Molecular Genetic Etiology of Prostate Cancer

Vaijayanti V. Pethe[1,2] and Bharati Bapat[*,1,2,3]

[1]*Samuel Lunenfeld Research Institute and* [2]*Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Canada*

[3]*Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada*

Abstract: Prostate Cancer (PCa) is the most frequently diagnosed non-skin cancer and second leading cause of cancer deaths after lung cancer in the western industrialized countries. It varies widely by geographic location and ethnicity. It is clinically heterogeneous, complex and indeed a multi-factorial disease. While the majority of PCa is sporadic, as much as up to 40% of the cases are associated with some form of genetic susceptibility. It is clear today that etiology of PCa involves several genetic loci with no single major gene accounting for a large proportion of susceptibility to the disease. In particular, allelic variations in four genes, namely *RNASEL* (1q25), *MSR1* (8p22), *ELAC2* (17p11) and *EphB2* (1p36) have been shown to be associated with increased susceptibility to PCa. Also, tumors harboring mutations in these genes present with more aggressive clinical features and poor outcome. Recently, novel genetic alterations in prostate cancer patients have been identified – these include gene fusions involving the prostate-specific gene transmembrane protease, serine 2 (TMPRSS2) and members of the erythroblastosis virus E26 transforming sequence (ETS) family of transcription factors. This predominant molecular subtype is considered to be an early event in PCa, and emerging evidence demonstrates its potential in prostate cancer detection, stratification and treatment. In addition to gene fusions, there is compelling evidence demonstrating 8q24 region as a prostate cancer susceptibility locus and markers at this locus are statistically significantly associated with an increased PCa risk in different ethnic groups. Genotyping of SNPs / markers in a predefined 8q24 region as well as genome-wide association studies have implicated several polymorphisms (rs7008482, rs1447295, rs16901979, rs698367) in this region as risk factors for PCa. In additions to genetic alterations, frequent epigenetic aberrations such as DNA hypermethylation of tumor suppressor genes has been observed in PCa affecting the expression and function of a battery of genes leading to tumorigenesis, tumor progression and metastasis. In this review, we highlight some of the recent advances in molecular genetic etiology of PCa including promising candidate hereditary PCa susceptibility genes, novel gene fusions in acquired PCa, 8q24 susceptibility locus, as well as examine current literature regarding epigenetic changes leading to prostate cancer development and progression.

INTRODUCTION

Prostate Cancer (PCa) is the most frequently diagnosed non-skin cancer and second leading cause of cancer deaths after lung cancer in the western industrialized countries [1, 2]. It is predominantly a disease of elderly men, its incidence increasing steeply in the 7th decade of life. An estimated 40% of men over 50 years of age have slow growing and well-differentiated prostate cancer that can be histologically diagnosed. The incidence of prostate cancer varies widely by geographic location, and race/ ethnic background. The highest rates are reported in the US, Canada, Sweden, Australia and France. Global incidence patterns indicate that European countries have intermediate rates and Asian countries have lowest rates [3]. When stratified by ethnicity, African-Americans have higher incidence (40% of all PCa cases) and mortality rates compared to other ethnic groups in the US [4, 5] The traditional and widespread use of Prostate Specific Antigen (PSA) and digital rectal exam for PCa screening has resulted in earlier disease detection in the last decade. Treatment options including radical prostactomy, external beam radiation and brachytherapy are being increasingly used to control localized disease. Despite these attempts, PCa continues to be a significant health problem in most western countries. The treatment of PCa is difficult, in that only a small proportion of men diagnosed will have the aggressive form of the disease. Prostate tumors can be very slow growing and as such many men die *with* and not *because of* their PCa [6]. Prostate cancer is a complex and multifactorial disease and factors such as lifestyle, environment, hormones, and occupation have long been recognized as contributors of the disease [7]. Risk factors for overall incidence of PCa include increasing age, body-mass index (BMI), cigarette smoking, a lack of physical activity, ethnic background (e.g. African ancestry), and family history of PCa [8, 9]. Role of different dietary factors in PCa development has been studied; these include -supplementation with minerals like Calcium, Selenium, Zinc, Vitamins such as A, D, E; intake of soy, green tea, tomato-rich products (lycopene) and alpha-lenoleic acid, as well as dietary lipids [9-13]. Diet that includes fruits, vegetables (tomatoes, legumes), selenium, vitamin E and D have been suggested to decrease the risk of PCa. Moreover, there is emerging evidence that low-grade infection may have a role in prostate cancer development [14]. Predisposition to prostate cancer is

