH, but its sensitivity is low. Most PrCas arise in the PZ, and it is established that tumors of at least 0.2 mL located in this area can be detected by DRE. An abnormal DRE result implies higher risk of a tumor with higher GS,

and it is a clear indication for prostatic biopsy. The use of combined PSA blood test and DRE is widely used, and taking into account PSA levels can increase the positive predictive value of DRE.

- TransRectal UltraSonography

  TRUS is an important imaging method to guide prostate needle biopsies, localize the possible tumors and in some cases it can be helpful to distinguish between BPH and PrCa.

- Magnetic Resonance Imaging

  MRI is a good technique to initially assess the risk of clinical significant disease. For patients suspected to have low-risk disease, MRI can determine whether there is a need to perform a prostate biopsy, with all its possible side effects; thus significantly reducing the morbidity of these patients. At the same time, it can be useful to guide subsequent prostate biopsies. The detection rates of PrCa by MRI depend on several factors like the tumor volume or the GS; having great detection rates for GS ≥ 7 tumors. However, MRI has some limitations in terms of inter-reader variability and the definitions of positive or negative results.

The indications for a prostate biopsy depend on the different diagnostic tests mentioned above and the overall patient's medical history. High PSA levels should be confirmed by repeated PSA tests, and be in concordance with abnormal DRE and imaging results. Taking into account the patient's history and risk factors is important to avoid unnecessary morbidities. Prostate biopsies can be made by transrectal, perineal, or transurethral method; and around 12 biopsy cores should be taken, bilaterally from apex to base, as far posterior and lateral as possible from the peripheral gland. Also, additional cores should be taken when suspicious areas have been identified through imaging techniques. The histopathological study of the biopsied samples will determine the final diagnosis. Notably, some PrCa cases are incidentally diagnosed when patients undergo medical procedures due to other causes, for

example biopsies due to bladder cancer. The clinical significance of the incidentally diagnosed tumors is still a matter of controversy.[21,29,33]

2.4. Prostate cancer classification

2.4.1. Clinical Staging

The clinical staging of PrCa is performed according to the internationally accepted criteria tumor-node-metastasis (TNM) system[34,35], and includes clinical stages I- IV.

The TNM system assesses both the pathological stage and the spread of the disease, categorizing the cancer according to the size and local growth of the primary tumor (pathological stages T1-T4) **[Figure 5]**, the absence or presence of metastases in regional lymph nodes (N0-N1) and of distant metastases (M0-M1). **[Table 1]**

Localized disease	
Tx	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Clinically inapparent tumor neither palpable nor visible by imaging
T1a	Tumor incidental histologic finding in ≤ 5% of resected tissue
T1b	Tumor incidental histologic finding in > 5% of resected tissue
T1c	Tumor identified by needle biopsy (eg, because of elevated PSA level)
T2	Tumor confined within prostate
T2a	Tumor involves one-half of one lobe or less
T2b	Tumor involves more than one-half of one lobe but not both lobes
T2c	Tumor involves both lobes
Local extension	
T3a	Extracapsular extension (unilateral or bilateral)
T3b	Tumor invades seminal vesicle(s)
T4	Bladder invasion, fixed to pelvic side wall, or invasion of adjacent structures
Metastatic disease	
N1	Positive regional lymph nodes
M1	Distant metastasis

Table 1. 2010 TNM staging system of Prostate Cancer.
From Edge *et al*[34], Ann. Surg. Oncol. 2010

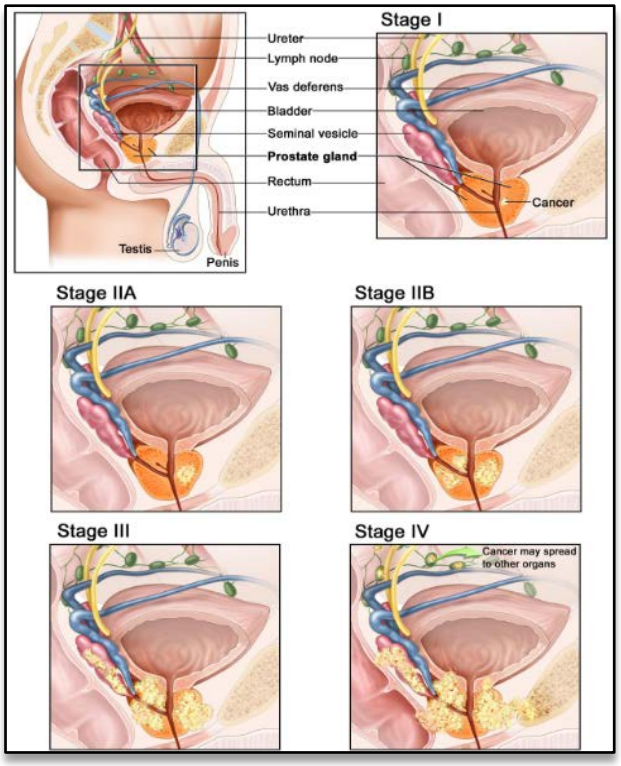

Figure 5. Pathological stages of PrCa.
From the website of the National Cancer Institute.
(https://www.cancer.gov)

The clinical stages are thus defined by the grouping of the tumors according to the TNM classification as well as taking into account the histological Gleason grade, which will be explained in the next section, and include:

- Clinical stage I:

  Defined by a tumor clinically unapparent (T1a), with no regional lymph node (N0) nor distant (M0) metastases, and Gleason grade 1.

- Clinical stage II:

  Including clinically unapparent tumors (T1a-c) or tumors confined to the prostate (T2a-c), with no presence of regional lymph node (N0) nor distant (M0) metastases, and Gleason grades 2-4 in the case of T1a, or any Gleason grade in the cases of T1b-c or T2a-c.

- Clinical stage III:

  The tumors extend through the prostate capsule (T3), still there is no presence of metastases in regional lymph nodes (N0) or distant metastasis (M0), and any Gleason grade can be present.

- Clinical stage IV:

  In this stage, there can be cases characterized by a fixed tumor or a tumor invading adjacent structures other than the seminal vesicles (T4), with no apparent lymph node (N0) or distant (M0) metastases, or other cases that can include any T and any N affectation with presence of distant metastases (M1), or other cases with any T, N1, and no distant metastases (M0). Any Gleason grade can be present.

2.4.2. Histological grade of prostate cancer:

The Gleason grading system was created by Dr. Donald Gleason in 1966[36–38], and although it has been refined with some modifications over the years[39–41], it is still the dominant method used for the histological grading of PC in both daily clinical practice and in research.

This system has been demonstrated to be related to many histopathological and clinical end points, and is frequently used to predict responses to therapies[42]. It is entirely based on histological patterns in hematoxylin-eosin staining. And the main characteristics assessed to classify the tumors into the different Gleason grade patterns are: the tumor shape, the tumor borders, the invasion of the stromal component and the way the tumor cells are arranged. **[Figure 6 and Figure 7]**

There are five possible Gleason grade patterns[42]:

- Gleason grade 1 is characterized by well-differentiated, rounded to oval uniform glands which are close but separated, and with a well-defined tumor margin and no stromal invasion.

- Gleason grade 2 is very similar to Gleason grade 1, but shows less well-defined gland shape and more separation between glands (up to one gland diameter). The tumor margins are less well-circumscribed, with minimal potential for stromal infiltration.

Both grade 1 and grade 2 are rare and usually incidentally found.

- Gleason grade 3, instead, is moderately-differentiated and is the most common pattern identified. It is defined by individual separated glands (usually greater than one gland diameter) with irregular shape; papillary and/or by cribriform epithelium; with infiltrating edges of irregular extension into the stroma, with smooth pushing borders.

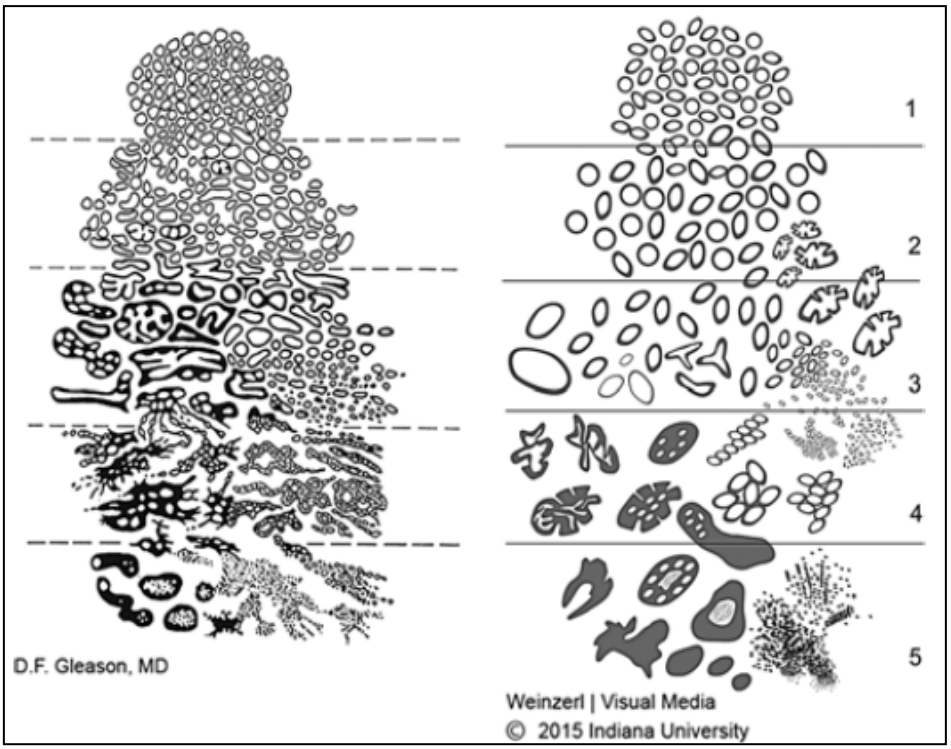

Figure 6. Histological Gleason grade patterns diagrams for PrCa.
Original (left) and 2015 Modified ISUP Gleason (right).
From Epstein J.I. *et al,* Am. J. Surg. Pathol. 2016[41]

Both Gleason grade 4 and 5 are high grade and poorly differentiated carcinomas.

- Gleason grade 4 is raggedly infiltrative, with fused glands creating masses, cords or chains and diffusely invading the stroma.
- Gleason grade 5 defines the most poorly differentiated epithelium, consisting of papillary, cribriform or solid masses with central necrosis, or ragged sheets of anaplastic adenocarcinoma cells with very few glands.

Since PrCa is a multifocal disease, the histological grades are used to generate a GS, which is calculated according to the sum of the two most common Gleason grades in the sample. GS can theoretically range from 2 to 10. However, the lowest score currently assigned is 6 and thus, for practical purposes, GS ranges from 6 to 10.

Consequently, a new grading system for PrCa aiming to simplify and better stratify the tumors was very recently described[41,43–45]. This classification simplifies the number of grading categories from GS 2 to 10 to Grade Groups 1 to 5 **[Figure 6]**, with potential to reduce overtreatment of indolent cancer, and it has been approved by the International Society of Urological Pathology (ISUP) and by the World Health Organization (WHO). This new grading system would be used in conjunction with GS.

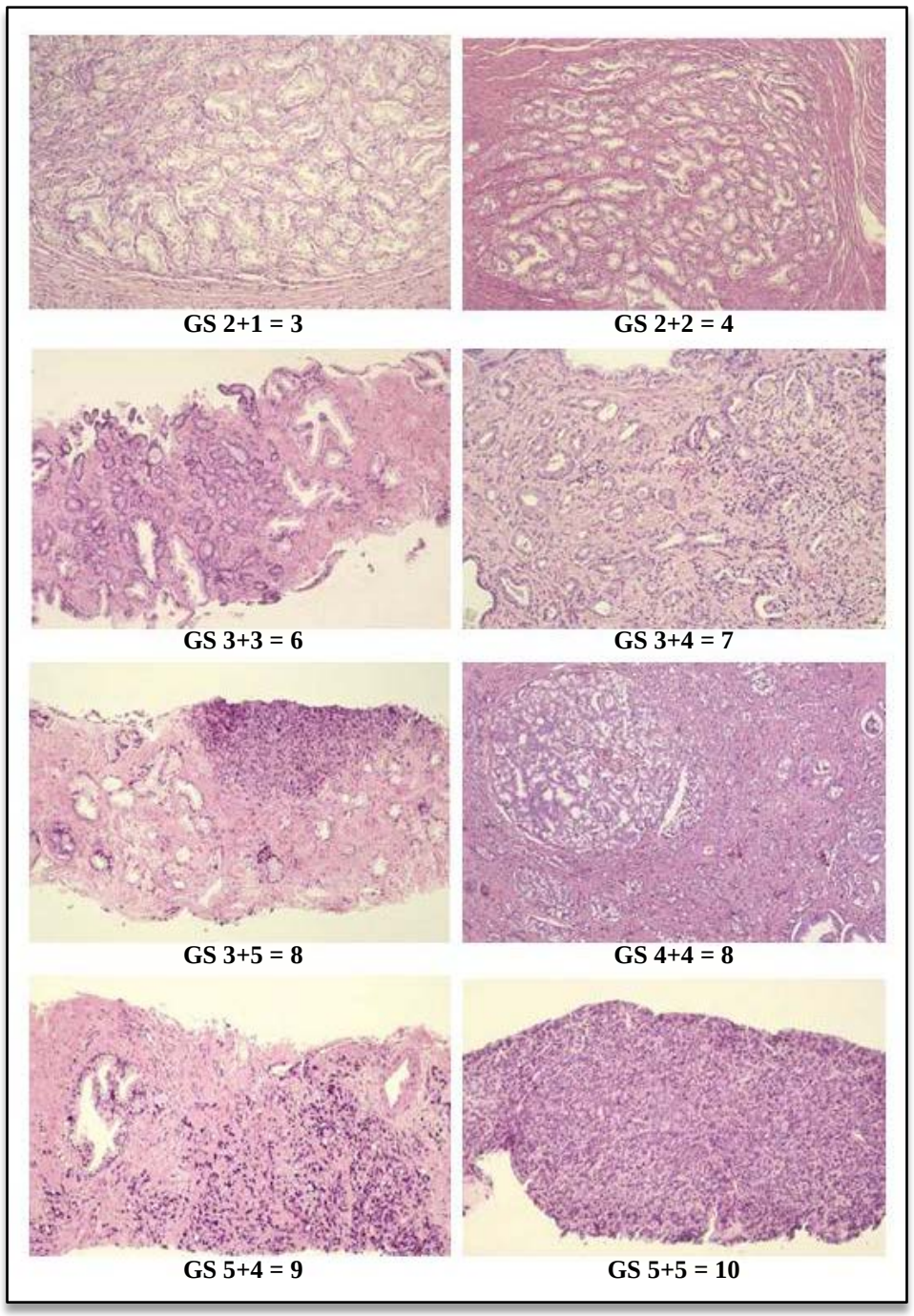

Figure 7. Histological Gleason grade patterns images.
Adapted from Humphrey, P.A. Modern Pathol. 2004[42]

