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The Molecular Biology of Prostate Cancer: Diagnosis, Resistance and Virulence

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Abstract

Prostate cancer is the most common form of non-skin cancer in men and the second leading cause of cancer death among them. The estimated incidence of prostate cancer worldwide was 1,414,259 in 2020, making it the fourth most commonly diagnosed malignancy, with the majority of cases seen in those over 65 years of age. Several genes have been implicated in prostate cancer development using many molecular methods to elucidate genes related to its development and progressions. Prostate cancer gene variants can be categorised based on many factors, such as the type of gene they affect (tumour suppressor, oncogene) or the type of mutation observed in the development of the illness (SNP's, gene fusions, translocations, deletions, duplications etc) epigenetic changes and even alternative splice variants are also observed in the development of prostate cancer. Understanding these various genetic connotations can then have implications for diagnosis and treatment.

Keywords: Prostate; Cancer; Molecular; Genes; Alternative splicing; Epigenetics.

Introduction

Prostate cancer is the most common form of non-skin cancer in men and the second leading cause of cancer death among men [1]. The estimated incidence of prostate cancer worldwide was 1,414,259 in 2020, making it the fourth most commonly diagnosed malignancy, with the majority of cases seen in those over 65 years of age [2]. Prostate cancer appears to affect different populations at different rates, with persons of African origin having higher rates of the disease than other groups. Sub Saharan African and Caribbean countries also have the highest rates of prostate cancer deaths on earth, with Zimbabwe leading with 41.7 deaths per 100000 followed by Barbados at 40.3. This contrasts with the world average of 7.7 deaths per 100000 [3]. The difference in the rates of prostate cancer across groups may in fact point to very real differences in germline mutation load between different populations.

Development of prostate cancer: The human prostate is subdivided into various tissue subsections, including the central, transitional and peripheral zone. The majority (60-75%) of prostate cancers arise in the peripheral zone [4]. Prostate cancer development follows a particular pattern starting with Prostatic Intraepithelial Neoplasia (PIN) then progressing to localised prostate cancer followed by advanced prostate adenocarcinoma and ending in metastatic prostate cancer [5]. This metastasis is often associated with the bones and lymph nodes and may have many different cellular and molecular basis behind its development. Prostate cancer is also known to be categorised based on how well it responds to hormonal treatment. The link between testosterone and prostate cancer development has been well documented and castration has been shown in some cases to be an effective treatment for the advancement of prostate cancer [6], after which Androgen Receptor Therapy (ART) has now become the standard of care. Further to this, some forms of prostate cancer has been found to be resistant to ART and castration, termed Castration Resistant Prostate Cancer (CRPC) or even more advanced metastatic Castration Resistant Prostate Cancer (mCRPC) which has presented itself as a new challenge

in the clinical setting. These AR negative (AR-) or AR low prostate cancers may develop as a result of natural selection during AR antagonist therapy and result in the development of what has been termed AR independent tumours [7]. Based on this understanding of the development of prostate cancer, a new picture emerges that includes the categorisation of various stages of the disease from initiation to metastasis as well as the molecular mechanisms underlying these processes of development, treatment and prognosis. It is through this understanding of the biochemistry, cell biology and molecular biology of prostate cancer that a realistic understanding of the development of prostate cancer then begins to emerge.

Several genes have been implicated in prostate cancer development using many molecular methods to elucidate this [8-10]. Many of these gene conformations and mutations such as single nucleotide polymorphisms, translocations and duplications have been implicated in both primary prostate cancer and mCRPC development.

Prostate cancer variants can be categorised based on many factors, such as the type of gene they affect (tumour suppressor, oncogene) or the type of mutation observed in the development of the illness (SNP's, gene fusions, translocations, deletions, duplications etc) epigenetic changes are also observed in the development of prostate cancer.

Gene deletions: There are many gene deletions that have been implicated in the development of prostate cancer. In many studies conducted using various techniques. For example, deletions at the APC gene have been found to be implicated in about 5% of prostate cancers [11], though other studies have only found them in as little as 3% [12]. The APC gene is a tumour suppressor gene, which works in many processes, including the control of cell signalling, cell migration, adhesion among others. This has also been demonstrated in mouse models in which the deletion has been replicated in conjunction with TGF Beta deletion (another gene implicated in prostate cancer development) leading to the development of prostate cancer in these models [13].

