

Decoding the heterogeneous landscape in the development prostate cancer (Review)

YENIFER YAMILE SEGURA-MORENO^{1,2}, MARÍA CAROLINA SANABRIA-SALAS¹,
RODOLFO VARELA^{3,4}, JORGE ANDRÉS MESA⁵ and MARTHA LUCIA SERRANO^{1,2}

¹Cancer Biology Research Group, National Institute of Cancerology, Bogota 110411;

²Department of Chemistry, Faculty of Sciences, National University of Colombia, University City, Bogota 111321;

³Department of Urology, National Institute of Cancerology, Bogota 110411;

⁴Department of Urology, National University of Colombia, University City, Bogota 111321;

⁵Department of Pathology, National Institute of Cancerology, Bogota 110411, Colombia

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Abstract. Prostate cancer (PCa) is characterized as being histologically and molecularly heterogeneous; however, this is not only incorrect among individuals, but also at the multiple foci level, which originates in the prostate gland itself. The reasons for such heterogeneity have not been fully elucidated; however, understanding these may be crucial in determining the course of the disease. PCa is characterized by a complex network of chromosomal rearrangements, which simultaneously deregulate multiple genes; this could explain the appearance of exclusive events associated with molecular subtypes, which have been extensively investigated to establish clinical management and the development of therapies targeted to this type of cancer. From a clinical aspect, the prognosis of the patient has focused on the characteristics of the index lesion (the largest focus in PCa); however, a significant percentage of patients (11%) also exhibit an aggressive secondary foci, which may determine the prognosis of the disease, and could be the determining factor of why, in different studies, the classification of the subtypes does not have an association with prognosis. Due to the aforementioned reasons, the analysis of molecular subtypes in several foci, from the same individual could assist in determining the association between clinical evolution and management of patients with PCa. Castration-resistant PCa (CRPC) has the worst prognosis and develops following androgen ablation therapy. Currently, there are two models to explain the development of CRPC: i) The selection model and ii) the adaptation model; both of which, have been found to

include alterations described in the molecular subtypes, such as Enhancer of zeste 2 polycomb repressive complex 2 subunit overexpression, isocitrate dehydrogenase (NAPD⁺)1 and forkhead box A1 mutations, suggesting that the presence of specific molecular alterations could predict the development of CRPC. This type of analysis could lead to a biological understanding of PCa, to develop personalized medicine strategies, which could improve the response to treatment thus, avoiding the development of resistance. Therefore, the present review discusses the primary molecular factors, to which variable heterogeneity in PCa progress has been attributed.

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1. Introduction

According to the report by the International Agency for Research on Cancer of 2018 (1), prostate cancer (PCa) has the second highest estimated age-standardized incidence rate worldwide (29.3 per 100,000) and is the sixth cause of cancer-associated death in men (7.6 per 100,000). The problem with the management of PCa is due to the difficulty in stratifying between indolent and aggressive cases. Although <5% of patients exhibit advanced disease, up to 40% of patients will eventually develop metastatic disease despite local therapy (2,3). Other patients with PCa undergo hormonal therapy treatment, radical prostatectomy (RP) or radiotherapy, so, numerous cases of PCa only require expectant management; thus, in these patients, an overtreatment may result in significant morbidity.

High Grade Prostatic Intraepithelial Neoplasia (HGPIN) is a prostate preneoplastic lesion, which may develop towards an invasive PCa (25 to 30%) during a process, which may take over 10 years (4), according to a study conducted at

Correspondence to: Dr Martha Lucia Serrano, Cancer Biology Research Group, National Institute of Cancerology, Calle 1, 9-85, Bogota 110411, Colombia
E-mail: mlserranol@unal.edu.co

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Johns Hopkins University School of Medicine, in patients of Baltimore (Maryland, United States of America). Both, HGPIN and PCa are multifocal, and HGPIN foci coexist in adjacent areas of PCa sharing chromosome deletions and interstitial translocations that originate the TMPRSS2-ERG fusion gene and genetic alterations, such as hypermethylation of the π -class glutathione S-transferase (GSTP1) promoter, which suggests a common origin (5). The heterogeneous and multifocality nature of the disease makes it difficult to understand prostate carcinogenesis (6). Currently, there is no adequate method to differentiate patients with poor prognosis of PCa from those with indolent disease, who should only have a controlled follow-up. The primary method of determining the suitable treatment option for a patient with PCa is based on the Gleason classification (5), an assessment of its morphological heterogeneity, which is associated with prognosis. Pathologists can classify each focus of PCa using Gleason patterns (GP) ranging from 1 to 5, and assigning a Gleason score (GS); or using the updated Gleason grade group, which includes the two most representative GPs in the tumor (7,8). Despite the association between Gleason classification and tumor behavior (the degree of differentiation of the neoplastic cells) the association between morphological heterogeneity and molecular heterogeneity has not been elucidated (9).

Molecular studies of PCa have revealed numerous recurrent DNA alterations associated with deregulating genes involved in the development of the prostate, such as the deletions and interstitial translocations that originate the TMPRSS2-ERG fusion gene, chromatin modification, cell cycle regulation and androgen signaling (10,11). Over the last decade (12), the investigation into PCa has focused on identifying the exclusive molecular events in the development of PCa, which could represent early and divergent events and could direct the course of the disease (13); thus, it is crucial to elucidate the carcinogenesis of PCa and utilize the information in the treatment of patients.