2.5. Natural history of prostate cancer

The main proliferative prostatic diseases are chronic disorders that require a long time to develop and their prevalence increases along with age. Non-neoplastic affectations of the prostate are very frequently diagnosed, being prostatitis (mostly affecting the PZ and the TZ) and BPH (commonly affecting the TZ) the most common findings. Nonetheless, these affectations are not considered to be precursor lesions of PrCa[46].

On the other hand, as review by De Marzo *et al* [47], there are some processes that have been identified as putative precursor lesions for PrCa. High-grade prostatic intraepithelial neoplasia (HGPIN) is a well-accepted and the main precursor lesion of prostatic adenocarcinoma, but adenosis (atypical adenomatous hyperplasia) and proliferative inflammatory atrophy (PIA) have also been proposed as alternative precursor lesions. **[Figure 8]**

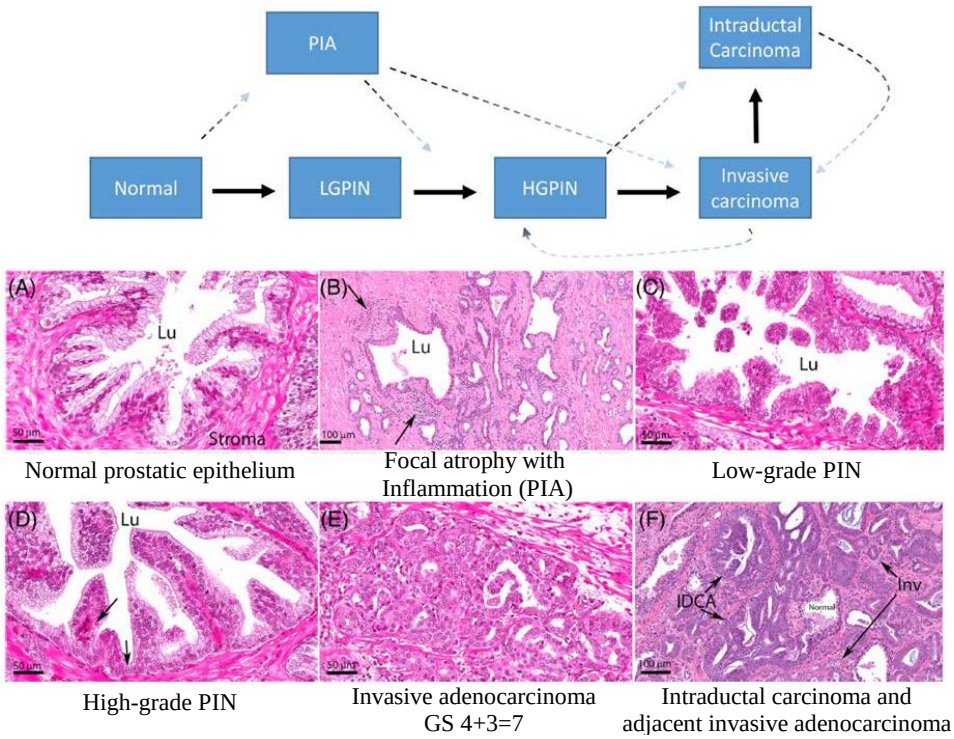

Figure 8. Model of the natural history of PrCa.
Adapted from De Marzo *et al*, A.M. CAPR. 2016[47]

prostatic intraepithelial neoplasia (PIN) was characterized for the first time by McNeal and Bostwick in 1986[48] to define an atypical proliferation of the secretory epithelium and, depending on the cell morphology and the grade of maintenance of the basal cells layer, it can be of low or high grade.

Low grade prostatic intraepithelial neoplasia (LGPIN) is characterized by minimal enlargement of the cell nuclei with minimal nucleoli; whereas in the case of HGPIN the nuclei are enlarged with marked nucleoli, and the basal cell layer is normally discontinuous, similar to adenocarcinoma cells. HGPIN is commonly found in the PZ and it is considered the pre-stage of PrCa, on the basis of cell morphology, nucleolar enlargement, zonal colocalization and frequent multifocal occurrence, phenotypic features and somatic genomic changes shared between HGPIN and PrCa. For example, *TMPRSS2-ERG* rearrangement has been described in 5-20% HGPIN of men of European descent [47,49].

PrCa itself has been further subdivided into different clinical-pathological subgroups, which might not be different entities but different stages of the natural history of PrCa.

- Latent PrCa

  Undiagnosed silent tumors, which never caused symptoms or death, found at autopsy of patients dying from unrelated causes and so called latent PrCa. With the introduction of PSA screening, its incidence decreased 3-fold, especially in men older than 70 years old.[50]

- Incidental PrCa

  This subgroup includes cases detected at cystoprostatectomy for urothelial carcinoma. Although it has been identified in 14-65% of the patients undergoing radical cystoprostatectomy, the detection of PrCa rarely impacts the management of these patients, as many cases overlap with the criteria for clinically insignificant PrCa.[51–53]

- Clinically insignificant PrCa

Equated by some authors with the similar definition of minute carcinoma, this category includes tumors that fulfill the following criteria at radical prostatectomy: low grade (GS ≤ 6; without Gleason patterns 4 or 5), low volume (<0.5cm^3) and low stage (organ confined), asymptomatic and negative on DRE. These tumors may be indolent and are unlikely to acquire clinical or biological significance without treatment. In concordance with the decrease on the detection of latent PrCa, the frequency of clinically insignificant tumors increased with the widespread use of PSA screening.[54,55]

- Clinically significant and metastatic PrCa

The clinical PrCa causes related symptoms and/or death, and may be androgen-dependent or may become hormone- resistant. This group includes all the tumors detected clinically and subjected to therapy; characterized by GS > 6, volumes > 0.5 cm^3, that might have progressed extending outside the prostate capsule and/or metastasizing in regional nodes or in distant metastasis, with an early and particular affinity for bones, and later, more ominous to dissemination to lungs, liver or brain[56,57].

2.6. Treatment

The decision making on the treatment of patients with PrCa depends on whether the patient presents a localized or a metastatic disease. As reviewed by Mottet N *et al*[29] and Attard G *et al*[58], treatment options and new therapies have evolved substantially in the past years. Nonetheless, the paradigm of personalized treatment for PrCa has not yet been optimized, probably due to the heterogeneous nature of this disease.

Indeed, patients with localized tumors can have very different outcomes/prognosis and treatment options, ranging from watchful-waiting to radical surgery, radiotherapy, and even chemotherapy in more advanced cases. Therefore, it is important to make a decision on the best approach in each case. For this purpose tools for risk assessment have been developed, that take into account patient's age, clinical tumor stage, PSA levels or GS, among others, as well as the benefits and side effects of each therapy. It is currently assumed that low grade tumors (GS $\leq$ 6) are harmless and patients can safely avoid treatment just following active surveillance through PSA tests, imaging techniques and repeated biopsy series. On the other hand, high-risk locally-advanced PrCa is mainly treated with long-term androgen deprivation combined with radical radiotherapy, or radical prostatectomy, or brachytherapy depending on the clinical situation. Treatments are usually decided involving the patient himself. New treatment modalities like cryotherapy or high intensity-focal ultrasound are also beginning to have a more widespread use.

Historically, the treatment for advanced and metastatic PrCa has involved androgen deprivation therapy (ADT) either by medical or surgical castration. Unfortunately, and despite the initial response to treatment, many patients progress with rising PSA levels after castration. Some of these patients with recurrences have no signs of metastasis (M0), and it is unclear what the best option for treatment is in this case. Others recur with metastasis (metastatic castration-resistant PrCa (mCRPC)), which is usually a lethal stage of the disease. Next-generation hormone therapies such as abiraterone or enzalutamide have been approved for the therapy of these patients, improving the overall survival of the patients for up to 5 months[59,60]. It has been estimated that the natural history of PrCa spans up to 40 years if the preclinical phase is included and about 15 years from the diagnosis to the last phases.

2.7. Molecular alterations in prostate cancer

The use of "-omic" technologies for the molecular characterization of PrCa has brought, despite technical challenges, further insights into the genomic processes driving the development and evolution of this highly heterogeneous and multifocal disease. Indeed, The Cancer Genome Atlas (TCGA) research network published in 2015 an extensive comprehensive molecular study of primary prostate carcinomas[61]. Importantly, they found that 74% of the cases could be classified into one of the seven different molecular subtypes identified, which were defined by ETS fusions (*ERG*, 45.6%; *ETV1*,8.4%; *ETV4*,4.2%; and *FLI1*,1.2%) or mutations in *SPOP*,11.1%; forkhead box A1 (*FOXA1*), 2.7%; and *IDH1*, 0.9% genes. The results also reaffirmed the highly heterogeneous nature of PrCa (26% of the tumors could not be categorized), showed the diverse genomic, epigenomic and transcriptomic patterns; and in turn identified potential targets for therapeutic approaches.

PrCa is overall characterized by low frequency of gene mutations, and much higher prevalence of copy-number variations (CNVs) and chromosomal rearrangements. Deregulation of several important pathways has been described in prostatic carcinogenesis and there are several reviews[58,62–67] dealing with this topic. AR, phosphoinositide-3-kinase (PI3K), cell cycle, deoxyribonucleic acid (DNA) repair, Wingless-related integration site (Wnt) and mitogen-activated protein kinase (MAPK) signaling are the most commonly dysregulated pathways in PrCa. Moreover, alterations in chromatin regulatory pathways, epigenetic alterations and chromoplexy have also been described to play an important role as well. **[Figure 9]**

2.7.1. *ETS* rearrangements

In 2005, Petrovics *et al* [68] identified one of the earliest and more prevalent genetic alteration in PrCa, reporting overexpression of the *ERG* oncogene in about half of prostate tumors. Later that same year, Tomlins *et al* [69] applied a bioinformatics approach and detected outlier overexpression of *ERG* and