Transforming Growth Factor Beta (TGF-Beta) is another oncogene with an interesting function in the development of prostate cancer. It has been shown in previous studies to provide opposite roles in prostate tumorigenesis, in that its dysregulation leads to the inhibition of normal growth in early stage disease and as a promotor in advanced disease. This dysregulation can be involved in many cellular processes linking it to prostate cancer development such as upregulated cellular proliferation and decreased apoptosis just to name a few [14].

BRCA1 and BRCA2 are well known tumour suppressors not only in prostate cancer development, but in other cancers like breast cancer as well. Deletions in these genes can lead to the over proliferation of cells in the prostate and the development of cancer as these genes are involved in the regulation of transcription, DNA repair and recombination [15]. Germline mutations in BRCA genes has been linked to an 8.6 fold increase in the risk of prostate cancer over the lifetime and has also been linked to the development of more aggressive disease and poorer prognosis [16]. It should be noted that these genes play a central role in DNA repair. Other deletions and various other types of mutations have also been found in other DNA repair genes such as ATM, PLAB2, RAD51D and CHEK, which has also been linked to prostate cancer in other studies [17].

In addition to the previously mentioned genes, another gene deletion that is known to be relevant to the development of prostate cancer is that involving the PTEN (Phosphatase and Tension Homolog) gene. This gene is known to act as a tumour suppressor by stopping the over proliferation of cells and being involved in apoptosis. This gene deletion is very significant to the development of prostate cancer as some studies place it in as much as 20% of primary prostate tumors and 50% of castration resistant prostate cancers [18]. This gene acts by producing enzymes that dimerise to form their active form and acts on the PI3K-AKT-mTOR pathway which is involved in many cellular processes such as cellular division control and apoptosis.

In addition to the previously mentioned deletions, others linked to prostate cancer are those of the ERF (found in around 1.5% of prostate cancers,) CDH1 (found in around 7%), ATM (7%), NKX3.1 (17%) and RBI (0.9%) among others, all of whose products have various roles from cellular proliferation and differentiation to Androgen Receptor (AR) cosuppression.

Gene fusions: In addition to these previously mentioned deletions, fusions gene transcripts have also been shown to contribute to the development of prostate cancer. One of the most relevant gene fusion is that between the Transmembrane Protease Serine 2 (TMPRSS2) and the erythroblastosis virus E26 oncogene homolog (ERG) gene has been implicated. Overexpression of the ERG gene due to this gene fusion has been identified in as many as 55% of prostate cancer cases [19]. The ERG gene is present on chromosome 21 and is a transcriptional regulator, having effects on various cellular processes such as vasculogenesis, angiogenesis, haematopoiesis and bone development. The TMPRSS2 is a prostate specific androgen receptor responsive oncogene which codes for a transcription factor involved in various processes such as cell proliferation, differentiation, angiogenesis, inflammation and apoptosis [20].

Single Nucleotide Polymorphisms (SNPs)

In addition to these fusion genes and their resulting effect on prostate cancer progression, Single Nucleotide Polymorphisms (SNPs) also appear to play a significant role in the development and progression of prostate cancer. SNP's have also been linked to Androgen Deprivation Therapy (ADT) resistance and even relapse of prostate cancer [21]. Genome Wide Association Studies (GWAS) have been conducted which have associated as many as 100 SNPs with prostate cancer development [22], one example being gene 8q24 polymorphisms which have been shown to be associated with prostate cancer susceptibility [23]. These markers have been found to have low to moderate contribution to prostate cancer development when single, but their effects increase measurably when seen in combination [24]. This has been used in theory by some researchers who used a combination of about 14 SNPs to be able to predict the development of prostate cancer in a small high-risk population [25]. Another study found SNPs related to three genes involved in prostate cancer progression HSD3B1, CYP19A1 and HSD17B4 associated with prostate cancer formation, estrogen conversion from testosterone and high Gleason score respectively [26-28]. These genes not only appear to play a role in risk and prognosis individually but also appear to demonstrate a cumulative effect on prostate cancer risk.