*Address correspondence to this author at the Samuel Lunenfeld Research Institute, Mount Sinai Hospital, R No. L6-304 B, 60 Murray St., Toronto, Ontario, Canada M5T 3L9; Tel: (416) 586-4800, Ext 5175; Fax: (416) 361-2655; E-mail: bapat@mshri.on.ca

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most likely caused by altered expression of some known and novel genes, and different models of Mendelian inheritance of germline mutations in candidate susceptibility genes [15-20]. The Androgen Receptor (AR) gene has also been implicated as a dominant oncogene in a subset of PCa cases; phosphorylation, somatic alterations, polymorphisms, amplification and over expression of Androgen Receptor are detected in PCa cases that progress despite hormonal treatment [21-25]. In addition to genetic mechanisms, epigenetic changes leading to transcriptional inactivation and loss of expression of tumor suppressor genes significantly contribute to PCa progression [26-28].

GENETIC SUSCEPTIBILITY TO PROSTATE CANCER

It is estimated that one in six men will have PCa in their life time and the risk of death due to metastatic disease is estimated to be 3-4% [16]. The majority of PCa is sporadic and displays an age-related increase in prevalence. It has been estimated that as much as 40% of PCa cases are likely to be associated with some form of genetic susceptibility. The underlying genetic factors include contribution of rare highly penetrant alleles, more frequently occurring weakly penetrant alleles, and gene-gene interactions. The terms "familial" and "hereditary" imply increased risk due to genetic susceptibility but are not synonymous. A diagnosis of hereditary prostate cancer includes early age of onset (<55 years of age) and having three or more family members diagnosed with PCa, consistent with the inheritance of a highly penetrant rare susceptibility gene. Familial PCa cases show evidence of aggregation of PCa, but not necessarily inherited in Mendelian fashion.

Familial Prostate Cancer

Familial aggregation of PCa has been recognized since 1958. Familial prostate cancer, which accounts for up to 20% of all cases of the disease in general population, refers to the occurrence of multiple cases (clustering) of PCa within a family. It is commonly defined as a family in which there are two first-degree (father, brother, son) relatives or one first-degree and at least two second-degree (grandfather, uncle, nephew) relatives with PCa. This clustering may be due to shared environment or chance occurrence given high frequency of PCa in general population, or may be due to genetic susceptibility. Populations of different origins including US Caucasian, Canadian, European, Asian, African-Americans exhibit this type of clustering [29-34]. Evidence also indicates that the risk of PCa increases proportionally to the number of relatives affected, the degree of relationship to the proband and is inversely related to the age at diagnosis of PCa. Epidemiologic studies employing different study designs and/or populations suggest that a family history of PCa that includes an affected father or brother is associated with at least a 2-fold increase in the disease risk among the relatives [31]. Men with 3 or more first -degree relatives with PCa are at a 5 to 11-fold increased risk of disease, than men without family history [31, 35]. Hemminki *et al.* (2008) [36] recently studied a total of 34 cancer sites among 205, 638 cases from the Swedish Cancer Registry, and reported that PCa showed the highest familial proportion (20.15%), followed by breast (13.8%) and colon (12.8%) cancer. A recent study involving a cohort of 179 patients from Quebec, Canada, reported familial clustering, defined as having at least one affected relative in the family, in 25% of cases [34].

Hereditary Prostate Cancer

Hereditary prostate cancer accounts for 5-10% of all PCa [37]. It is marked by a pattern consistent with passage of a rare highly penetrant susceptibility gene *via* Mendelian inheritance as an autosomal dominant susceptibility trait [20]. Hereditary PCa families are characterized by at least one of the following criteria, originally defined by Carter *et al.* 1993 [37]. These include: 3 or more first-degree relatives with PCa, three successive generations with PCa (either through paternal or maternal lineage), and two siblings with prostate cancer diagnosed at a relatively young age (< 55 y/o). Although inherited forms of PCa tend to develop at an earlier age compared to sporadic cases, the differences in terms of biological potential for PCa progression, biochemical recurrence, pathological characteristics of tumors between inherited and sporadic forms of the disease are less evident [37-39]. The genetics of hereditary prostate cancer is complex and several genes have been proposed as susceptibility factors in this syndrome. In the following section, we briefly describe promising candidate hereditary PCa susceptibility genes. We also summarize candidate genes that are known to alter overall PCa risk in different ethnic populations (Table **1**).