The present review will describe an updated review of intratumoral heterogeneity in multifocal PCa, to understand the carcinogenic process and its implication in the management of the disease; as the vast majority of molecular studies in PCa performed are single focused, and do not take into account molecular heterogeneity, which could contribute to limiting the use of molecular subtypes in the prognosis and treatment of the disease.

2. Molecular heterogeneity of PCa

The origin of PCa could be defined by the occurrence of chromosomal rearrangements that simultaneously, and in a coordinated manner, cause the inactivation of tumor suppressor genes (TSG) and the creation of oncogenic fusions, which would support a model of punctuated development in PCa (11,14). This could in turn be associated with the appearance of canonical alterations, and with the molecular subtypes involved in a broad genomic and transcriptomic diversity within and among intraprostatic PCa foci. Several studies have shown potential for their utilization as prognostic biomarker signatures (15-17). Recently published data from The Cancer Genome Atlas (TCGA) (17) supports the division of the major molecular subclasses of localized PCa into

erythroblast transformation specific (ETS)-rearrangement PCa [PCa with rearrangements and overexpression of ETS transcription factor ERG (ERG), ETS variant transcription factor (ETV)-1, -4, or other ETS family transcription factors], SPOP-mutated and CHD1-deleted [speckle type BTB/POZ protein (SPOP)/chromodomain helicase DNA binding protein 1] altered cancers (17), and several smaller categories, such as FOXA1 or IDH1 deletion, which have been described in Table I. The use of molecular classifiers to personalize treatment shows promise; however, it is still in its infancy and additional validation and optimization are required to ensure it can be used in a clinical setting.

Multiple complex chromosomal rearrangement, as a cause of molecular alterations in PCa. There are several molecular alterations in the PCa, such as copy number changes, gene fusions, single nucleotide mutations and polymorphisms, methylation, microRNAs and long non-coding RNAs, one of the most characteristic involves multiple complex chromosomal rearrangement processes (Fig. 1A), which has been reported in 63% of PCa cases (18). These rearrangements can be classified as chromothripsis or chromoplexy (Fig. 1B and C), and some coordinated structured rearrangements may have intermediate chromothripsis and chromoplexy properties (11), for instance both chromothripsis and chromoplexy display random breakage and fusion of genomic segments with low copy numbers, most likely mediated by non-homologous end-joining.

In addition, Dzamba *et al* (18) used the CouGaR statistical method, in 63% of PCa, and in 27% of bladder cancers, which is in contrast with other types of cancer such as rectal, breast and thyroid cancer, where these types of alterations have not been identified. The CouGaR method is a novel method for predicting the overall genomic configuration resulting from characterizing the genomic structure of amplified complex genomic rearrangements, leveraging both depth of coverage (DOC) and discordant pair-end mapping techniques, to identify multiple chromosomal rearrangements. The results of Dzamba *et al* are noteworthy, as it has been reported that bladder and PCa may both develop in the same patient (19).

It has been found that the breaking points of DNA rearrangements are more likely to occur near specific DNA sequences, where the androgen receptor (AR) binds as a transcription factor, known as androgen response elements (ARE), compared with that in other randomly predicted locations, that is anywhere else in the genome (20,21). This finding suggests that AR-ARE complexes may be predisposed to genomic rearrangements through transcriptional stress, since androgen signaling promotes co-recruitment of androgen receptor and topoisomerase II β (TOP2 β) to sites of TMPRSS2-ERG genomic breakpoints, triggering recombinogenic TOP2 β -mediated DNA double-strand breaks. For example, it has been found that transmembrane serine protease 2 (TMPRSS2)-ERG fusion is induced by the interaction of androgens with AR (14,22).

In the development of PCa, chromothripsis is relatively rare and occurs as one clonal early event; in contrast, chromoplexy is a common and sequential event, which is detected at clonal or sub-clonal level (14,18,23). In a study performed using 57 patients with PCa, Baca *et al* (11) identified over 5,000