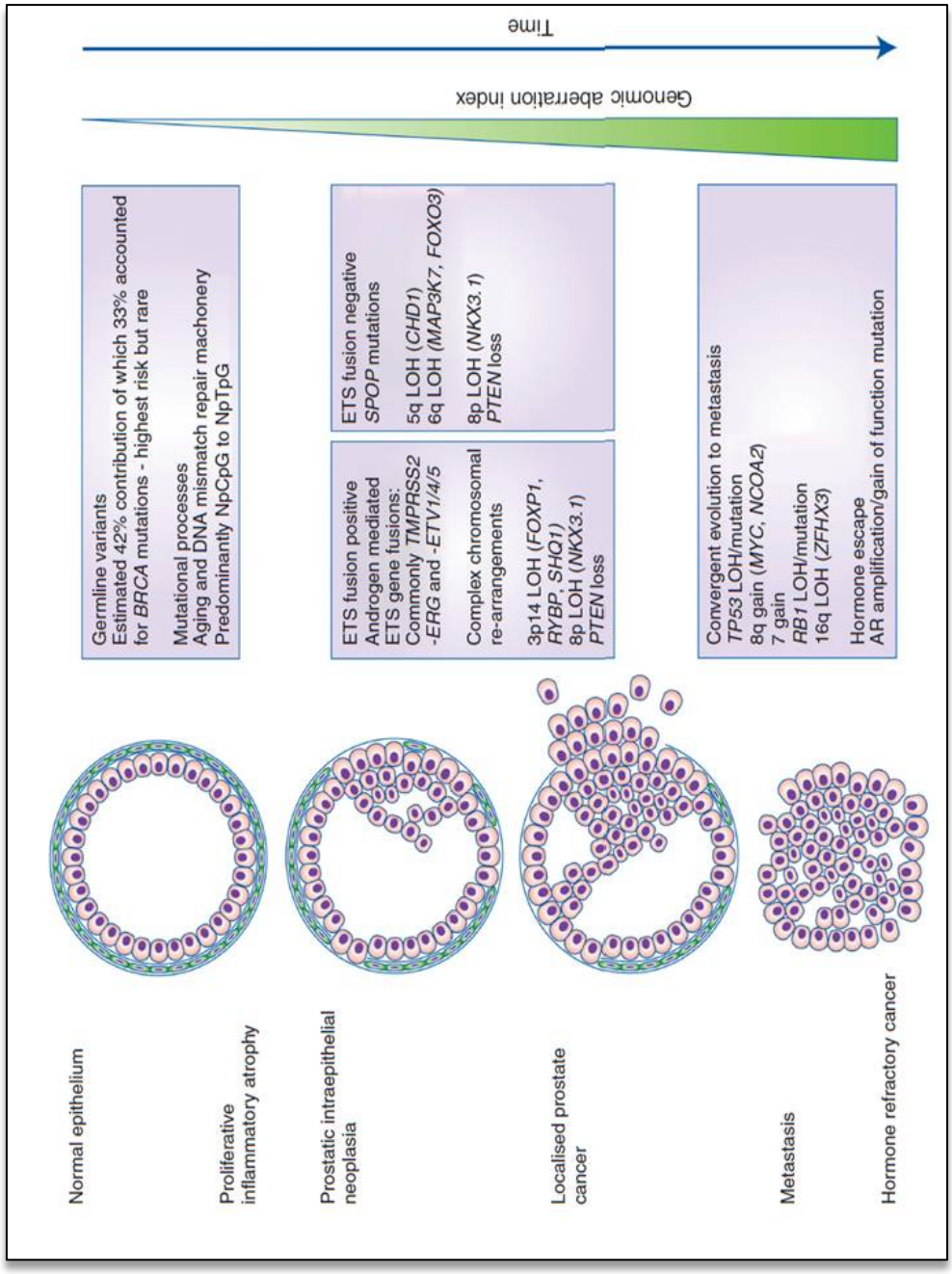

Figure 9.Genomic alterations involved in PrCa.

The pathological stages of prostate cancer are depicted on the left hand side of the figure, with corresponding genomic mutations that equate to cancer progression on the right. Cytobands are annotated according to which driver genes are most strongly implicated by the corresponding aberration

From Mitchell, T. *et al*, Br. J. Cancer. 2015[67]

ETV1 genes. Even more, they discovered the presence of recurrent gene fusions in PrCa, involving the 5'untranslated region (5'UTR) of transmembrane protease, serine 2 (*TMPRSS2*) and several members of the erythroblast transformation-specific (ETS) family of transcription factors, mainly the v-ETS avian erythroblastosis virus E26 oncogene related (*ERG)* but also ETS avian erythroblastosis virus E26 variant 1 (*ETV1*). These discoveries represented a milestone in the field of prostatic carcinogenesis research.

The involvement of *ETS* genes in gene fusions is a common alteration in human cancers, and leads to the formation of either chimerical fusion proteins or altered expression of the ETS protein. In prostate tumors, the *TMPRSS2-ERG* genetic rearrangement results in the juxtaposition of the androgen-regulated *TMPRSS2* promoter to the proto-oncogene *ERG*. In most cases, the fusion product is an almost full-length ERG protein[70]. **[Figure 10]**

ERG gene (GENE ID: 2078) is located on chromosome 21q22.2 and belongs to the ETS family of transcription factors which are involved in the control of embryonic development, cell proliferation, differentiation, metastasis, apoptosis, angiogenesis and inflammation. Interestingly, this gene is commonly detected as part of many chromosomal translocations in human cancer, including PrCa (*TMPRSS2-ERG*), Ewing's sarcoma (*EWS-ERG*) and acute myeloid leukemia (*FUS-ERG*). Indeed, *ERG* is the most commonly overexpressed and translocated oncogene in PrCa.

The *ERG* gene structure includes at least 12 exons and its locus expands around 300 kb. Zammarchi *et al*[71] addressed the inconsistencies found in the literature regarding *ERG* nomenclature. There are 30 major *ERG* transcript variants, arising from the presence of three alternative promoters, two common alternative splicing sites, three alternative polyadenylation sites and several translation initiation sites; which can give rise up to 15 *ERG* transcript variants. Nonetheless, some *ERG* transcripts are more common than others

and actually the more abundantly expressed *ERG* isoforms are *ERG-1a.Δ7b* (*ERG2*, NM_004449) and *ERG-1c* (*ERG3*, NM_182918). **[Figure 11]**

The ERG protein is mainly expressed in the cell nucleus and contains an ETS DNA-binding domain, an alternative domain (AD), a pointed (PNT) regulatory domain responsible of the self-association of chimeric oncoproteins, and a transactivational domain (TAD).[71–73] **[Figure 11]**

Several co-regulators of ERG mediated transcription have been described, including for example Ewing sarcoma breakpoint region 1 (EWS)[74], mitogen-activated protein kinase 1 (MAPK1/ERK2)[75] or bromodomain-containing protein 4 (BRD4)[76] as co-activators and polycomb repressive complex 2 (PRC2) and histone deacetylases, class I (HDACs) as co-repressors[77,78].

Moreover, ERG transcriptional activity is also regulated by post-translational modifications. For instance, it has been described that speckle-type POZ protein (SPOP), an E3 ubiquitin ligase substrate-binding protein, promotes ubiquitination and proteasome degradation of wild-type ERG. However, in cases involving *SPOP* mutations or cases harboring *TMPRSS2-ERG* fusions encoding N-terminal truncated ERG proteins, ERG is resistant to SPOP degradation and thus overexpressed.[79,80]

As mentioned above, ERG induces gene expression programs that contribute to oncogenesis, such as genomic damage, epigenetic reprogramming, differentiation and inflammatory pathways[81]. Notably, it has a key role on the regulation of genes involved in cell migration and invasion, epithelial-mesenchymal transition (EMT) and metastasis. For example, ERG promotes frizzled class receptor 4 (*FDZ4*) upregulation, loss of E-cadherin (CDH1), activation of *vimentin* (*VIM*), activation of *myc protooncogene* (*myc*) and activation of the Wnt-signaling pathway; all contributing to the EMT. At the same time, ERG also represses many epithelium-specific genes causing the dedifferentiation of the epithelium.[82–85]

Interestingly, Tan SH *et al*[86] performed an analysis of the ERG responsive proteome (ERP) both in FFPE PrCa samples from a Caucasian American cohort and in the *TMPRSS2-ERG* positive VCaP cell line. Concordant results from both analyses identified a group of proteins involved in cytoskeleton modulation and actin reorganization, cell migration, protein biosynthesis, and proteasome and endoplasmic-reticulum-associated protein degradation pathways.

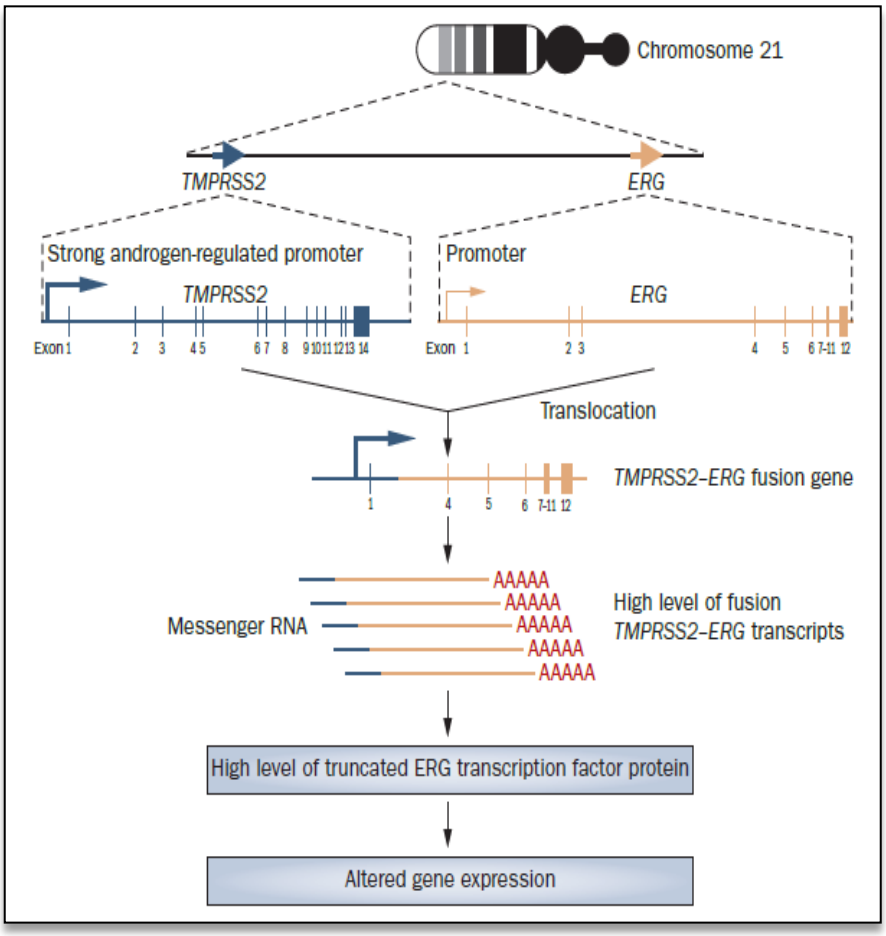

Figure 10. *TMPRSS2-ERG* rearrangement in PrCa.
The strong androgen-regulated *TMPRSS2* gene transcriptional promoter becomes fused to the *ERG* gene to form an androgen-regulated *TMPRSS2–ERG* fusion gene (middle). Under the influence of androgens this fusion gene is transcribed to produce high levels of *TMPRSS2–ERG* gene transcripts and encoded protein. High level production of truncated ERG transcription factor proteins is believed to cause alterations in the expression of target genes.
From Clark, J.P. *et al*, Nat. Rev. Urol. 2009[70]

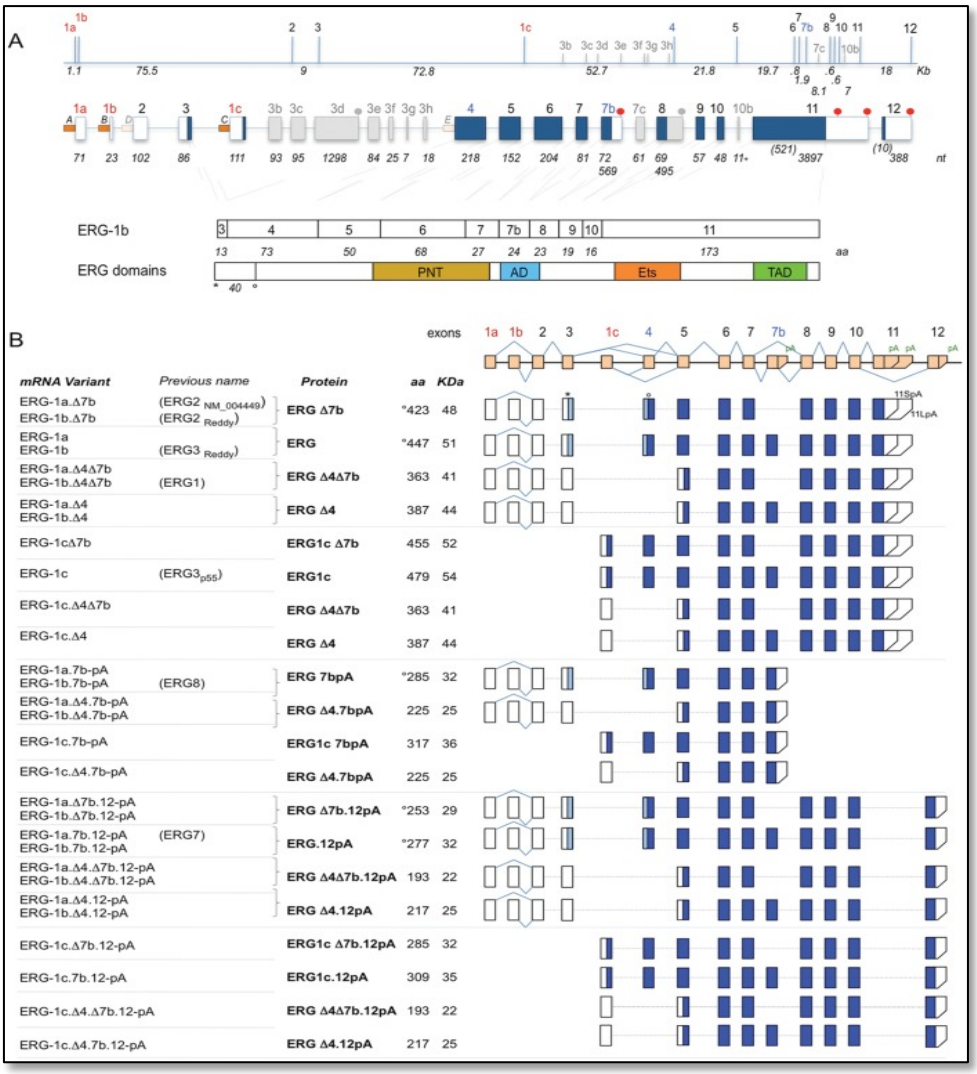

Figure 11. Human *ERG* gene structure and main isoforms.