PROSTATE CANCER SUSCEPTIBILITY GENES

Over the past decade, there have been many published studies suggesting linkage of PCa susceptibility to different chromosomal regions including 3p,5q,8q,13q,15 and X [40]. Predisposition to PCa is probably polygenic, which can be explained by different models of Mendelian inheritance or incomplete penetrance. Segregation analyses also support autosomal recessive or X-linked, as well as multifactorial mode of inheritance. It is clear that PCa etiology involves several genetic loci with no major or a single gene accounting for a large proportion of susceptibility to the disease. However, germline mutations have been found in a few candidate genes for hereditary prostate cancer. These include *RNASEL* at 1q25, *MSR1* at 8p22, *ELAC2* at 17p11 and more recently *EphB2* at 1p36. The frequency of nonsense and missense mutations within these candidate genes varies significantly across different ethnic populations. The mutations within these genes, in addition to common polymorphisms, are also known to contribute to sporadic disease in different populations [41-44].

Hereditary Prostate Cancer 1 (HPC1)

To accelerate progress in the field of gene discovery, the International Consortium for Prostate Cancer Genetics (ICPCG) was formed in 1995, and this group reported linkage to susceptibility locus on chromosomal region 1q24-q25 to PCa, which they named Hereditary Prostate Cancer 1 (HPC1). HPC1, was implicated in PCa susceptibility by generating the first genome-wide linkage scan using 772 families affected by hereditary prostate cancer and this locus was found to account for about a third of highly penetrant early prostate cancer cases [45]. Importantly, the gene RNASEL is localized to HPC1 and several recent studies have linked RNASEL germline mutations to PCa susceptibility [42, 46-48]. Functional studies revealed that RNASEL 1 regulates

Table 1. Genes Influencing Prostate Cancer Risk

Gene/(Locus)	Alterations Associated with PCa Risk	Function [Citations]
Annexin A7 (10q21)	Reduced expression/ Loss of heterozygosity (LOH)	Encodes for Ca- activated GTPase implicated in both exocytotic secretion in cells and control of growth [131]
ATBF1 (16q22)	Deletion of codon 3381 (3381 del)	Gene coding for cell cycle active protein [132, 133]
CDKN1B (12p11-13) p27/Kip	Reduced expression and SNP variant in codon 109	Inhibits cyclin-dependent kinases and blocks cell proliferation [134, 135]
CHEK2 (22q12.1)	Truncating mutation 1100delC	Important regulator of p53 in the DNA-damage-signaling pathway [136, 137]
CYP17 (10q24.3)	Polymorphic T (A1 allele) to C (A2 allele) in 5' promoter region	Encodes enzyme P-450c17α which functions in androgen biosynthesis pathway [138]
CYP1B1 (2p21-22)	SNP at codon 119 (G→T), Haplotype CGCCG of-1001C/T, -263G/A, -13C/T+142C/G and +355G/	Involved in androgen metabolism [139]
CYP3A4 (7q21.1)	CYP3A4 A>G variant in 5' promoter	Member of cytochrome P450 family involved in oxidation of testosterone for deactivation of hormone [140, 141]
GSTP1 (11q13.3)	313 A>G variant	Metabolism of carcinogens and defense against Reactive Oxygen Species (ROS) [142]
KLF6 (10p15)	Intronic SNP - IVS1 –27 G>A [IVS A allele] & reduced expression	Zinc finger transcription factor with a role in cell proliferation and differentiation [143, 144]
PTEN (10q23.3)	Reduced expression Somatic mutations/deletion Hypermethylation	Acts as tumor suppressor and codes for protein that regulates cell cycle and prevents cell proliferation [145-147]
NKX3.1 (8p21)	Loss of heterozygosity (LOH), Reduced expression, T164A	Homeodomain containing transcription factor [148-152]

Genetic alterations (SNPs*, deletions, LOH*, copy number aberrations) and/or altered gene expression patterns affecting prostate cancer risk in different ethnic populations.
*SNP – Single Nucleotide Polymorphism *LOH- Loss of heterozygosity.