In addition to SNPs being related to prostate cancer risk and progression, some SNPs have also been found to be associated with relapse during and after Androgen Deprivation Therapy (ADT) [29]. Some of the genes involved have been linked to sev-

eral oncogenic signalling processes such as Epidermal Growth Factor (ERG) [30], genes encoding androgen transporter proteins such as SLCO2B1 and SCCO2B3 [31]. In a study conducted in Taiwan, some SNPs have even been found to be associated with increased risk of prostate cancer mortality, namely those associated with the ARRDC3, TACC2, SKAP1, FLT1 and BLT2 genes [32].

Some SNPs are also associated with response to treatment of anti cancer drugs in Castration Resistant Prostate Cancer (CRPC). CRPC is very often treated by docetaxel, a well known anti cancer agent. Decreased response to docetaxel treatment has been related to a GC (Guanine-Cytosine) variant of the CYP1B1 gene in the 4326 position as opposed to the CC (Cytosine-Cytosine) variant [33]. Three SNPs associated with another gene, ABCB1 2677, 1235 and 3435 are thought to have a cumulative effect on resisting treatment with docetaxel and increased risk of subsequently developing neuroplastic prostate cancer [34].

Despite our understanding of the contribution of SNPs to prostate cancer on various levels, it is important to note that many SNPs are located on introns and intergenic sequences, outside of coding regions. As such, many SNPs that have been associated with prostate cancer may not even contribute to the actual aberration in function or expression that leads to prostate cancer development and morbidity [35]. Other studies have shown however that some SNPs are in fact involved in the control of expression and function of some genes such as the previously mentioned TMPRSS2 [36,37].

Epigenetic changes linked to prostate cancer: In addition to previously mentioned gene aberrations, epigenetic changes, or changes in the expression of existing wildtype genes through histone acetylation and DNA methylation has also been seen. Promoter hypermethylation leading to silencing of onco-suppressive genes and global hypomethylation leading to greater expression of proto oncogenes are some of the most common initial examples of epigenetics involved in the development of cancers. One good example of hypermethylation of a promotor contributing to the development of prostate cancer is that of the GSTP1 gene. This gene is normally involved in the prevention of cancer formation via the protection of DNA from oxidants and carcinogens. Hypermethylation of its promotor thus leads to the loss of its function in cells and thus increased vulnerability of cells to DNA damage. In some studies, it has been found that this gene's promotor has been methylated in as many as 75% of pre invasive high grade prostatic intraepithelial neoplasms and in over 90% of prostate tumours [38].

The DNA methylation process is catalysed by DNA Methyl Transferase (DNMT) which can consist of DNMT1, DNMT3A and DNMT3B [39]. DNMT1 has been shown to be a tumour suppressor in early stage but an oncogene in late stage prostate cancer, particularly as it relates to the regulation of the EMT (Epithelial Mesenchymal Transition) gene [40]. DNA Histone demethylases which catalyse the removal of methyl groups have also been implicated in prostate cancer. For example, one gene, JMJD1A has been shown to regulate alternative splicing in AR-V7 (Androgen Receptor splice variant 7), [41] this splice variant of the gene is shown to confer resistance to various anticancer agents such as enzalutamide and abiraterone [42].

In addition to methylation via DNMT, histone modification (through acetylation, methylation and phosphorylation) has also been shown to have an effect on prostate cancer formation and severity. Certain key histone epigenetic modifiers have

been linked to the formation of primary prostate cancer and metastatic CRPC (mCRPC). Mutations in epigenetic regulators and chromatin re modellers such as ASXL1, KMT2C (MLL3), KMT2D (MLL2) and KMT2A among others have been implicated in as many as 20% of prostate cancers [11].

Alternative splicing in prostate cancer: The previous mention of the effect of the Androgen Receptor Splice Variant 7 (AR-V7) leads naturally to a greater discussion of the increasingly apparent effect on alternative splicing on prostate cancer. Several other genes, such as VEGFA, KLF6, BCL2L1 and ERG among others have been demonstrated to have alternative oncogenic isoforms that aid in various stages of prostate cancer [43-45].