A. Top: The ~300 Kb human ERG locus, drawn roughly to scale. Approximate intron sizes are indicated, along with exons position (bars). Red indicates first exons, blue common alternative ones and gray uncommon ones. Middle: Exon structure, with exon sizes at the bottom. Blue boxes indicate the main predicted ORFs, white boxes the untranslated regions and gray the uncommon exons. Red circles indicate polyA sites. Bottom: alignment of the exons forming the main ORF (ERG-1b) with the protein's domains. Numbers indicate size in amino acids. Asterisk and circle indicate position of the first and second ATG. **B.** Human ERG main variants. Alignment of exons forming the 30 main RNA variants of human ERG. Blue indicates the ORF, light blue the additional region from the ATG in exon 3. For each variant, the proposed name is indicated next to previous nomenclature (if available). The proposed protein name is reported along the predicted size in aa and KDa. Variants derived from the alternative usage of promoter 1a and 1b are paired as they lead to related mRNAs and identical proteins.

From Zammarchi, F. *et al*, Plos One. 2013

The unique ERPs for ERG positive tumors involved cell growth and survival pathways, whereas for ERG negative tumors the proteasome and redox function pathways were enriched; confirming the roles of ERG in inhibiting cell differentiation and activating cell growth.

The *TMPRSS2* gene (Gene ID: 7113) is located on chromosome 21q22.3, very close to the *ERG* locus. There are different isoforms of this gene that arise from events of alternative splicing. *TMPRSS2* is preferentially expressed in normal prostate tissue and it is under androgenic control. It encodes for a protein of the serine protease family, which contains a type II transmembrane domain, a receptor class A domain, a scavenger receptor cysteine-rich domain and a protease domain[72].

Complex intra and interchromosomal rearrangements in *TMPRSS2* and *ERG* are a common and specific feature in a substantial portion of prostate adenocarcinomas[81,87,88]. *TMPRSS2-ERG* rearrangement is the most frequent genetic alteration described in human solid tumors and accounts for more than 90% of the *ETS* rearrangements detected in PrCa[89,90].

The most common mechanism of fusion is through interstitial deletion of the chromosomal region between *TMPRSS2* and *ERG*[91–96]. The fact that these two genes are closely located on chromosome 21, at a distance of around 3Mb, could explain its high prevalence in comparison to other rearrangements. *TMPRSS2* and *ERG* breakpoints arise nonrandomly, and AR signaling seems to be involved in the generation of the gene fusion[97,98]. Besides, *ERG* expression might be related to AR as a consequence of the *TMPRSSS-ERG* rearrangement in PrCa, since the *TMPRSS2* promoter is the portion included in the fusion, and it is under androgenic control.[99–101]

In addition to the *ERG* fusion product overexpression, recent papers have reported that normal *ERG* gene allele can be controlled by the expression of the *TMPRSS2-ERG* product, resulting in overexpression of the native ERG protein. A positive feedback loop involving TMPRSS2-ERG and native ERG

that could lead to androgen independence has been proposed by some authors.[71,102]

The complexity of the *TMPRSS2-ERG* fusion in PrCa is further increased by the presence of several fusion variants, which are believed to occur through alternative splicing. [69,89,91,103–111]

Two major types of *TMPRSS2-ERG* fusion variants have been described[112]. The most common type I variants encode for a full length ERG protein (ERG1, M21535; ERG2, NM004449; ERG3, NM182918) containing both protein-protein interacting domain (PNT/SAM) and ETS DNA-binding domain. The most common protein encoded is ERG3, which is truncated in the N-terminus part (first 32 amino acids), but still retains both PNT and ETS domains. On the other hand, type II variants encode truncated ERG proteins (ERG8, AY204742; or TEPC, EU432099) which lack the ETS DNA binding domain and also the first 32 amino acids of the N-terminal part of ERG. Type II variants are more abundant than type I, and interestingly type I variants showed a trend of correlation with worse pathology and outcomes in the study by Hu Y *et al*, but more studies are needed to clarify this observation.

Overall, the most frequent *TMPRSS2-ERG* transcripts are T1-E4 (exon1 of *TMPRSS2* fused to exon 4 of *ERG*), T2-E4 and T1-E5, which encode N-terminus truncated ERG proteins that still retain PNT and ETS domains. Even more, T1-E4 and T1-E5 variants have been shown to coexist in the same cancer sample.[73,104,113] Some authors have suggested the implication of distinct fusion variants to tumor aggressiveness. For instance, the fusion of exon 2 of *TMPRSS2* and exon 4 of *ERG* (T2-E4) are associated with PSA recurrence and seminal vesicle invasion.[106,107]**[Figure 12]**

The *TMPRSS2-ERG* fusion is considered to be an initial event in prostate oncogenesis. Cerveira *et al* [108] described for the first time the presence of this alteration in a subset (21%) of HGPIN. This observation was confirmed with frequencies ranging from 10% to 21%[114–118]. Some authors have suggested

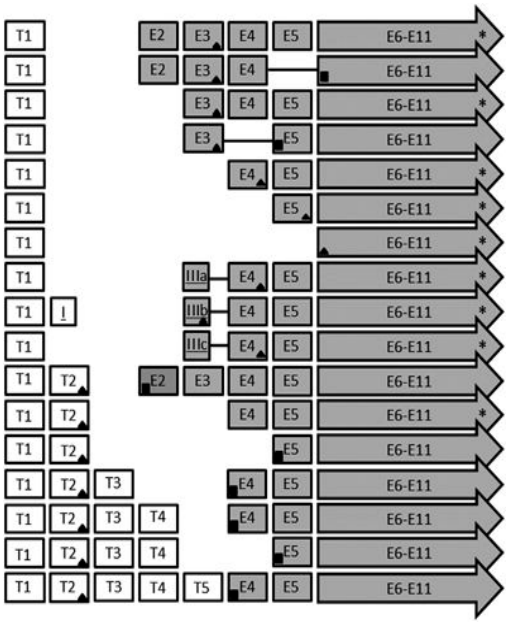

Figure 12. *TMPRSS2-ERG* fusion types in PrCa.
White boxes represent the TMPRSS2 exons (labelled T1–T4), grey boxes represent ERG exons (E2 to E11), white boxes with underlined numbers indicate a retained fragment of TMPRSS2 intron I and underlined numbers in grey boxes signify different variants of ERG retained intron III. Black triangles indicate translation start and * ERG's normal translation stop site. Black rectangles indicate early stop sites created by frameshifts.
From Adamo P and Ladomery MR. Oncogene. 2016

that the assessment of ERG expression in cases with HGPIN could be extremely useful to improve risk stratification of the patients, as patients presenting HGPIN with ERG overexpression were more likely to develop PrCa[117,119]. In this sense, mechanistic studies have proved that *ERG* and *TMPRSS2-ERG* overexpression promote cell migration and invasion in benign prostatic epithelial cells[87,120,121]. Furthermore, *ERG* overexpression under androgenic control leads to the development of pre-neoplastic lesions in mice[87,121]. It seems that *TMPRSS2-ERG* plays an important role in the transition from HGPIN to invasive carcinoma[121]. Nonetheless, the gene rearrangement itself is not sufficient to drive prostate cancer, and additional aberrations would be needed[122]. As discussed below, combined *ERG* overexpression and loss of phosphatase and tensin homolog (*PTEN*) cooperate in the development of prostatic adenocarcionma[116,123].

Notably, the study of *TMPRSS2-ERG* has been useful not only to reaffirm that PrCa is a multifocal disease but to prove that, in the context of *TMPRSS2-ERG* fusion, metastases probably arise from a unique tumor focus[93,124,125]. Indeed, Mehra *et al* [93] described complete uniformity in the rearrangement

status of different prostate metastases in the same patient, suggesting the clonal evolution of PrCa to metastasize. Nonetheless, other authors have recently stated that PrCa metastases might arise from a single tumor clone in some cases, but also from several tumor foci in others[126].

There is still a lot of controversy regarding the clinical and prognostic implications of the *TMPRSS2-ERG* rearrangement and of the subsequent ERG overexpression. Some authors have suggested an association of the rearrangement to indolent disease and favorable clinical-pathological outcomes[68,116,127,128] while others favor its association to more advanced and aggressive tumors[129,130]. Yet other series have not found any association between *TMPRSS2-ERG* and GS, tumor stage or patient survival[129,131–133]. It is important to mention that the inter- and intra-heterogeneity nature of PrCa, the acquisition of samples, different study cohorts and the techniques used, as well as additional genetic or epigenetic alterations, could explain these discordances.

Given the importance of *TMPRSS2-ERG* rearrangement in PrCa, several studies have dealt with its possible use in urine-based tests. *TMPRSS2-ERG* alone is highly specific but lacks sensitivity. Nonetheless, in combination with other urinary markers could be potentially useful as a diagnostic tool and also as a biomarker for PrCa aggressiveness.[90,134]

The *TMPRSS2-ERG* rearrangement is the main mechanism to achieve ERG overexpression under androgenic control. Nonetheless, other *ERG* 5'partners have been identified, including the solute carrier family 45 member 3 (*SLC45A3*) and the N-myc downstream regulated 1 (*NDRG1*) genes, which are preferentially expressed in the prostate and turn to be under androgen regulation as well[95].

SLC45A3 is, at a much lower frequency than *TMPRSS2-ERG*, the second most common 5'partner for *ERG* rearrangements in PrCa. Also, other less

common rearrangements have been described between *SLC45A3* and different 3'partners such as for example *SLC45A3-ETV5*[135] and *SLC45A3-ELK4*[136].

SLC45A3 gene, also known as prostein (Gene ID: 85414), is located at 1q32.1, consists of 9 exons, it is preferentially and almost exclusively expressed in the prostate; and it is under both androgenic and estrogenic regulation. Prostein is a prostate-specific marker of benign and malignant prostatic epithelial cells, although its expression is significantly lower in PrCa.

The *SLC45A3-ERG* rearrangement was first described by Han *et al*[137] and it leads to the formation of a truncated ERG protein. Moreover, the presence of *SLC45A3-ERG* rearrangement leads to the loss of prostein expression and may as well affect ERG expression.[72,95,138–141] Indeed, Perner *et al*[141] have reported that loss of SLC45A3 protein as a result of the rearrangement is associated with shorter PSA progression-free survival and high GS.

Interestingly, the presence of concomitant *TMPRSS2-ERG* and *SLC45A3-ERG* rearrangements has been found in a subset of PrCa[95,140]. Importantly, the study by Esgueva *et al*[95] was the first to assess the frequency of *SLC45A3-ERG* rearrangements in a large clinical cohort. Even more, the authors showed for the first time that concurrent *TMPRSS2-ERG* and *SLC45A3-ERG* rearrangements could happen within the same tumor focus. Actually, about 11% of the *ERG* rearranged cases harbored concurrent *TMPRSS2* and *SLC45A3* rearrangements in this cohort. Nonetheless, the meaning of this association is still unknown and no previous study has analyzed its significance in PrCa.

2.7.2. AR signaling pathway

AR signaling is crucial for the normal growth and differentiation of the prostate. AR is a transcription factor that belongs to the steroid hormone receptor family. Upon the binding of androgen steroids, importantly testosterone and its metabolite dihydrotestosterone (DHT), AR homodimerizes, gets phosphorylated and translocated into the nucleus where

it can bind to androgen responsive elements (AREs) located in promoter and enhancer regions of its target genes, hence regulating their expression. The binding of AR to AREs is dependent on different important cofactors, among them: transcriptional co-activators (nuclear receptor co-activator 2 (NCOA2), E1A binding protein p300 (EP300)), transcriptional co-repressors (Nuclear receptor co-repressor 2 (NCOR2)) and chromatin regulatory elements (FOXA1, forkhead box P1 (FOXP1), forkhead box O1 (FOXO1) and forkhead box O3 (FOXO3)).[142,143]

AR signaling is commonly deregulated in the prostatic carcinogenesis. Since Huggins and Hodges[144] first showed in 1941 the importance of AR signaling in PrCa by describing tumor regression in patients undergoing orchiectomy, AR signaling pathway has been extensively studied.[145]

Alterations in AR itself including amplification, mutations or splice variants; and also in its co-activators and co-repressors, along with aberrant activation and post-translational modifications have been and continue to be extensively studied. AR alterations tend to be more common during advanced stages of the disease, and have been shown to promote PrCa progression.[62,146–148]

2.7.3. PI3K signaling pathway

Besides the main role of the AR signaling pathway in prostate carcinogenesis, alterations in the PI3K signaling pathway, which is involved in many cellular processes like the regulation of cell proliferation, survival and apoptosis, metabolism, motility and angiogenesis, are of great importance and frequently found in this disease.[149–151]

Several factors, like tyrosine kinase growth factor receptors or other molecules like for example oncogenic Ras, stimulate the tyrosine kinase receptor (TKR), which in turn activates PI3K. PI3K, a heterodimer consisting of a catalytic and a regulatory subunit, then promotes the phosphorylation of phosphatidylinositol (4,5) bisphosphate (PIP2) into of phosphatidylinositol (3,4,5) trisphosphate (PIP3). Next, PIP3 is able to bind to the serine/threonine-

specific protein kinase AKT, promoting its phosphorylation and consequently its translocation into the nucleus, where it controls a wide variety of cellular processes. On the other hand, *PTEN* gene encodes for a lipid phosphatase that reverses the actions of PI3K by dephosphorylating PIP3 into PIP2, which prevents AKT activation and thus negatively regulates this signaling pathway.[152]

Alterations in different components of the PI3K pathway and also downstream targets of this pathway have been described.[147,153] As an example, Robinson *et al*[153] found that 49% of mCRPC had somatic alterations in the PI3K pathway. These included loss of *PTEN*; *PI3K* alterations, primarily in *PIK3CA* subunit (mutations, amplifications and fusions) but also, for the first time, in other catalytical subunits (*PIK3CB*), and activating mutations in *AKT1*.