cell proliferation and apoptosis through interferon-regulated 2-5A pathway and is a candidate tumor suppressor gene [49]. In particular, Arg 462 Glu [R462Q], a common and well documented missense variant, with reduced enzymatic activity compared to wild type, has been implicated in prostate cancer risk from several epidemiologic and functional studies [42, 48]. A large, controlled sib-pair study (family-based case-control study) implicated the R462Q variant in up to 13% of unselected prostate cancer cases [42]. One mutated copy of R462Q increased the risk of PCa by about 50%. In addition, rare mutations of RNASEL are associated with different ethnic groups. A founder and deleterious frame shift mutation 471delAAAG was identified in a single Ashkenazi Jewish cohort [50]. In another study, the 471del AAAG mutation was detected in a single male with Prostate cancer (1/294, 0.3%), in two ovarian cancer patients (2/141, 1.4%) and in one of 242 healthy controls (0.41%) [51]. A truncating mutation, E265X, found in Finnish hereditary PCa families, showed an association with increased risk of prostate cancer [48]. PCa patients carrying 471del AAAG and E265X mutations showed loss of heterozygosity of wild type allele in microdissected prostate tumor DNAs [46, 47]. Despite these reports supporting the involvement of RNASEL in PCa etiology [42, 46-48], others have reported no link of RNASEL to PCa [52-54]. This discrepancy may be attributable to either population differences and/or distinct study designs directly or indirectly modulating the impact of RNASEL on prostate carcinogenesis.

Further intriguing is the role of RNASEL in viral defense as an important effector of antiviral action of interferons. Urisman *et al.* (2006) [14] explored a possible link between incidence of viral infection and RNASEL genotypes in prostate cancer patients. Using DNA microarray-based strategy (DNA ViroChip) they identified the presence of novel gammaretroviral (XMRV) sequences in cDNA samples from seven of 11 (60%) R462Q homozygous (QQ) cases, and in one of eight (10%) heterozygous (RQ) and homozygous wild-type (RR) cases.

HPC2 (ELAC2) – [tRNA Processing Endoribonuclease]

HPC2 located on chromosomal region 17p11, harbors ELAC2, which is the first candidate gene identified for human prostate cancer based on linkage analysis and positional cloning [55]. ELAC2 encodes a 3'processing endoribonuclease, associated with gamma tubulin, which is a component of mitotic apparatus suggesting a possible role of ELAC2 in cell cycle control [56]. A number of germline variants including mutations have been identified in this gene. Specifically, two common missense alterations Ser217Leu (S217L) and Ala541Thr (A541T), are associated with increased risk of PCa in men belonging to hereditary prostate cancer families [43, 44, 55, 57]. A number of polymorphic variants have also been described linked to PCa risk in various populations [44, 58-63].

MSR1 [Macrophage Scavenger Receptor 1]

The chromosomal 8p22 region is one of the loci that are frequently deleted in PCa and also linked to hereditary prostate cancer [64-66]. MSR1 is a candidate PCa susceptibility gene that was identified by a combination of family-based linkage and association studies and systematic evaluations of genes at 8p22-23 region by screening for mutations in

probands of 190 hereditary prostate cancer families [65]. The MSR gene encodes proteins that function with responses to infections, which may play a role in susceptibility to prostate cancer [16]. It can also bind to bacteria and modified lipoproteins [67] and functions in several processes relevant to prostate carcinogenesis [68]. Mutations in MSR1, including truncating mutations have been shown to be associated with PCa risk both in hereditary and sporadic cancers. Xu *et al.* [65, 66] carried out a comprehensive genetic study using a large number of subjects from multiple populations (men from hereditary prostate cancer families, non- hereditary prostate cancer men, and case-control studies conducted using African-American men) and screened for germline variants in MSR1 among probands in each group. Six rare missense mutations [Pro36Ala, Ser41Tyr, Val113Ala, Asp174 Tyr, Pro275Ala, Gly369Ser] and one nonsense [Arg 293X] mutation within MSR1 were observed to co-segregate with the disease in hereditary prostate cancer (HPC) families, (p=0.0007) [65]. Furthermore, the prevalence of MSR1 mutations in European and African American probands was substantially higher compared to unaffected men [65]. Arg 293X and Ser 41 Tyr were the most common mutations detected among PCa patients of European and African-American descent, respectively [66]. Seppala *et al.* [69] screened the youngest affected member from each of 120 hereditary prostate cancer families for MSR1 mutations by Single-strand conformational polymorphism analysis. Three MSR1 variants (R293X, P275A, -1473A>G) were identified and they reported no significantly elevated or lowered risks for PCa for the carriers of these variants. However, the mean age of diagnosis of R293X mutation carriers among the herediatary prostate cancer probands was significantly lower compared with noncarriers (55.4 versus 65.4 years; t test, p=0.04).