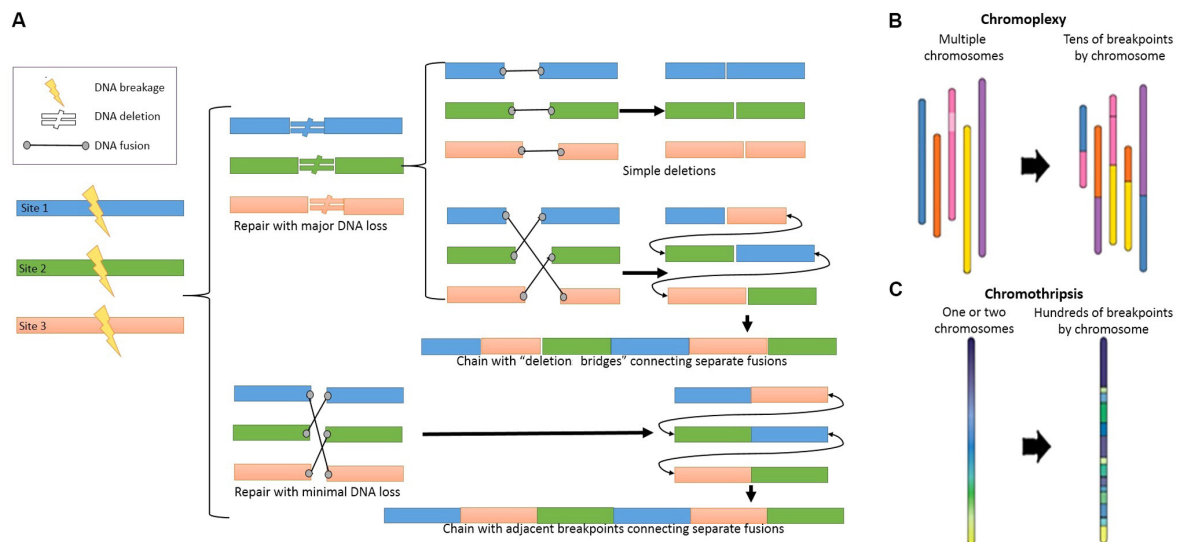


Figure 1. Multiple chromosomal rearrangements in prostate cancer. (A) Mechanisms for the formation of multiple chromosomal rearrangements. DNA ds breaks can be repaired by DNA- breakage, deletion or fusion. Loss of DNA at sites of ds breaks may result in simple deletions (top) or 'deletion bridges' (middle) which span breakpoints from distinct fusions. Adjacent breakpoints or deletion bridges may provide evidence for multiple rearrangements. Concerted repair with minimal loss of DNA results in fusion breakpoints which map to adjacent positions (indicated by the punctuated arrows). (B) Chromoplexy. Multiple breakpoints, including several chromosomes (C) Chromothripsis. Hundreds of breaking points grouped in one or two chromosomes. DS, double strand.

somatic rearrangements associated with this type of chromosomal rearrangements. Several cancer genes were repeatedly deleted or rearranged by chromoplexy, including PTEN, NK3 homeobox 1, cyclin dependent kinase inhibitor 1B, tumor protein p53 (TP53), and RB transcriptional corepressor 1. These multiple complex chromosomal rearrangements are one of the primary reasons for the high molecular heterogeneity in PCa.

Molecular subtypes in PCa. Over the last decade, several studies have focused on determining the excluding molecular events in the development of PCa, which are able to establish different subtypes, and are associated with the prognosis of the disease, and have the potential to be developed into targeted therapies (11,16,17,24). Therefore, the current status of PCa subtypes, will be subsequently discussed, using TCGA study (17) as a reference for the majority of the comparisons, the prognostic involvement, and the therapies of inhibitors of target oncogenes associated with subtypes, such as EZH2 or ERG (Table I), which have been used or are currently in the experimental phase, in this way some treatments that apply to cases that are ETS(+) will not work for those that are ETS(-), such as blocking function of ERG regulating co-factors, such as PARP1 (25-27) (Table I). Table I summarizes the main clinical-pathological characteristics of the subtypes and treatments that have been investigated as a development of personalized medicine (13,17,24-26,28-63).

ETS(+) subtypes. The ETS family of transcription factors consists of phosphorylated proteins with DNA-binding domains (ETS domain) that act as either activators or repressors of transcription. The family consists of 30 identified genes, 28 of which are found in the human genome. Previous studies have found that between 50 and 70% of patients with PCa overexpress ETS, following gene fusion, which causes ETS to be controlled by ARE (12). Due to the prevalence of these

types of rearrangements, efforts to molecularly characterize PCa begin by separating the cases which have gene fusion from those that do not, termed ETS(+) and ETS(-), respectively in numerous studies (20,38,64). TCGA study (17) has characterized four different ETS(+) subtypes, depending on the type of ETS involved in the fusion: ERG, ETV1, ETV4 and Fli-1 proto-oncogene, ETS transcription factor ERG is the most frequently overexpressed ETS. TMPRSS2 the most frequent fusion gene in all ETS fusions; however, fusions with other androgen-regulated genes have also been described, including solute carrier family 45 member 3 and N-myc downstream regulated 1 (65).

The TMPRSS2-ERG gene fusion causes ERG overexpression, which initiates a cascade of events that continues with Enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2) overexpression and decreased NKX3-1 expression (Fig. 2) (20,28). EZH2 is a methyltransferase from the polycomb group, involved in tissue-specific differentiation by histone methylation (H3K27), while NKX3-1 is an androgen-regulated, prostate-specific gene, which encodes a critical transcription factor during prostate development by downregulating epithelial cell growth, and is considered a TSG (20,28,66).

Other common molecular alterations in PCa are deletions and point mutations of PTEN, leading to activation of Akt, which can subsequently lead to over-activation of AR signaling; and in the presence of TMPRSS2-ERG gene fusion, it can increase the activation of a cascade involving ERG, EZH2 and NKX3-1 (Fig. 2) (20,31,33).

ETS(-) subtypes. ETS(-) are PCa subtypes with a canonic alteration, which is different to that in the ETS fusion genes. Whereas rearrangements occurring in ETS(+) tumors display features of chromoplexy, ETS(-) tumors display a higher frequency of chromothripsis (11). Among the ETS(-) subtypes, different studies have found the existence of a highly-expressed EZH2

Table I. Prostate cancer molecular subtypes, prognosis significance and target therapy.