Moreover, it has been reported that there is a crosstalk between the PI3K pathway and the AR signaling pathway in PrCa, not only by the direct phosphorylation of AR via AKT but also through other mechanisms[150,154,155].

Nonetheless, the most common alteration involving this pathway in PC is the loss of *PTEN*. The *PTEN* gene is located in chromosome 10q23, it can undergo several aberrations during tumorigenesis, and it is an important tumor suppressor in PrCa. This alteration has been extensively studied in PrCa, and heterozygous deletion is the most frequently reported alteration. About 40% of primary prostate cancers present it, and its incidence increases in metastatic advanced cases (70%); whereas inactivating mutations are rare events. [148,156–159]

PTEN alteration has been associated with poor prognosis in PrCa. In that sense, it has been reported that *PTEN* loss is infrequent in clinically insignificant tumors (around 2%) and GS=6 tumors of large volume (13%), but much prevalent in GS≥7 PC (around 46%). Thus, detection of aberrant

PTEN in GS=6 suggests a greater likelihood of clinically significant disease.[160]

Interestingly, there seems to be interplay between *PTEN* loss and the presence of *ETS* rearrangements, particularly the *TMPRSS2-ERG*. The presence of *TMPRSS2-ERG* fusion and *PTEN* loss seems to be associated to worse outcomes but there are some conflicting results described in the literature.

Overall, many studies have reported the concomitant occurrence of both *TMPRSS2-ERG* fusion and *PTEN* loss in a subset of PrCa[96,160–165]. Besides, it has been shown that the heterogeneity in multifocal prostate cancer is also present at the level of *PTEN* losses and presence of *TMPRSS2-ERG* rearrangement[166]. Even more, some authors[167] have suggested that *PTEN* alterations might be driven by ERG expression.

In a study by Kim S.H. *et al*[168] a positive ERG immunohistochemistry (IHC) was associated with favorable biochemical-free survival. Instead, *PTEN* loss IHC results associated with unfavorable biochemical-free survival. However, the patients with worse prognosis were those harboring both alterations. Nagle R.B. *et al*[169] described for first time that concomitant ERG overexpression and *PTEN* deletion imply a higher risk of capsular penetration.

In contrast, a study analyzing the effect of both alterations in lethal PrCa, suggested that *PTEN* loss alone is associated with higher risk of lethal progression, mainly in tumors not harboring *ERG* rearrangement.[170]

Some mechanistic studies have also been reported, highlighting the importance and interrelation of these two alterations in PrCa. Carver B.S. *et al*[116] demonstrated, through *in vitro* and *in vivo* studies, that aberrant ERG expression cooperates with *PTEN* haploinsufficiency promoting the progression of HGPIN to invasive carcinoma. In a very recent study by Linn D.E. *et al*[96], the authors used a mouse model with the background of prostate *PTEN* deficiency and found that only the mice with interstitial deletion between *TMPRSS2* and *ERG* developed poorly differentiated tumors with

EMT. The authors found that some genes comprised in this deleted region act as tumor suppressor genes in PrCa, and their loss is associated with tumoral progression and lethal disease.

2.7.4. MAPK signaling pathway

Although the involvement of this pathway in the prostatic carcinogenesis has not been as well characterized as in other cancers, some alterations have been described in a subset of PrCa. For instance, the v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*) has been described as mutated or fused (sometimes to *SLC45A3*) in only about 2.5% of the tumors.[61]

2.7.5. Cell cycle

The majority of human cancers are characterized by alterations in cell cycle regulatory genes. This is also the case in PrCa, particularly in mCRPC, which is also characterized by aberrations in many genes controlling cell cycle and proliferation. Tumor suppressor genes, by excellence tumor protein p53 (*TP53*), which is the most commonly mutated gene in human cancer; and also retinoblastoma (*RB1*) are altered in prostate tumors either through mutations or deletions.[61,153]

2.7.6. DNA repair

DNA repair pathway has been demonstrated to play a crucial role in prostate carcinogenesis, in both localized PrCa and particularly in high grade and mCRPC. Many genes involved in the DNA damage response are altered in PrCa, including breast cancer 1 gene (*BRCA1)*, *BRCA2*, cyclin dependent kinase 12 gene (*CDK12)* and ataxia telangiectasia mutated gene (*ATM)*, among others. The prevalence of aberrations in this pathway is relatively high, especially in mCRPR where 23% of cases harbor an alteration[153,171]. Interestingly, not long ago, frequent (6-13% of PC) recurrent non-synonymous point mutations in *SPOP* were described in PrCa. These

mutations are mutually exclusive with *ETS* rearrangements and have been proven to crosstalk with AR signaling pathway[156,172,173].

2.7.7. Wnt signaling pathway

Wnt pathway is involved in the embryological development of the prostate[174–176]. There are several studies describing the crosstalk between AR and Wnt/β-catenin signaling pathways and it has been shown that Wnt signaling pathway is reactivated during prostatic carcinogenesis[176]. Alterations in this pathway, such as mutations in β-catenin (*CTNNB1*) and adenomatosis polyposis coli tumor suppressor (*APC*) genes, are commonly found in PrCa[148,153].

2.7.8. Chromatin regulatory pathways and epigenetics

Epigenetics is an important field of study that, besides genetics, has the potential to significantly contribute to the development and/or progression of the prostatic carcinogenesis and represents a promising new target for the therapeutics of PrCa. Many epigenetic changes have been described in PrCa[177–180].

As an example, enhancer of zeste homolog 2 (EZH2), a subunit of the PRC2, has been shown to play an important role in mCRPC. The physiological activity of EZH2 is to act as a histone methyltransferase to silence gene expression as part of the PRC2 complex, playing a critical role in chromatin regulation. Nonetheless, it has been demonstrated that EZH2 is overexpressed in aggressive tumors and mCRPC. In this scenario, EZH2 becomes phosphorylated, undergoing a functional switch and acting as a transcriptional coactivator for some transcription factors, including AR. These observations suggest that EZH2 could be a potential target for the treatment of advanced PrCa.[181]

On the other hand, Berger M.F. *et al*[182] have suggested that genomic rearrangements in PrCa could emerge from transcriptional or chromatin

aberrations. In this study, they found that rearrangement breakpoints were more commonly found near regions of open chromatin, AR and ERG binding sites in the subset of tumors harboring *TMPRSS2-ERG* rearrangement, whereas these regions were inversely correlated in tumors without the rearrangement.

2.7.9. Chromoplexy

Chromoplexy, complex genome restructuration, was first described by Baca *et al*[183] in 2013. The authors performed whole genome sequencing (WGS) and DNA copy number profiling and identified abundant DNA translocations and deletions arising in an interdependent manner; supporting the thought that PrCa primarily arises from CNVs and chromosomal rearrangements. Through chromoplexy, PrCa gains chained chromosomal rearrangements and deletions, dysregulating cancer genes.

AIM, HYPOTHESIS AND OBJECTIVES

1. AIM

PrCa is the most frequent malignant tumor (after non-melanoma skin tumors) and one of the main causes of death by cancer in men. Due to the anatomical and histological peculiarities of the prostate, there is a lack of information about the prostatic carcinogenesis. In addition, as the tumors that remain stable for years are morphologically very similar to the ones that progress, the natural history of PrCa is still incompletely understood.

The aim of this study has been to analyze the potential use of the recurrent *ERG* rearrangements and *PTEN* alterations in the stratification and prognosis of patients with PrCa.

2. HYPOTHESIS

The presence of *TMPRSS2-ERG* is crucial in a subset of prostatic tumors, but by itself is not enough to predict the shift of the tumor from a relatively quiescent phase to more aggressive phenotypes, and other relevant alterations, including additional fusions or tumor suppressor gene inactivation, must concur in this process.

The key molecular events in the progression of the prostatic carcinomas, from a quiescent and low activity, to more aggressive and faster phases, could be identified more accurately through detecting the differences between tumors of different histological grades, diverse clinical-pathological subtypes or different tumor volume, features that can reflect different evolutionary stages of the tumor.

The molecular changes in low grade prostate tumors that convey a more aggressive behavior are key events in prostate carcinogenesis. The combination of several critical molecular changes, rather than individual molecules, is essential in understanding PrCa carcinogenesis. Their detection

in tumor samples could allow a fine monitoring and a personalized treatment for each patient.

In this project, we have hypothesized that careful analysis of several recurrent alterations that have been described in PrCa could give some insights on the pathogenesis of this disease. *TMPRSS2-ERG* and *SLC45A3-ERG* rearrangements, along with ERG and *PTEN* expression could be useful prognostic tools for the stratification and prognosis of the patients.

3. OBJECTIVES

Thus, the main objectives of this project are:

- To investigate the role of *TMPRSS2-ERG* rearrangement in the pathogenesis and prognosis of prostate cancer.

- To analyze the alterations of *PTEN* in prostate cancer and its possible relationship with the presence of *ERG* rearrangement/s.

- To determine if the alterations in *TMPRSS2-ERG* and *PTEN*, alone or in combination, could be useful as molecular markers of aggressive prostate cancer.

- To investigate the role of *SLC45A3-ERG* rearrangement in the pathogenesis and prognosis of prostate cancer. To analyze the effect of *SLC45A3-ERG* rearrangements on *ERG* gene expression.

- To analyze the impact of combined *SLC45A3-ERG/TMPRSS2-ERG* rearrangements, and PTEN loss on prostate cancer.

- To analyze the relationship of these alterations and the clinical-pathological features of the patients.

- To establish the potential application of these molecules in the stratification, prognosis and treatment of the patients.

RESULTS

The results from the research conducted during this thesis have been reflected in the following publications:

1. Association of *ERG* and *TMPRSS2-ERG* with grade, stage, and prognosis of prostate cancer is dependent on their expression levels. **Font-Tello A**, Juanpere N, de Muga S, Lorenzo M, Lorente JA, Fumado L, Serrano L, Serrano S, Lloreta J, Hernández S. Prostate. 2015 Aug 1;75(11):1216-26. doi: 10.1002/pros.23004.

2. Concurrent *TMPRSS2-ERG* and *SLC45A3-ERG* rearrangements plus *PTEN* loss are not found in low grade prostate cancer and define an aggressive tumor subset. Hernández S*, **Font-Tello A***, Juanpere N, de Muga S, Lorenzo M, Salido M, Fumadó L, Serrano L, Cecchini L, Serrano S, Lloreta J. Prostate. 2016 Jun;76(9):854-65. doi: 10.1002/pros.23176.

Association of *ERG* and *TMPRSS2-ERG* with grade, stage, and prognosis of prostate cancer is dependent on their expression levels.

BACKGROUND. There is controversy in the literature on the role of the fusion *TMPRSS2-ERG* in the pathogenesis and progression of prostate cancer. The quantitative differences in *TMPRSS2-ERG* fusion expression have received very limited attention in the literature.

METHODS. We have quantitatively analyzed the mRNA levels of *TMPRSS2-ERG, ERG, PTEN,* and *AR* (n=83), as well as ERG immunostaining (n=78) in a series of prostate tumors.

RESULTS. Among the *TMPRSS2-ERG* cases (n=57), high fusion levels were associated with GS≥8 (*P*=0.025). *ERG* mRNA overexpression was associated with GS ≥8 (*P*=0.047), and with stage T3–T4 tumors (*P*=0.032). Among the *ERG* overexpressing cases (n=54), higher expression levels were found in 92.3% of GS ≥8 tumors (*P*=0.02). ERG immunostaining, regardless of staining intensity, was also associated with high stage (*P*=0.05). There was a statistical association between ERG immunostaining and PSA progression-free survival (Log Rank test, *P*=0.048). Decreased *PTEN* expression was associated with *TMPRSS2-ERG* (*P*=0.01), *ERG* mRNA overexpression (*P*=0.003) and ERG immunostaining (*P*=0.007). Furthermore, decreased *PTEN* expression, alone (*P*=0.041) and also combined with *TMPRSS2-ERG* (*P*=0.04) or with *ERG* overexpression (*P*=0.04) was associated with GS ≥7 tumors.