One good example of this is seen with the Vascular Endothelial Growth Factor (VEGFA) gene. This gene is involved in angiogenesis and has as many as 8 isoforms in humans brought about by alternative RNA splicing. Of these many forms, some are pro or anti angiogenic. The pro angiogenic isoforms are normally upregulated and anti-angiogenic forms downregulated during tumour formation.

Another good example is seen in Kruppel Like Factor 6 (KLF6) which is a gene coding for a group of transcription factors involved in cancer cell apoptosis and tumour suppression [46]. In its normal form, it acts as a tumour suppressor but a single nucleotide polymorphisms in intron 1 leads to errors in alternative splicing of the functional forms leading to truncated forms of the protein that then become associated with prostate cancer [47].

The two previously mentioned examples are just a few of many other genes where alternative splicing may lead to prostate cancer development, increased aggressiveness or resistance to treatment. Other relevant alternatively spliced genes that have been found so far include Cyclin D1 (CCND1), while another gene, Myc, has been implicated in the control of the alternative splicing of pre mRNA in as many as 147 relevant oncogenes [48].

Genes linked to drug resistance: Androgen Receptor (AR) and androgen deprivation therapy has been used for many years as the main mode of action of treatment for prostate cancers. This is because the androgen plays a significant role in the development of the prostate as a whole, but also in the development of prostate cancer. As prostate cancer develops and progresses, androgens and androgen receptors begin to take more of a proliferation and less of a differentiation role in the prostate. As such, most anti-cancer prostate therapy has been geared towards androgen deprivation or androgen receptor blocking [49].

This method of treatment has then led to the development of Castration Resistant Prostate Cancer (CRPC) and even more seriously, metastatic Castration Resistant Prostate Cancer (mCRPC). This mainly arises through the activity of the Androgen Receptor (AR) and mutations related to it such as SNPs, AR gene amplifications and splice variants.

Androgen receptor gene amplifications are involved in the majority of castration resistant prostate cancer [15]. This is due to the fact that the effectiveness of AR antagonists very often depends on the concentrations of AR receptors, agonists and antagonists an increase in the amount of AR may render AR antagonist drugs unable to effectively block them [50]. In addition to this, mutations in many transcription factors known to interact with the AR enhacer are already known to affect AR signal-

ling and increase prostate cancer risk such as HOXB13, NKX3.21, GATA2 [51].

AR point mutations have also been shown to be involved in prostate cancer drug resistance. One significant example is the previously mentioned SNP associated with the CYP1B1 gene. Single Nucleotide Polymorphisms (SNPs) found on the 4326 position (GG genotype) has been shown to have a significantly reduced survival rate compared to the GC genotype of the same gene after treatment with docetexal [33]. SNPs can also result in gain of function mutations affecting Ligand Binding Domain (LBD) of the AR. This leads to the AR becoming sensitive to a wider array of steroids, and can even lead to previously antagonistic molecules becoming agonists, as in the case of enzalutamide and leading to AR activation [52,53]. This phenomenon then shows potential as a treatment marker as known point mutations in the LBD such as F877L, H875Y/T878A and F877L/T878A [54], which confer some resistance go enzalutamide, can then be identified and another treatment used.

In addition to this, the importance of the splice variant AR V7 in conferring resistance to AR therapy has already been discussed. Another splice variant, Arv567es, has also been found to contribute to resistance. Both of these splice variants lack the LBD and so cannot be bound by most AR inhibitors leading them to be able to continue androgen receptor driven gene expression as normal. This leads to most AR based treatment being ineffective for cancers containing these variants [55].

Conclusion

Prostate cancer has been shown to be a multifaceted condition with several points of origin and various genetic bases. These molecular and genetic bases affect development, virulence as well as resistance. Gene deletions, single nucleotide polymorphisms, splice variants and other mutation forms all contribute to prostate cancer elucidation. Understanding these various genetic connotations can then have implications for diagnosis and treatment.

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