A, ETS(+) subtypes (50-70% prevalence in PCa)			
Molecular subtype (% prevalence in PCa)	Characterization of the subtype	Prognosis	Targets for treatments
ERG (46) ETV1 (8) ETV4 (4) FLI1 (1)	Overexpression of EZH2, which facilitates formation of heterochromatin and the decreased expression of NKX3-1 (28). PTEN ^{Mut} is frequently found, and has been associated with subsequent activation of Akt, which leads to upregulation of AR and ERG signaling in TMPRSS2-ERG (Fig. 2) (29).	Metastatic phenotype due to an increase in cellular migration, decrease in anoikis and CRPC (24,28). PTEN ^{Mut} has been associated with a higher GS and a lower disease-free survival rate (30,31).	Directed to overcome epigenetic silencing caused by EZH2: -DZNep: A global histone methylation inhibitor that reactivates developmental genes not silenced by DNA methylation, in <i>in vitro</i> assays (32). -HDAC and DNMT inhibition in <i>in vitro</i> assays (33). Inhibitors of EZH2: -GSK 126: <i>In vitro</i> , overcomes resistance to enzalutamide (antagonist of AR) in CRPC (34). -CPI-1205: Combined with enzalutamide/abiraterone (inhibitor of androgen biosynthesis) is currently being tested for patients with CRPC (35). Directed to inhibit ERG function: -ERG direct inhibition is difficult. -Blocking function of ERG regulating co-factors, such as PARP1, is more feasible in pre-clinical assays <i>in vitro</i> and <i>in vivo</i> (25-27).
B, ETS(-) subtypes (50-30% prevalence in PCa)			
Molecular subtype (% prevalence in PCa)	Characterization of the subtype	Prognosis	Targets for treatments
Highly expressed EZH2 but independent of ERG expression (31)	Overexpression of EZH2 can be caused by MYC overexpression, miR-26a, miR-26b and miR-101 loss (36-38).	Similar to ETS(+), but with worse prognosis (39).	The same that for ETS(+), but without ERG inhibition. <i>In vitro</i> assays with aristeromycin and DZNep cause growth inhibition of PCa via induction of mir-26a (40) Inhibitor of MYC (against MYC-driven PCa including CRPC): -AZD1208 (oral PIM1 kinase inhibitor): Effective at downregulating MYC activity, inhibiting the growth of MYC-driven PCa, inhibits tumorigenesis in tissue recombinants, Myc-PCa, and human PCa xenograft models (41).

Table I. Continued.

Molecular subtype (% prevalence in PCa)	Characterization of the subtype	Prognosis	Targets for treatments
SPOP ^{Mut} / SPINK-1 overexpression (11-15)	<p>-SPOP is a TSG, which codifies for a protein involved in both proteasomal degradation and DNA repair (13).</p> <p>-Increase of SPOP substrate levels (AR, ERG, DAXX, DEK and SRC-3) and the NHEJ pathway (43).</p> <p>-High DNA methylation levels have been associated with the presence of CHD1 mutations and MAP3K7 (44,45).</p> <p>-SPINK-1 overexpression could act as a growth factor, stimulating signaling through EGFR/MAPK (46).</p>	<p>-Less adverse pathologic features and favorable prognosis (47).</p> <p>-The high expression of SPINK-1 could be associated with high GS and a possible biochemical or clinical recurrence (48,49).</p> <p>-SPINK-1 induces EMT, which increases migratory and invasive capacity <i>in vitro</i> (46).</p> <p>Androgen-deprivation therapy induce SPINK1 upregulation associates with neuroendocrine prostate cancer phenotype in mice and patients (50).</p>	<p>-CX-6258 (pan-PIM kinase inhibitor) with CX-5461 (inhibitor of RNA polymerase I): Preclinical efficacy of therapies that combine to target MYC-directed signaling to the ribosome (42).</p> <p>Directed to SPOP's substrates:</p> <p>-Lestaurtinib: Inhibitor for PRK-1 (serine/threonine kinase of the PKC family). PRK-1 was shown to play an important role in the AR complex. Pre-clinical model <i>in vitro</i> of treatment directed to AR and SRC-3 (51,52).</p> <p>-Abiraterone (inhibitor of androgen biosynthesis): CRPCs with SPOP^{Mut} and CHD1 loss appear highly sensitive (53).</p> <p>Mab anti-SPINK-1 (54).</p>
FOXA1 ^{Mut} (3)	<p>-FOXA1 is important to differentiate epithelial cells in the prostate; it binds the enhancer regions in the genome, increases local chromatin accessibility, and facilitates the recruitment of AR. However, due to its dual roles as re-programming factor of AR, as well as functioning as a collaborating factor, FOXA1 has been shown to exhibit both oncogenic and tumor suppressive roles in prostate cancer (55).</p> <p>-FOXA1 mutations have been associated with the SPOP^{Mut}/SPINK-1 subtype (13). mRNA, SCNA and methylation profiles of this subtype were similar to SPOP (17).</p>	<p>-It promotes progression of the cell cycle in CPRC in pre-clinical assays both <i>in vitro</i> and <i>in vivo</i> (17,58,59).</p> <p>-Mutations in FOXA1 Wing2 domains (50% of all mutations) were detected in PCa at all stages, whereas mutations at the highly conserved DNA-contact residue R219 (~5%) are enriched in metastatic tumors with neuroendocrine histology (60).</p>	<p>-FOXA1 downregulation induces TGF-β signaling, EMT, and cell motility, which is blocked by the TGF-β receptor I inhibitor, LY2157299. Combination LY2157299 treatment sensitized PCa cells to enzalutamide, leading to synergistic effects in inhibiting cell invasion <i>in vitro</i>, and xenograft CRPC tumor growth and metastasis <i>in vivo</i> (58).</p> <p>-<i>In vitro</i> assays show that PARP-2 inhibitors suppress AR signaling and PCa growth through disruption of FOXA1 function (59).</p>

Table I. Continued.