CONCLUSIONS. Although more studies are needed to further clarify their role, our findings emphasize that the expression levels of the *TMPRSS2-ERG* fusion and *ERG* mRNA, rather than their mere presence, are related to a more aggressive phenotype, have an effect on prognosis and could be molecular markers of progression for prostate cancer. Furthermore, ERG immunohistochemistry could be also a potentially useful prognostic factor.

Hernández S, Font-Tello A, Juanpere N, de Muga S, Lorenzo M, Salido M, Fumadó L, Serrano L, Cecchini L, Serrano S, Lloreta J. Concurrent TMPRSS2-ERG and SLC45A3-ERG rearrangements plus PTEN loss are not found in low grade prostate cancer and define an aggressive tumor subset. Prostate. 2016 Jun;76(9):854-65. doi: 10.1002/pros.23176

Concurrent TMPRSS2-ERG and SLC45A3-ERG rearrangements plus PTEN loss are not found in low grade prostate cancer and define an aggressive tumor subset.

BACKGROUND. *SLC45A3* is the second most common *ERG* partner in prostate cancer (PC). Coexisting *TMPRSS2* and *SLC45A3* rearrangements are found in a subset of cases, but the meaning is still unknown.

METHODS. *SLC45A3-ERG* and *TMPRSS2-ERG* rearrangements and their association with *ERG* and *PTEN* expression and with clinical and pathological features have been analyzed in 80 PrCa (PSMAR-Biobank, Barcelona, Spain). *ERG* and *PTEN* mRNA were assessed by qRT-PCR; *TMPRSS2-ERG* and *SLC45A3-ERG* by RT-PCR, FISH, and direct sequencing; and ERG expression by IHC. The endpoints were Gleason score (GS), stage, and PSA progression-free survival.

RESULTS. Single *TMPRSS2-ERG* was found in 51.6% GS ≤7 and 22.2% GS≥8 tumors (*P*=0.027). *SLC45A3-ERG* was found in 25 cases, 20 of them with concurrent *TMPRSS2-ERG* rearrangement: 11.5% GS=6, 22.2% GS=7, and 50% GS≥8 tumors (*P*=0.013). Double rearrangements were associated with higher levels of *ERG* mRNA (*P*=0.04). Double rearrangement plus *PTEN* loss was detected in 0% GS=6; 14.7% GS=7, and 29.4% GS≥8 tumors (*P*=0.032). Furthermore, this triple change was present in 19.2% stage T3-4 but not in any of stage T2 tumors (*P*=0.05). No relationship was found with PSA progression-free survival.

CONCLUSIONS. Single *TMPRSS2-ERG* translocation is associated with low grade PC. Subsequent development of *SLC45A3-ERG* results in higher *ERG* expression. The combination of double rearrangement plus *PTEN* loss, according to our series, is never found in low grade, low stage tumors. These findings could be potentially useful in therapeutic decision making in PrCa. Tumors with combined *TMPRSS2-ERG/SLC45A3-ERG* fusions plus PTEN

loss should be excluded from watchful waiting and are candidates for intensive therapy.

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DISCUSSION

PrCa is a highly heterogeneous disease, and this is obvious at clinical, histological and molecular level, both among different patients and among different tumor foci in the same patient. Consequently, the prognosis, diagnosis and management of this disease are still controversial.

Despite the huge efforts and great advances of PrCa research in recent years, there is still the need to better understand the mechanisms underlying this highly prevalent malignancy. Recently, the use of next-generation sequencing (NGS)[64] technology has open the opportunity for a much better understanding of the biology of prostatic carcinogenesis, and has refined the subclassification of tumors and the stratification of patients, bringing PrCa closer to the goal of personalized treatment.

The discovery of the high prevalence of *ETS* rearrangements in PrCa by Tomlins *et al*[69] represented a huge breakthrough in the understanding of prostatic carcinogenesis, but its significance and utility in terms of diagnosis, prognosis and treatment of patients has been the focus of intensive research, often with paradoxical results. The fact of the matter is that the complete map of prostatic carcinogenesis remains to be settled.

As extensively reviewed in the introduction of this thesis, *TMPRSS2-ERG* rearrangement is the most prevalent event in PrCa and constitutes more than 90% of the *TMPRSS2-ETS* family rearrangements. This prostate cancer specific rearrangement has been reported in a high percentage of tumors[89,93,95,103,106,108–111,184–191], although there is controversy about its association with prognostic factors[69,87,88,192].

Currently, the debate about the role of the *TMPRSS2-ERG* in the development and progression of prostate cancer and its association with prognostic factors is still ongoing [127,133,187,193,194]. Some previous studies found an association with low grade prostate tumors[128,131], whereas other authors reported an association with high GS[194]. In fact, in a previous study[132] we did not find any

association between GS and *TMPRSS2-ERG* rearrangement, detected either by FISH or IHC.

With all these previous concepts in mind, it was hypothesized that not only the presence but also the levels of the rearrangement, the isoforms, and the addition of other changes should have a decisive role in the pathogenesis of a subset of prostate tumors. Thus, in the present study (first study in this thesis) on a series of frozen and FFPE prostate tumors, we analyzed the mRNA levels of *TMPRSS2-ERG*, *ERG* and *PTEN* by quantitative RT-PCR and the ERG protein immunohistochemical expression. Our main goal was to determine if any of these parameters, alone or in combination, could be a potential molecular marker of poor prognosis and aggressive phenotype in prostate cancer.

Most of the studies have focused on detecting the presence or absence of the rearrangement using techniques such as FISH and IHC. *ERG* rearrangement is almost invariably associated with intense nuclear ERG immunostaining[193,195] and also with *ERG* mRNA overexpression. Therefore, RT-PCR and qRT-PCR techniques have been used previously for detection of *TMPRSS2-ERG* rearrangement[106,113,196,197]. In addition, some studies have shown high concordance between *TMPRSS2-ERG* fusion transcript analysis by RT-PCR and FISH[198–200], as well as between *ERG* mRNA overexpression by qRT-PCR or RT-PCR and the presence of *ERG* rearrangement[69,113,196].

The reported results from the first study presented in this thesis revealed that about 69% of the tumors expressed *TMPRSS2-ERG* and 65% overexpressed *ERG* mRNA by quantitative RT-PCR analysis. The quantitative levels of *TMPRSS2-ERG* and *ERG* were statistically related, supporting the fact that the rearrangement controls *ERG* mRNA expression. However, it has been reported that as a result of the *TMPRSS2-ERG* rearrangement, *ERG* expression might be related to the AR in prostate cancer[99–101]. Different studies have found a significant association between ERG and AR immunostaining[133,201,202]. Moreover, it has also been suggested that AR

signaling may be integrally involved in generating *TMPRSS2* and *ERG* rearrangements[203].

In our series of tumors, we analyzed the quantitative mRNA expression of *AR*, *TMPRSS2-ERG* and *ERG* genes. Whereas there was a perfect correlation between *TMPRSS2-ERG* and *ERG* levels, there was no relationship between *AR* and *TMPRSS2-ERG* or *ERG* mRNA levels, suggesting that at least in our series of prostate tumors the main regulatory mechanism that lead *ERG* mRNA overexpression could be the rearrangement itself. In this regard, some studies have reported a feed-forward mechanism where expression of endogenous *ERG* is controlled by overexpression of the *TMPRSS2-ERG* fusion product[204]. In addition, Zammarchi *et al.*[71] proposed that a positive feedback loop involving *ERG* and *TMPRSS2-ERG* could lead to androgen independence.

Important insights were elucidated when analyzing the different alterations assessed in this first study in relation to the clinical-pathological features of the tumors. In fact, there was no correlation between the presence or the absence of the rearrangement or the *ERG* mRNA overexpression (both assessed by qRT-PCR) and ISUP-GS. However, when the rearrangement was assessed quantitatively, high rearrangement levels (2+) showed a statistically trend to be associated with ISUP-GS ≥8 cases, and high *ERG* mRNA levels (2+) were statistically associated with ISUP-GS ≥8 prostate tumors. Moreover, if only positive cases were considered, high levels (2+) of both, *TMPRSS2-ERG* and *ERG* mRNA showed a clear association with ISUP-GS ≥8 prostate tumors. In addition, *ERG* mRNA overexpression and ERG positive immunostaining (P=0.05) were associated with high stage tumors. Decreased *PTEN* expression was associated with *TMPRSS2-ERG* rearrangement, *ERG* mRNA overexpression and ERG immunostaining. Furthermore, decreased *PTEN* expression was associated with ISUP-GS≥7 tumors and showed a marginally significant association with high stage.

The combinations *TMPRSS2-ERG/PTEN* loss and *ERG* overexpression/*PTEN* loss were also associated with ISUP-GS ≥7 and showed a marginally significant association with stage classification. Also concurrent ERG positive immunostaining/*PTEN* loss showed a marginally significant association with high stage tumors.

On the other hand, there was a statistical association between ERG immunostaining and PSA progression-free survival, suggesting that ERG IHC could be a potentially useful prognostic factor, a fact that deserves further studies to determine its clinical applicability. No relationship was found between either single or combined *TMPRSS2-ERG*, *ERG* mRNA overexpression or decreased *PTEN* expression and PSA progression-free survival.

In addition, the association with younger age at diagnosis suggested that cases with *TMPRSS2-ERG* fusion, *ERG* mRNA overexpression and ERG immunostaining could be earlier onset tumors, and in this regard, our results concur with those reported by Schaefer *et al* [205] and Steurer *et al* [206].

This suggested that such high levels could be useful markers of tumor aggressiveness or differentiation. In addition, the increased levels of both *TMPRSS2-ERG* and *ERG* mRNA expression in more aggressive tumors could be an indication that the tumor cell clones that are expanding could be those that harbor the rearrangement. Nevertheless, more studies are needed to clarify their role in PrCa progression and the mechanisms underlying the differences in the expression of ERG and its rearrangements.

Similar to our studies, a range of expression levels has been reported by other authors[68,69,118,196,200,207], but the role of these differences in the pathogenesis of prostate tumors remains unsettled. Petrovics *et al* [68] analyzed quantitative *ERG* and *ERG1* isoform mRNA expression in a set of matched tumor and benign prostate samples. They established a more than two-fold cut-off for overexpression. Overall *ERG* overexpression was found in more than 70% of

the tumors, and *ERG1* isoform was overexpressed in about 63%. They also classified overexpression in different categories. High (more than two-fold) *ERG1* expression levels were associated with favorable clinical-pathological features, such as longer PSA recurrence-free survival, low and intermediate ISUP-GS, or lower stage. Also, Tomlins *et al*[69] reported a high range of *ERG* overexpression in prostate tumors. In their study, *ERG* expression was higher in tumor than in normal prostate samples, but the authors did not report differences in the expression levels across the GS groups. Very recently, Svensson *et al*[200] analyzed the presence of the *TMPRSS2-ERG* rearrangement considering cases as positive when the ratio of the *TMPRSS2-ERG variant III/PSA* mRNA copies x100,000 was ≥1, but they did not assess the role of the different expression levels. Smit *et al*[196] analyzed *ERG* mRNA expression with GeneChip exon 1.0 ST arrays as well as *TMPRSS2-ERG* gene expression with qRT-PCR in a set of fresh-frozen prostate tumors. They considered cases as rearranged when normalized *TMPRSS2-ERG/HPRT* ≥10 copies, and 61% of their cases had the rearrangement. Although the presence of rearrangement was higher in castration resistant prostate cancer, the expression levels were significantly lower than in primary tumors. They did not find any correlation between percentage of tumors with rearrangement and GS, and they did not analyze the relationship between *TMPRSS2-ERG* mRNA levels and GS. Van Leenders *et al*[118] also assessed *ERG* mRNA expression through qRT-PCR, and found 68% of prostate tumors with *ERG* overexpression. They considered *ERG* mRNA overexpression when *ERG/GAPDH* normalized levels were greater than 5, but they did not analyze the relationship with clinical-pathological features. In concordance with our results, and also using qRT-PCR, Hagen *et al*[207] have recently reported that *ERG* mRNA is significantly upregulated in stage T3 cancer compared with stage T2. Moreover, high levels have been also significantly associated with seminal vesicle invasion (pT3b) and biochemical recurrence. However, they did not find any relationship with the different GS prostate tumors. In our series, we found an association between ERG immunostaining and biochemical recurrence.

With regard to the immunohistochemical expression of ERG, the results of our first study had not identified any relationship between ERG positivity or intensity and ISUP-GS; however, there was an association with high stage tumors. In addition, there was no relationship between *TMPRSS2-ERG* or *ERG* mRNA levels and the intensity of ERG immunostaining.

PTEN loss, detected either by FISH or IHC, is a well-known event in prostate cancer and it has been reported to be associated with high tumor grade, recurrence, and progression[166,208–210]. In fact, PTEN loss in GS=6 biopsies has been found to identify a subset of prostate tumors with increased probability of upgrading at radical prostatectomy[211]. In a similar way, PTEN loss and chromosome 8 alterations in Gleason pattern 3 PrCa cores have been shown to predict the presence of un-sampled pattern 4 component[212]. Several papers also suggested that Gleason pattern 3 tumors containing pattern 4 differ at the genomic level from those having only pattern 3[211–214].

ERG rearrangements and *PTEN* loss may cooperate in prostate cancer pathogenesis and progression. Previous studies have described *PTEN* deletion as a late subclonal event occurring after *ERG* gene fusion within a given established prostatic carcinoma clone[94,116,165–167,188,215]. Combined *TMPRSS2-ERG* fusion and *PTEN* loss has been found to predict early recurrences[188] and capsular penetration of prostate cancer[169]. Furthermore, Krohn *et al*[208] reported that genomic deletion of *PTEN* is associated with tumor progression and early recurrence in both *ERG* fusion-positive and fusion-negative prostate cancer. However, Leinonen *et al*[216] reported that loss of PTEN expression was associated with shorter progression-free survival only in ERG-positive cases. Also, in a study by Kim *et* al[168] it has also been shown that patients with ERG overexpression and normal PTEN had the best, and patients with ERG negativity and PTEN loss the worst biochemical recurrence-free survival.

In keeping with the previous literature cited above, we found loss of *PTEN* expression to be more common in prostate tumors with ISUP-GS $\geq$7 and marginally associated with high stage (T3–T4) tumors.

In our study, we also found a very good correlation between presence of the rearrangement and *PTEN* loss at mRNA level. Decreased *PTEN* expression was more often present in tumors with the *TMPRSS2-ERG* rearrangement, *ERG* mRNA overexpression, and ERG protein expression. Moreover, concurrent *TMPRSS2-ERG* rearrangement or *ERG* overexpression and *PTEN* loss were also associated with ISUP-GS ≥7 prostate tumors. These combinations, and also ERG positive immunostaining/*PTEN* loss showed a statistical trend to be associated with high stage tumors, indicating that *PTEN* loss could be a molecular marker of prostate tumor progression, particularly in cases with *TMPRSS2-ERG* rearrangement.

To the best of our knowledge, this was the first study to show an association between high levels of *TMPRSS2-ERG* and *ERG* and high grade (ISUP-GS ≥8) prostate tumors. In addition, it also showed that *ERG* overexpression at mRNA level and also its immunohistochemical detection were associated with high stage tumors. Moreover, ERG immunostaining was associated with PSA progression-free survival, suggesting that ERG IHC could be a potentially useful prognostic factor, a fact that deserves further studies to determine its clinical applicability. Loss of *PTEN* expression, either alone or combined with *TMPRSS2-ERG* rearrangement or *ERG* mRNA overexpression, were associated with intermediate or high grade prostate tumors (ISUP-GS ≥7), and showed also a trend to association with high stage. Although more studies are needed to further clarify their role, our findings emphasized that the expression levels of the *TMPRSS2-ERG* fusion, *ERG* mRNA, and ERG protein, rather than their mere presence, were related to a more aggressive phenotype, had an effect on prognosis and could be molecular markers of progression for prostate cancer.

Our next objective was to study the role of the second most common *ERG* rearrangement in PrCa: *SLC45A3-ERG*. There are few papers dealing with the role of *SLC45A3-ERG* rearrangement in PrCa[95,137,140,217,218]. The coexistence of *TMPRSS2-ERG* and *SLC45A3-ERG* rearrangements has

been found in a subset of prostate tumors[95,140] and it has been shown that multiple simultaneous rearrangements can occur within the same tumor foci[95,140,219], but the meaning of this finding is still unknown. To the best of our knowledge, our paper was the first to report the association between single *TMPRSS2-ERG* and low GS, and between double rearrangement and high GS. In addition, we reported for the first time an association between double rearrangements and higher levels of *ERG* mRNA.

Concretely, in the second study presented in this thesis, we analyzed the mRNA expression of *TMPRSS2-ERG* and *SLC45A3-ERG* rearrangements and their relationship with the quantitative mRNA levels of *ERG* and *PTEN*, as well as with ERG protein IHC, in a series of prostate tumors. Our goal was to determine the distribution of single and concurrent *TMPRSS2-ERG* and *SLC45A3-ERG*, the relationship of the *SLC45A3-ERG* rearrangement with ERG expression, their association with *PTEN* loss and with the clinical-pathological features of the respective prostate tumors. To confirm the *ERG* rearrangements detected by RT-PCR, FISH was also performed in a subset of cases.

The proportion of *SLC45A3-ERG* rearrangements in our series was higher than previously reported[95,140]. The frequency of concurrent *SLC45A3-ERG* and *TMPRSS2-ERG* rearrangements was also higher. In fact, the two previous papers dealing with the prevalence of *TMPRSS2-ERG* and *SLC45A3-ERG* in the same subset of tumors[95,140], reported a lower proportion of double rearrangements. This could be explained by their lower proportion of GS $\geq$ 8 and perhaps also by the fact that they performed FISH in TMA sections only and this could also lead to a reduced detection rate. Interestingly, in keeping with one of these series[95], we also found a much lower proportion of isolated *SLC45A3-ERG*, than of the concurrent *SLC45A3* and *TMPRSS2* rearrangements.

The high concordance between *TMPRSS2-ERG* rearrangement and *ERG* mRNA overexpression observed in our first study was also confirmed in

this second paper: *TMPRSS2-ERG* rearrangement was associated with *ERG* mRNA overexpression in 91% of the cases. As discussed above, different techniques have been used to study *ERG* rearrangements in PrCa, including FISH, RT-PCR, qRT-PCR and IHC, among others. Overall, taking into account the results from both papers presented in this thesis, it is remarkable mentioning that in our first study we used qRT-PCR to assess the *TMPRSS2-ERG* rearrangement levels whereas in our second paper, the rearrangement status was analyzed by RT-PCR. We found a very good concordance between these two techniques, as 68.7% of the cases harbored *TMPRSS2-ERG* rearrangement by qRT-PCR while 70% had this rearrangement when using RT-PCR.

The rearrangement analyses in this second study showed that single *TMPRSS2-ERG* rearrangement was strongly associated with *ERG* mRNA overexpression, reaffirming our previous observations. *SLC45A3-ERG* rearrangement alone was a minor event with only five cases and our results suggested that it was not associated with *ERG* transcription upregulation nor with ERG protein expression, because only one of the five cases showed *ERG* mRNA overexpression and positive immunostaining, and the other four cases were negative for both. However, although *SLC45A3-ERG* by itself did not result in *ERG* overexpression, there was an obvious statistical association between double rearrangements and higher levels of *ERG* mRNA. From our results and the current literature, it is not known whether the second rearrangement has a causal role in ERG overexpression or if it is rather the consequence of the increase in ERG protein. More studies are needed to clarify this association.

The controversy about the role of *TMPRSS2-ERG* in the development and progression of PrCa has been addressed in detail in the introduction of this thesis[117,127,133,187,194,220]. As discussed, previous papers have reported an association with low grade prostate tumors[128,131,221] while other studies support an association with high grade[194]. In a previous study analyzing

the *TMPRSS2-ERG* rearrangement by FISH[132] we did not find any association between the rearrangement and Gleason grade. But, as it was previously reported in our first study, the expression levels of *TMPRSS2-ERG* and *ERG* mRNA were related to more aggressive tumors[222].

In this second study, we found a statistical association between single *TMPRSS2-ERG* and GS <7 tumors. Double rearrangements were also associated with GS ≥ 8 tumors. The low incidence of *SLC45A3-ERG* fusion in GS = 6 tumors and its increase in the GS ≥ 7 cases could suggest that this superimposed fusion would be directly or indirectly related with the transition from Gleason pattern 3 to 4. Interestingly, *SLC45A3-ERG* is more likely to appear as a second fusion in cases already harboring the *TMPRSS2-ERG* fusion. Thus, according to our results, when a double fusion is found in a biopsy of an ERG+ GS = 6 tumor, it would suggest that either there is a higher probability of transition to GS = 7 or that pattern 4 is already present in other areas of the tumor. Other molecular mechanisms may be involved in the progression of a *TMPRSS2-ERG* positive case from GS = 6 to GS = 7 [94,116,122,165,167], and further research is needed to support the role of double fusions in this process, including a prospective study of GS = 6 biopsies to assess the probability of finding GS = 7 in the respective radical prostatectomy.

Regarding the *PTEN* alterations in our series, the coexistence of *TMPRSS2-ERG* rearrangement, either single or combined, and *PTEN* loss was associated with GS≥7 tumors. We did not find any case of single *SLC45A3-ERG* rearrangement associated with *PTEN* loss: all the cases harbored also the *TMPRSS2-ERG* fusion. The prevalence of the double rearranged cases showed a trend to be associated with the high grade tumors in the *PTEN* loss group, whereas in the *PTEN wt* cases the prevalence of *TMPRSS2-ERG* plus *SLC45A3-ERG* was similar in the different GS groups. However, our study did not investigate the mechanistic effect of *PTEN* loss on the association of single or double rearrangements with Gleason grade and with other prognostic

features of prostate cancer. Thus, we cannot know if *PTEN* loss drives the association of the double rearrangement with the most aggressive prostate tumors. Our feeling is that double fusions regardless of *PTEN* loss, may have by themselves an effect on grade and prognosis. In favor of this concept is the fact that isolated *PTEN* loss is a rare event, much less prevalent in our series than the double rearrangement.

A remarkable finding in our relatively short series was that the presence of *TMPRSS2-ERG* and *SLC45A3-ERG* rearrangements together with loss of *PTEN* expression, referred to as "triple hit," was not found in any GS = 6 or T2 stage tumor. This triple hit was statistically associated with higher Gleason grade and with T3-4 stage. Again, this could be used as an exclusion criterion in needle biopsy cases showing GS = 6 foci only: finding the triple hit in such cases would probably mean that these foci are Gleason pattern 3 areas in a case already having pattern 4. Similarly, the triple hit would be strongly suggestive of high stage. Thus, assessment of these three molecular changes could impact prognosis and therapeutic decision making in PrCa. This hypothesis was later tested in a prospective study detailed below.

In summary, the results of our second study suggested that *TMPRSS2-ERG*, when present as the only rearrangement, was associated with low grade PrCa, and developing *SLC45A3-ERG* as a second rearrangement, as well as *PTEN* loss would mark the transition to higher grade and stage. In addition, the higher *ERG* expression levels in cases with double rearrangement suggested that the *SLC45A3-ERG* rearrangement could contribute to or be the consequence of increased *ERG* expression. The coexistence of *TMPRSS2-ERG*, *SLC45A3-ERG* plus *PTEN* loss was not found in low grade or low stage PrCa and this could have potential impact in patient management.

Recent results from our group[224] have confirmed the role of the triple hit on the progression of PrCa (paper included in the annex of this thesis). In this study, we aimed to corroborate some of our previous results presented in this thesis, where we identified an aggressive tumor subset characterized by the

triple hit at the level of mRNA expression, which was strongly associated with higher GS and T3-4 stage. In keeping with our results, the triple hit could have a huge impact in the prognosis and therapeutic decisions in PrCa.

Thus, to validate our observations, a large independent cohort of 220 PrCa was selected from PSMAR-Biobank, Barcelona, Spain, and the end points were clinical-pathological variables and PSA progression-free survival.

IHC results, in concordance with previous literature, showed that 46.8% of the cases overexpressed ERG whereas 30% and 34% of the tumors had loss of SLC45A3 and PTEN expression, respectively. Both loss of prostein and PTEN were associated with higher GS, in concordance with previous studies[223,225,214,212].

As mentioned above, it is well established that different *ERG* rearrangements and loss of *PTEN* are frequent events in PrCa and can co-occur in the same tumor, most likely leading to PrCa progression. In this regard, a very recent paper[226] found that *TMPRSS2-ERG* rearrangement and PTEN loss are frequently found in association with heterogeneous loss of DNA repair factors and this has been suggested to reveal an unconventional DNA damage checkpoint regulation mechanism in prostate carcinogenesis that could have applications in new targeted therapy strategies in this tumor.

When analyzing the relationship between protein expression of the three genes involved in the triple hit, we found that loss of PTEN expression was statistically associated with ERG-positive PrCa and this was in concordance with previous studies[94,116,123,188,225,227,228]. On the other hand, decreased SLC45A3 expression, usually related to rearrangement, was statistically associated with ERG expression (indicative of *ERG* rearrangement).

Overall, our results showed that the increasing number of aberrant events and the triple hit were strongly associated with shorter PSA progression-free survival.

Indeed, GS=6 cases were associated with single ERG positive IHC (probably due to *ERG* rearrangement), which in turn showed an association with tumor progression. None of the GS=6 tumors harbored the triple hit, reaffirming its potential use as a prognostic marker. These findings support our hypothesis that the "triple hit" phenotype could be used as an indicator that cases with GS = 6/GG1 foci in needle biopsy harboring this triple change would be under-sampled cases, and that these cases would most likely be upgraded to at least GS = 7 or GG2/GG3 at radical prostatectomy. This fact could have an impact on patient management, as it could be used to select more precisely the optimal candidates for active surveillance.

Regarding GS=7 tumors, an association with double ERG overexpression/PTEN loss alterations was found.

Different combinations defined GS $\geq$ 8 tumors, depending on presence or absence of Gleason pattern 3. Concretely, tumors with this component were characterized by the triple hit, ERG overexpression and PTEN loss, whereas GS $\geq$ 8 tumors with no Gleason pattern 3 were characterized by single loss of SLC45A3. These findings seem to support the existence of two discrete pathways of prostate carcinogenesis: ERG positive cancer that progresses from pattern 3 towards pattern 5, and ERG negative cancer that more often arises as a *"de novo"* high grade tumor. PTEN and SLC45A3 alterations would be highly prevalent additional molecular changes associated with progression in ERG positive, but not in ERG negative cases.