Molecular subtype (% prevalence in PCa)	Characterization of the subtype	Prognosis	Targets for treatments
IDH1 ^{Mut} (1)	<p>-IDH1^{Mut} produces R-2-HG, which leads to hypermethylation of DNA by the inhibition of members of the α-KG-dependent dioxygenase protein family. It causes a shift towards glycolysis and angiogenesis, as an adaptation to hypoxia, and it changes the cellular redox environment by altering the ratio of NADPH to NADP⁺ (60).</p> <p>-Characterized by tandem focal duplications, gene fusions and a marked differential expression of genes. High load of neoantigens induced by fusion and increased infiltration of tumor T cells by clonal expansion (63).</p>	<p>-Due to its low frequency, there are no significant conclusions on its prognosis; however, IDH1 mutation is rare in located PCa (17,61).</p>	<p>-Use of blocking agents of D-2-hydroxyglutarate and hypomethylating agents in pre-clinical <i>in vitro</i> assays (62).</p> <p>-Knockdown of IDH1 decreased cancer cell proliferation and blocked the AR-mediated induction in IDH metabolic reprogramming in pre-clinical assays <i>in vitro</i> (62).</p>
Biallelic loss of CDK12 (6,9)	<p>-Characterized by tandem focal duplications, gene fusions and a marked differential expression of genes. High load of neoantigens induced by fusion and increased infiltration of tumor T cells by clonal expansion (63).</p>	<p>-Associated with CPRC (63).</p>	<p>-Immunotherapy by inhibition of the immune control checkpoint (anti-PD1 inhibitor) in a pilot clinical study (63).</p>

Mut, mutated; ETS, erythroblast transformation specific; ERG, ETS transcription factor ERG; ETV, ETS variant transcription factor; EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit; NKX3-1, NK3 homeobox 1; SPOP, speckle type BTB/POZ protein; SPINK1, serine protease inhibitor Kazal type 1; EGFR, epidermal growth factor receptor; FOXA1, forkhead box A1; IDH1, isocitrate dehydrogenase (NAPD⁺) 1; CDK12, cyclin dependent kinase 12; TSG, tumor suppressor gene. GS, Gleason Score; CRPC, castration-resistant prostate cancer.

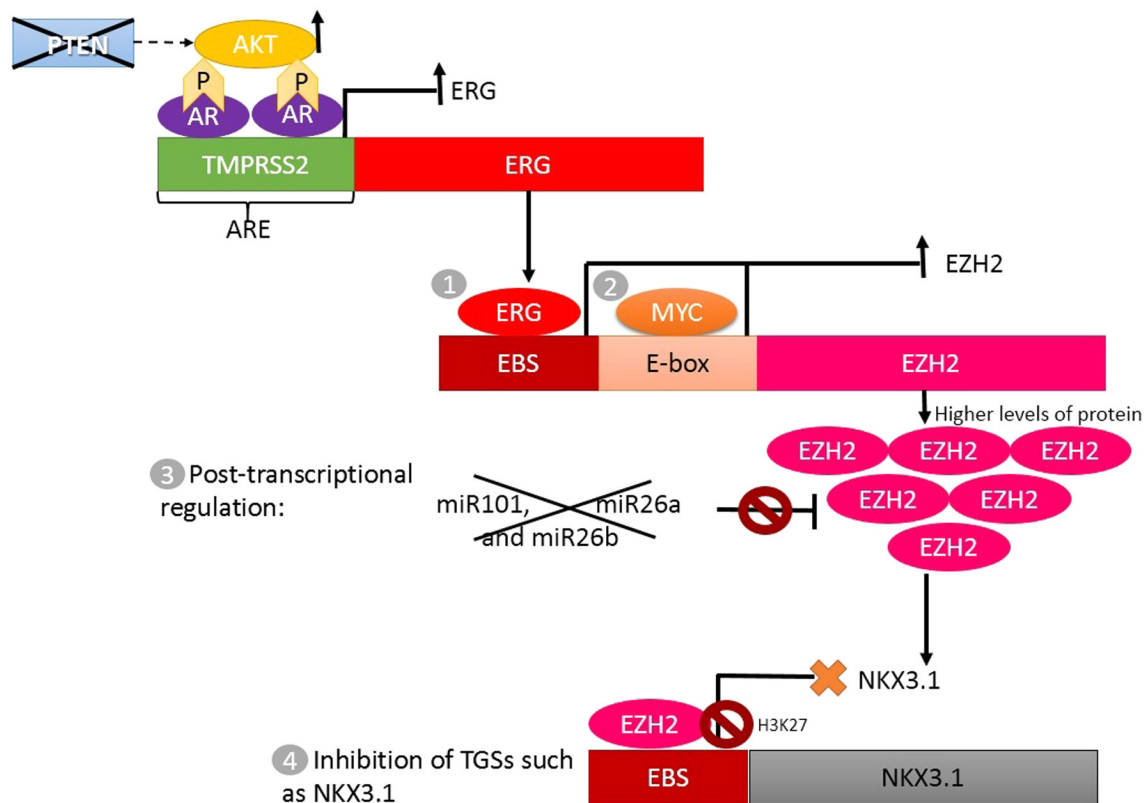


Figure 2. EZH2 overexpression in prostate cancer. The following steps are involved: 1. ERG-dependent mechanisms. Upregulation of the PI3K/Akt signaling pathway by PTEN mutations increases AR signaling and contributes to ERG overexpression in ETS(+) tumors. TMPRSS2-ERG fusion leads to overexpression of ERG, leading to overexpression of the EZH2 protein. 2. MYC oncogene binds to the E-box of the EZH2 promoter and induces its expression. 3. Post-transcriptional regulation of EZH2 by miRNAs. miR101, miR26a and miR26b reduces the translation of EZH2. Genomic loss of miR-101 and overexpression of MYC, which represses the transcription of miR-26a and miR-26b, increases EZH2 translation. 4. EZH2 is involved in gene silencing of TSG, NKX3-1. miR, microRNA; TSG, tumor suppressor gene; EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit; ERG, ETS transcription factor ERG; AR, androgen receptor; TMPRSS2, transmembrane serine protease 2; EBS, ETS binding site; NKX3-1, NK3 homeobox 1; ARE, androgen response elements.

subtype, which is independent of ERG expression (38,39). The overexpression of EZH2 has been described as well; it is caused by nuclear phosphoprotein MYC, which is involved in the cell cycle progression, apoptosis and cellular transformation. MYC oncogene also represses transcription of microRNA (miR)-26a and miR-26b, which are targets of EZH2, thus contributing to overexpression of EZH2 (36,38). In addition, the genomic or functional loss of miR-101, a TSG whose targets include the EZH2 gene, causes the overexpression of this gene (37) (Fig. 2).