In keeping with previous studies[94,114,219,229], our findings support the concept of *ERG* rearrangement being an early event in prostate carcinogenesis. Thus, the results of the present study seem to indicate that, in typical ERG positive low grade cases, *ERG* rearrangement would be the single main molecular event. PTEN loss would mark the transition to GS = 7 (GG2/GG3), i.e. the appearance of pattern 4, and loss of SLC45A3 expression would further determine progression towards highest grades (GS $\geq$ 8; GG4/GG5).

Keeping in line with the results from the second paper presented in this thesis, it is becoming clear that "triple hit" IHC phenotype has enormous potential as an exclusion criterion in needle biopsy cases containing GS = 6 foci only. Finding the "triple hit" in such cases would probably mean that these foci are Gleason pattern 3 areas in a case already having unsampled pattern 4 and could impact prognosis and therapeutic decision making in PrCa.

Perner *et al.*[218] hypothesized that SLC45A3 protein is a marker of prostatic differentiation, and hence SLC45A3 protein loss would be an independent sign of dedifferentiation of PrCa. According to our results, loss of SLC45A3 expression as part of the triple hit is associated with GS $\geq$ 8 containing pattern 3 areas, but when it is the only altered gene (with normal ERG and PTEN) it is independently associated with the opposite situation, i.e. GS $\geq$ 8 foci devoid of pattern 3 component. This could indicate that high GS prostate tumors without *ERG* rearrangement could evolve from an *ERG* independent pathway.

These results prove that ERG overexpression defines a distinct pathway of PrCa, and PTEN and SLC45A3 alterations add relevant prognostic information in the context of ERG aberrant expression. The triple hit clearly defines an aggressive PrCa phenotype that can be used to improve stratification, treatment and follow-up.

CONCLUSIONS

1. *TMPRSS2-ERG* is a very prevalent rearrangement in PrCa.

2. High *TMPRSS2-ERG* and *ERG* mRNA expression levels are related to a more aggressive phenotype in PrCa.

3. The outcome of the patients is influenced by both *TMPRSS2-ERG* and *ERG* alterations.

4. *TMPRSS2-ERG* and *ERG* alterations could potentially be useful markers of PC progression.

5. Single *TMPRSS2-ERG* is associated with low grade PrCa.

6. Subsequent development of *SLC45A3-ERG* results in higher *ERG* expression.

7. The triple hit, i.e. combination of *TMPRSS2-ERG* plus *SLC45A3-ERG* plus *PTEN* loss is not found in low grade nor in low stage tumors. This combination is associated with cases having Gleason pattern 4 and T3-4 stage.

8. Thus, the triple hit could be potentially useful in therapeutic decision making in PC.

9. Tumors with combined *TMPRSS2-ERG/SLC45A3-ERG* rearrangements plus *PTEN* loss should be excluded from watchful waiting and are candidates for intense therapy.

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ANNEX

Supplementary Table I – Font-Tello A. *et al,* Prostate, 2016.

Relationship of *TMPRSS2-ERG, ERG, PTEN* mRNA levels and ERG immunostaining with clinical-pathological features

Relationship of *TMPRSS2-ERG, ERG, PTEN* mRNA levels and ERG immunostaining with Gleason Score						
Type of alteration	**% GS = 6 Tumors**	**% GS = 7 Tumors**	**% GS ≤ 7 Tumors**	**% GS ≥ 7 Tumors**	**% GS ≥ 8 Tumors**	**P-value**
TMPRSS2-ERG (3-tier)	**63**	**72,2**	-	-	**70**	0.727 (Pearson Chi-Square)
High *TMPRSS2-ERG* (2+) (3-tier)	**22,2**	**27,8**	-	-	**50**	0.222 (Pearson Chi-Square)
High *TMPRSS2-ERG* (2+) (2-tier)	-	-	**25,4**	-	**50**	**0,079 (Pearson Chi-Square)**
Only *TMPRSS2-ERG* (2+) (2-tier)	-	-	**37,2**	-	**71,4**	**0.025 (Pearson Chi-Square)**
ERG overexpression (3-tier)	**59,3**	**69,4**	-	-	**65**	0.703 (Pearson Chi-Square)
High *ERG* overexpression (2+) (3-tier)	**33,3**	**38,9**	-	-	**60**	0.184 (Pearson Chi-Square)
High *ERG* overexpression (2+) (2-tier)	-	-	**36,5**	-	**60**	**0.047 (Fisher's Exact test)**
Only *ERG* overexpression (2+) (2-tier)	-	-	**56,1**	-	**92,3**	**0.02 (Fisher's Exact test)**
PTEN loss (3-tier)	**20**	**47**	-	-	**44,4**	0.123 (Pearson Chi-Square)
PTEN loss (2-tier)	**20**	-	-	**46,2**	-	**0,041 (Pearson Chi-Square)**
TMPRSS2-ERG/PTEN loss *(3-tier)*	**15**	**41,2**	-	-	**39**	0.121 (Pearson Chi-Square)
TMPRSS2-ERG/*PTEN* loss (2-tier)	**15**	-	-	**40,4**	-	**0.04 (Pearson Chi-Square)**
ERG overexpression*/PTEN* loss *(3-tier)*	**15**	**41,2**	-	-	**39**	0.121 (Pearson Chi-Square)
ERG overexpression/*PTEN* loss (2-tier)	**15**	-	-	**40,4**	-	**0.04 (Pearson Chi-Square)**
ERG positive IHC	**64,0**	**68,6**	-	-	**61,1**	0.850 (Pearson Chi-Square)
ERG high levels (2+) IHC	**43,7**	**58,3**			**63,6**	0.533 (Pearson Chi-Square)
ERG positive IHC/*PTEN* loss	**20,0**	**39,4**	-	-	**40**	0.298 (Pearson Chi-Square)
ERG positive IHC/*PTEN* loss	**20**	-	-	**39,6**	-	0.119 (Pearson Chi-Square)

Supplementary Table I (continued)– Font-Tello A. *et al,* Prostate, 2016.

Relationship of *TMPRSS2-ERG, ERG, PTEN* mRNA levels and ERG immunostaining with tumor stage			
Type of alteration	**% Stage T2**	**% Stage T3-T4**	**P-value**
TMPRSS2-ERG	**60**	**72,4**	0.263 (Pearson Chi-Square)
High *TMPRSS2-ERG* (2+)	**28**	**32,7**	0.667 (Pearson Chi-Square)
ERG overexpression	**48**	**72,4**	**0.032 (Pearson Chi-Square)**
High *ERG* overexpression (2+)	**28**	**48,3**	0.086 (Pearson Chi-Square)
PTEN loss	**22,2**	**44,4**	0.093 (Pearson Chi-Square)
TMPRSS2-ERG/PTEN loss	**16,6**	**39**	**0.083 (Pearson Chi-Square)**
ERG overexpression/*PTEN* loss	**16,6**	**39**	**0.083 (Pearson Chi-Square)**
ERG positive IHC	**50**	**72,2**	**0.05 (Pearson Chi-Square)**
ERG high levels (2+) IHC	**25**	**40,7**	0.181 (Pearson Chi-Square)
ERG positive IHC/*PTEN* loss	**16.6**	**40**	**0.072 (Pearson Chi-Square)**

ERG overexpression plus SLC45A3 (prostein) and PTEN expression loss: Strong association of the triple hit phenotype with an aggressive pathway of prostate cancer progression

TMPRSS2 and *SLC45A3* rearrangements may coexist in the same tumor. *ERG* rearrangements and *PTEN* loss are concomitant events in prostate cancer (PrCa), and can cooperate in progression. We have reported that mRNA expression of *TMPRSS2-ERG* and *SLC45A3-ERG* rearrangements plus *PTEN* loss define an aggressive tumor subset. The aim of this study has been to validate these results by immunohistochemistry in a large cohort of tumors. ERG, SLC45A3 and PTEN immunostaining and their association with pathological features and PSA progression-free survival were analyzed in 220 PrCa (PSMAR-Biobank, Barcelona, Spain). ERG protein expression was found in 46.8% and SLC45A3 and PTEN loss in 30% and 34% tumors, respectively. Single ERG positive immunostaining was associated with GS = 6 tumors (p = 0.016), double ERG+/PTEN loss with GS = 7 (p = 0.008) and Grade Group 2 (GG) or GG3 cases (p = 0.042), ERG+/SLC45A3 loss/PTEN loss ("triple hit") with GS ≥ 8 (p < 0.0001) and GG4 or GG5 tumors (p = 0.0003). None of GS = 6 nor = GG1 cases showed this combination. In the GS ≥ 8 group, ERG+ (p = 0.002), PTEN loss (p = 0.009) and "triple hit" (p = 0.003) were associated with Gleason pattern 3 component, and single SLC45A3 loss (p = 0.036) with GS ≥ 8 without pattern 3. The number of aberrant events and the triple hit were strongly associated with shorter PSA progression-free survival. In GS = 6 PrCa, single ERG+ was also associated with progression. ERG+ identifies a distinct pathway of PrCa. Additional assessment of PTEN and SLC45A3 adds relevant prognostic information. The triple hit phenotype (ERG+/SLC45A3 loss/PTEN loss) is associated with progression and could be used for patient stratification, treatment and follow-up.

Hernández-Llodrà S, Juanpere N, de Muga S, Lorenzo M, Gil J, Font-Tello A, Agell L, Albero-González R, Segalés L, Merino J, Serrano L, Fumadó L, Cecchini L, Lloreta-Trull J. ERG overexpression plus SLC45A3 (prostein) and PTEN expression loss: Strong association of the triple hit phenotype with an aggressive pathway of prostate cancer progression. Oncotarget. 2017 May 26;8(43):74106-74118. doi:10.18632/oncotarget.18266

Abbreviations

5’UTR	5’untranslated region
AD	alternative domain
ADT	androgen deprivation therapy
APC	adenomatosis polyposis coli tumor supresor
AR	androgen receptor
AREs	androgen responsive elements
ATM	ataxia telangiectasia mutated
BPH	bening prostatic hyperplasia
BRAF	v-raf murine sarcoma viral oncogene homolog B1
BRCA1	breast cancer 1
BRCA2	breast cancer 2
BRD4	bromodomain-containing protein 4
CaPr	càncer de pròstata
CDH1	E-cadherin
CDK12	cyclin dependent kinase 12
CNV	copy-number variation
CTNNB1	β-catenin
CZ	central zone
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DRE	digital rectal examination
EMT	epithelial-to-mesenchymal transition
EP300	E1A binding protein p300
ERG	v-ETS avian erythroblastosis virus E26 oncogene related
ERP	ERG responsive proteome
ERSPC	European Randomized Study of Screening for Prostate Cancer
ETS	erythroblast transformation-specific
ETV1	ETS avian erythroblastosis virus E26 variant 1
EWS	Ewing sarcoma breakpoint region 1

EZH2	enhancer of zeste homolog 2
FDZ4	frizzled class receptor 4
FOXA1	forkhead box A1
FOXO1	forkhead box O1
FOXO3	forkhead box O3
FOXP1	forkhead box P1
FZ	fibro-muscular zone
GS	Gleason Score
HDACs	histone deacetylases, class I
HGPIN	high-grade prostatic intraepithelial neoplasia
hk2	human kallikrein 2
HOXB13	homeobox B13
IHC	immunohistochemistry
ISUP	International Society of Urological Pathology
KLK	kallikrein
KLK3	kallikrein Related Serine Peptidase 3
LGPIN	low grade prostatic intraepithelial neoplasia
M	metastasis
MAPK	mitogen-activated protein kinase
MAPK1/ERK2	mitogen-activated protein kinase 1
mCRPC	metastatic castration resistant prostate cancer
MRI	magnetic resonance imaging
myc	myc protooncogene
N	node
NCOA2	nuclear receptor co-activator 2
NCOR2	nuclear receptor co-repressor 2
NDRG1	N-myc downstream regulated 1
NGS	next-generation sequencing
PI3K	phosphoinositide-3-kinase
PIA	proliferative inflammatory atrophy

PIN	prostatic intraepithelial neoplasia
PIP2	phosphorylation of phosphatidylinositol (4,5) bisphosphate
PIP3	phosphatidylinositol (3,4,5) trisphosphate
PLCO	prostate, Lung, Colorectal and Ovarian
PNT	pointed
PRC2	polycomb repressive complex 2
PrCa	prostate cancer
PSA	prostate-specific antigen
PTEN	phosphatase and tensin homolog
PZ	peripheral zone
RB1	retinoblastoma
SLC45A3	solute carrier family 45 member 3
SPOP	speckle-type POZ protein
T	tumor
TAD	transactivational domain
TCGA	The Cancer Genome Atlas
TKR	tyrosine kinase receptor
TMPRSS2	transmembrane protease, serine 2
TNM	tumor-node-metastasis
TP53	tumor protein p53
TRUS	transrectal ultrasonography
TURP	transurethral resection of the prostate
TZ	transition zone
USPSTF	States Preventive Services Task Force
VIM	vimentin
WGS	whole genome sequencing
WHO	World Health Organization
Wnt	wingless-related integration site