Since epigenetic abnormalities driven by MYC overexpression have been associated with a poorer prognosis of PCa (progression-free survival after radical prostatectomy), therapies targeting these abnormalities may favor patients with EZH2 overexpression (25-27) (Table I). Furthermore, TCGA describes 3 ETS(-) subtypes, characterized by mutations in genes, including SPOP, overexpression of serine protease inhibitor Kazal type 1 (SPINK1), forkhead box A1 (FOXA1) or isocitrate dehydrogenase (NAPD⁺)1 (IDH1) (17) (Table I). Previous studies have reported an overexpression of SPINK, as an independent subtype (66,67); however, its overexpression was associated with the mutated SPOP subtype in TCGA study (17). In 2018, Wu *et al* (63) reported a novel subtype, characterized by the inactivation of cyclin dependent kinase 12, which could benefit from immune checkpoint inhibition therapy (Table I).

In TCGA study, 26% of PCa cases could not be classified into any of the identified subtypes (17). There were three major groups of prostate cancers in the study, one with mostly unaltered genomes (referred to as quiet), a second group encompassing 50% of all tumors with an intermediate level of SCNAs, and a third group with a high burden of arm level genomic gains and losses (17). These PCa were clinically and genomically heterogeneous; low-pass and high-pass whole-genome sequencing (WGS) on 100 and 19 tumor/normal pairs, some of had numerous somatic copy number alterations (SCNA) and a high GS, which is an indicator of poor prognosis; 33% of them were genomically similar to the SPOP and FOXA1 subtypes, others were enriched for mutations of TP53, KDM6A, and KMT2D (lysine methyltransferase 2D) or specific SCNAs spanning MYC and CCND1 (cyclin D1) and other cases had a low GS (GS 6) with fewer genomic alterations (38% in the 'quiet' class vs. 8% in the class with the greatest burden of alterations), such as SCNA and DNA methylation patterns similar to those in normal tissue (17).

Table I organizes the different subtypes with their clinical significance and the treatment options that are being addressed for the future development of personalized medicine; however, this classification has not been clear or reproducible in numerous cases, nor has the response of patients to the treatments either, as ~75% of PCa are multifocal, and more than

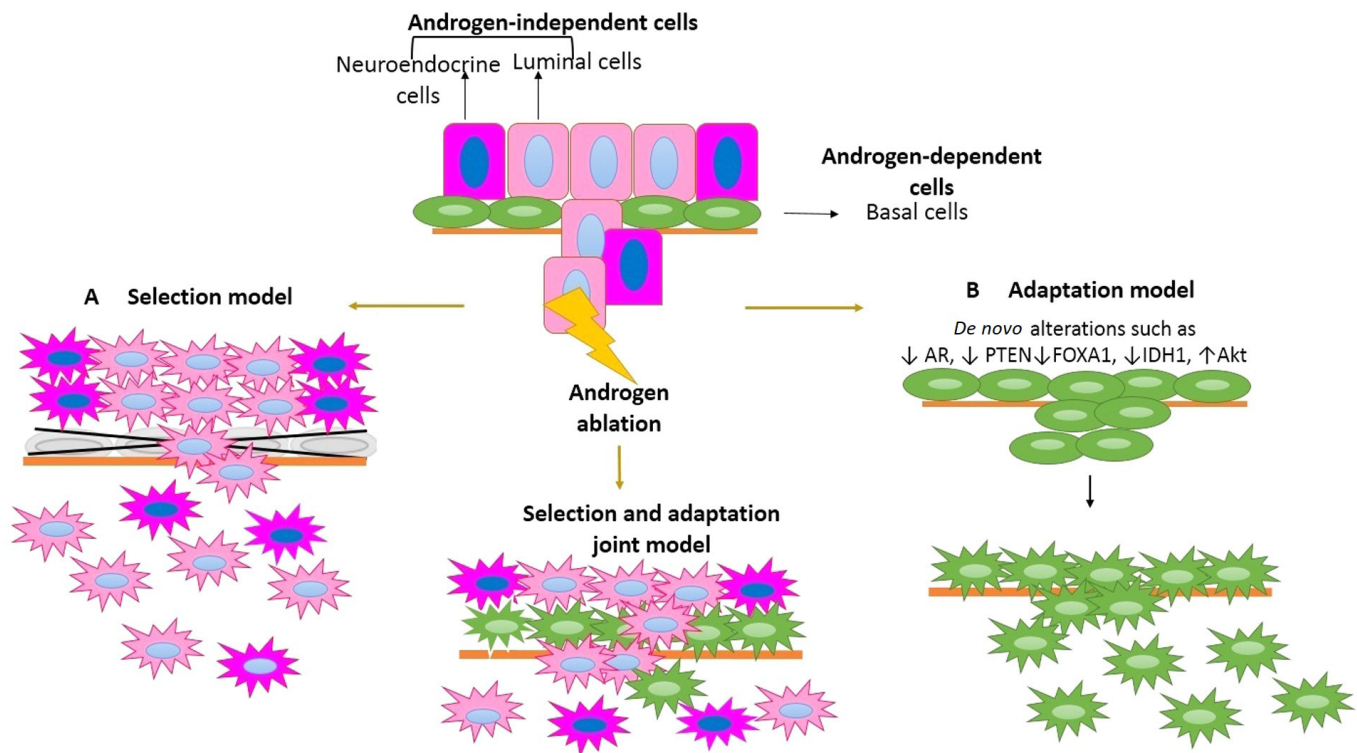


Figure 3. Models proposed for the origin of castration resistant PCa. (A) Selection model. Most luminal cells undergo apoptosis, following androgen ablation, while basal cells proliferate and neuroendocrine cells are resistant to castration. (B) Adaptation model. Following androgen ablation, androgen-dependent PCa cells acquire novel alterations which allow them become androgen-independent. However, it is also possible that both models, independently or cooperatively, contribute to CRPC growth in a patient, forming a selection and adaptation joint model. The orange line indicates the basal membrane. The grey cells with a black X represent cells that have undergone apoptosis. AR, androgen receptor; IDH1, isocitrate dehydrogenase (NAPD)⁺; PCa, prostate cancer.

one subtypes can coexist in the same patient with multifocal PCa (6,68).

Intratumoral molecular heterogeneity. The Gleason classification, using histological methods, that heterogeneity in multifocal PCa can be recognized, which is higher when analyzed from a molecular point of view (6,69). This intratumoral heterogeneity makes it difficult to associate specific molecular alterations, detected in a single focus, with the clinical behavior of a patient with multifocal PCa (6,69). In molecular studies, the pattern of the index tumor is typically obtained to assign molecular alterations and subtypes; however, other foci are not taken into account (6). The index tumor is the largest tumor focus, which in most cases (89%) can be associated with significant pathological parameters, such as the highest GS, the largest tumor volume and extraprostatic extension (70); however, this is not always the case (69). Numerous studies have found that a single clone is responsible for the dispersal of all metastatic foci (13,71,72); which suggests that identifying the 'deadly' clone is of utmost importance, and should not necessarily start from the index tumor.

Due to the heterogeneity caused by multifocality in PCa, it is necessary to perform studies, that analyze the molecular alterations in various foci, which would allow the identification of the impact of different molecular subtypes in the same patient (4,64). Some studies using the TMPRSS2-ERG fusion (73,74), PTEN deletion (75), SPINK1, ERG (67), and whole genome sequencing (6,75-77) have found a markedly

interfocal discordance, which was consistent with the concept that multiple foci of PCa have a multiclonal origins (6). Wei *et al* (6) also found this heterogeneity when using commercial PCa diagnostic kits (Decipher, Prolaris and Oncotype DX), which are based on the expression of various genes, including immune response genes like testis-specific basic protein and PBX homeobox 1 of Decipher, cell cycle-related genes CDC20 (Cell Division Cycle 2), CDKN3 (Cyclin Dependent Kinase Inhibitor 3), CDC2 (cell division control protein 2 homolog) of Prolaris, genes of androgen signaling AZGP1 (Alpha-2-Glycoprotein 1) and FAM13C (Family With Sequence Similarity 13 Member C) of Oncotype DX. These results suggest that applying a single subtype of the molecular taxonomy of PCa proposed by TCGA to a patient, i.e., studying a single focus, is an over simplified and incorrect view of the molecular landscape of PCa.

3. Molecular progression to castrate-resistant prostate cancer

As aforementioned, the origins of PCa begins as a pre-neoplastic lesion which progresses to localized cancer, and can subsequently metastasize. Elimination of androgens using surgical or chemical castration, in numerous cases, results in control of PCa (78,79). However, when relapse occurs despite treatment, PCa has progressed to an androgen-independent form of cancer or CRPC, which is considered the most aggressive form of PCa (80).

Most of the molecular alterations in PCa have been described from HGPIN (or associated with HGPIN). However, there are studies, which have found associations between some of these alterations with different stages of PCa progression. ETS fusions and FOXA1 mutations frequently occur in HGPIN (14,23). Overexpression of SPINK1 (~10-25%) and SPOP (~11%), TP53 (~25%), IDH1 (~1%), MAP3K7 and CHD1 (~10-20%) mutations (11,13,17,23,49,81) are more frequent in localized PCa, while the highest and lowest expression levels of EZH2 and NKX3-1, respectively, together with PTEN deletion, have been found in metastatic CRPC (11,23,82). Monoallelic loss of PTEN is present in up to 60% of localized prostate cancers and complete loss of PTEN in prostate cancer is linked to metastasis and androgen-independent progression (86). Alteration of the AR signaling pathway compared with that in other pathways in CRPC suggest that AR signaling continues being the 'master regulator' for PCa progression (45,55,84), including AR copy number gain (24% of CRPCs) or AR point mutation (20% of CRPCs). These results assist to define the sequence of the molecular events in the development of PCa, from the origins of the disease through to its progression into metastasis, resistance to treatment and death.

There are 3 types of cells which interact in PCa to survive androgen ablation treatment: Androgen-dependent, androgen producing, and androgen-independent cells (85). The interaction among them determines whether CRPC will develop or not. The pathway which leads from the development of androgen-dependent to androgen-independent cells is still unknown. However, there are two models which have been used to explain this process. Some studies suggest that there is a collection of androgen-independent preexisting cells following therapy (selection model) (86,87); in contrast, other studies postulate that cells acquire novel alterations which allow them to survive in the absence of androgens (adaptation model) (88) for developing CRPC (Fig. 3).

Selection model. In this model, primary PCa consists of a heterogeneous mix of luminal, neuroendocrine and stem cells. When the patient undergoes androgen ablation treatment, most of the androgen-dependent cells undergo apoptosis, while androgen-independent cells persist and survive due to their low androgen requirement (Fig. 3) (86,87,89).

The vast majority of PCa are luminally differentiated adenocarcinomas with the presence of neuroendocrine cells, and respond to hormonal therapy (90); however, there are some tumors, which consist of aggressive and highly proliferating neuroendocrine cells only, for example small cell neuroendocrine PCa, which do not respond to hormone therapy, therefore platinum-based chemotherapy (phase II trial) is used (90,91). These malignant neuroendocrine cells, which share their origin with normal prostatic neuroendocrine cells (93), express epidermal growth factor receptor (EGFR) and receptor tyrosine-protein kinase erbB-2; for these reasons, they are classified as androgen-independent cells (94), and their abundance is considered a promising prognostic marker for the development of CRPC (95).

A previous study compared global transcriptomic profiles of normal basal and luminal epithelial lineages from samples of patients with PCa and PCa cell lines (87). It was found that

PCa cells exhibited a gene expression profile similar to that found in a luminal cell and aggressive and neuroendocrine PCa were similar to basal cells (87).

In addition to cell type, in a few cases, point mutations in AR can cause cells, which were originally androgen-dependent, to become androgen-independent cells, and can be resistant to therapy, that is, those pre-existing mutations in the localized disease confer a selective advantage with therapy-resistant cells (84,95-97). The S646F mutation within AR, in the hinge region, has been associated with a short response to endocrine therapy, due to a markedly increased transcriptional activity on ARE-containing promoters (95). In addition, AR gene copies (two to four copies), due to polysomy of the X-chromosome, are present in a subgroup of localized PCa, and these specimens may have an advantage in low concentrations of androgens due to therapy (96), since the additional AR copies may be a factor leading to a poor clinical outcome of antiandrogen therapy as there is a compensatory mechanism allowing activation of the AR post-castration. Furthermore, that study concluded that high stage primary prostate cancer may be associated with increased frequencies of aneuploidies of the X chromosome resulting in an increased AR gene copies number.

Adaptation model. The adaptation model suggests that resistance to castration is the result of the acquisition of genetic and/or epigenetic alterations in response to therapy, which allows cells that were previously dependent on androgens to proliferate at low concentrations of androgens due to therapy (97). Adaptations to androgen ablation treatment include the occurrence of mutations with a copy number gain of the AR gene, changes in the expression of AR co-regulation molecules (48,84,98), and deregulation of key molecules in proliferation (99), such as Akt overexpression (29).

The evidence supports the selection model (100-102); however, it is difficult to establish either of these models as the definitive or exclusive mechanism. It is also possible that both models independently or cooperatively contribute to the development of CRPC (85,100). Some cells could be independent of androgens and be selected, or they can also gain adaptive advantages to proliferate at low concentrations of androgens; or there could be cells selected for their independence from androgens, and others that adapt and proliferate at low concentrations of androgens (100).

The aforementioned neuroendocrine PCa from small cells may arise *de novo*, which would support the selection model; however, these generally occur as recurrent tumors in men who have received hormone therapy for prostatic adenocarcinoma, suggesting that the neuroendocrine phenotype is driven by the hostile environment created by hormone therapy, or the adaptation model, and such tumors are composed of pure neuroendocrine cells that are highly proliferative and aggressive (88,90,103), due mutation in P53 (104) (allele of a missense transition converting G to A at position 747, changing negatively charged aspartic acid to hydrophilic amino acid asparagine at amino acid 184) and the inactivation of the interleukin-8-C-X-C motif chemokine receptor 2 signaling pathway (90,104). Differential epigenetic markers between the neuroendocrine and non-neuroendocrine CRPC

support a key role of the epigenome in the emergence and/or maintenance of neuroendocrine CRPC (105).

With the widespread use of novel drugs targeting the androgenic axis, such as abiraterone acetate and enzalutamide, there has been a rapid increase in the incidence of small cell neuroendocrine carcinoma (91,105), on the basis of autopsy series and other studies this type of PCa may represent approximately 25% of late stage of PCa (106), which will become a major challenge in the treatment of these patients. The clarification of the determining factors that lead to CRPC will be key to understanding the carcinogenesis process and guiding the clinical management of each patient.

4. Conclusions

The use of molecular subtypes in PCa to personalize treatment is promising; however, it is necessary to consider multifocality. The lack of an association between subtype and prognosis in PCa may be due to the fact that only the index tumor is investigated. It is important to analyze the subtypes in multiple foci, to elucidate the development of PCa, which could include different molecular subtypes; during the development of tumor foci, these would be selected according to their adaptive advantages, such as resistance to castration and the ability to metastasize.

The presence or absence of a specific alteration in any of the foci may be associated with the potential of PCa to progress to CRPC or to be the target for the development of targeted therapy, which does not necessarily have to be found in the index lesion.

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Availability of data and materials

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

Authors' contributions

YYSM and MLS were responsible for writing the manuscript, editing, acquisition, analysis, interpretation of the data, contributed to data acquisition and edited references. YYS, MCSS, RV, JAM and MLS contributed to the conception and design of the article and were involved in critical revision of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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