EphB2 [Eph Receptor B2]

The EphB2 gene encodes for a receptor tyrosine kinase. It was recently identified as a tumor-suppressor gene in DU145 PCa cell line and in primary prostate tumor specimens using a combination of strategy of nonsense –mediated mRNA decay microarray profiling and array-based CGH [70]. EphB2 maps to chromosomal region 1p36 previously shown to be linked to hereditary prostate cancer among ethnically diverse sets of families [71-73]. Somatic mutations in EphB2 occur in ~10% of sporadic prostate tumors. Kittles *et al.* recently (2006) [74] evaluated the contribution of EphB2 to inherited PCa susceptibility in African Americans (AA) by screening for germline polymorphisms. Ten coding sequence variants were identified, including the K1019X (3055A to T), a germline nonsense mutation, which was present in 15.3% of the African –American hereditary prostate cancer probands, but only 1.7% European American control samples. This mutation increased the risk for PCa over two-fold [p = 0.003]. Although the functional significance of K1019X mutation is unknown, it suggests a pathogenic role for EphB2 in PCa, which warrants further investigation.

BRCA1/BRCA2

Germline mutations of tumor suppressor genes BRCA1 (17q21) and BRCA2 (13q12) are linked to hereditary breast cancers. While multiple studies have excluded a potential role for BRCA1 in prostate cancer, an association with prostate cancer is reported in breast-ovarian cancer families with BRCA2 mutations accounting for about 2-5% of early-onset prostate cancers [75-77]. A common founder mutation (6174delT) has been identified in Ashkenazi Jewish population and this allele showed a significant association with prostate cancer risk [78].

Androgen Receptor (AR)

Androgens, which exert their effect *via* Androgen Receptor (AR), are essential for the prostate development and maintenance [[79]. The AR gene located on the X chromosome contains polymorphic trinucleotide repeats (CAG or GGC) in exon 1 and these encode for a variable length of glutamine and glycine tract, respectively, in the AR protein. There is an inverse relationship between repeat length and AR transcriptional activity [80]. Reduced repeat length is associated with prostate cancer recurrence and early-onset disease [39, 81]. The number of CAG repeats ranges from 8-31 [82]. Decreased transactivation activity and binding affinity for androgens is associated with increased number of repeats and it may confer a protective effect in terms of prostate cancer risk. Whereas somatic alteration of the repeat length is very rare, a shorter CAG repeat has been shown to be associated with increased risk and more aggressive tumor features (high tumor stage and grade, metastasis, mortality)[83] The GGC repeat also appears to be associated with prostate cancer risk [84].

The association between AR and PCa has been well established and AR expression is sustained even at the most advanced phases of androgen-independent disease [85]. Therefore, androgen- ablation and antiandrogen therapy form important treatment regimen of the disease, though most patients go on to develop and will die of androgen-independent prostate cancer. There is a plethora of literature available explaining the possible mechanisms of AR and AR-regulated gene expression in recurrent disease. Some of the mechanisms include: 1. AR plays a role as a potent oncogene; 2. AR activity is enhanced by genomic amplification in approximately one-third of tumors even in the relative absence of androgen [25] 3. In some prostate carcinomas, somatic AR mutations alter the specificity of the AR receptor, enhancing its hormone sensitivity [21, 25, 86, 87]. Germline polymorphisms in the trinucleotide repeat of the AR gene, which probably affect AR activities, have been linked to increased prostate cancer risk. Others have reported that activation of AR in androgen-independent disease may also be accomplished by induction of co-activators such as β-catenin and p160 family members [88-90]. β-catenin is rarely mutated in PCa, but can activate AR cause its co-localization to the nucleus and enhance hormone sensitivity [89, 90].

8q24 REGION AND PROSTATE CANCER RISK

To date multiple chromosomal aberrations [91] as well as certain chromosomal regions (1q, 17p, 8p) have been identified as likely harboring PCa susceptibility genes [40]. Mutations and sequence variants in many candidate genes from these regions have been reported to be associated with PCa risk [46, 55, 65, 68]. Emerging evidence indicates linkage of both 8p and 8q regions to PCa and frequent genomic rearrangements are observed at 8p [53, 72, 92-95] as well as 8q [96, 97] regions in prostate tumors.

It has been known for some time that amplification, or gain of chromosomal region 8q24 (including the c-MYC region) is a frequent event in PCa [98]. Genome-wide association [99, 100] studies and studies involving Single Nucleotide Polymorphism(SNP) genotyping have identified several SNPs associated with PCa risk [101, 102]. Recently, Amundadottir *et al.* (2006) [103] localized a region at 8q24 locus *via* linkage analysis and identified specific variants in a region spanning from 128.54 – 128.62 Mb that were associated with increased risk of prostate cancer in Icelandic families. Further analysis of this region led to the identification of several common nucleotide variants associated with PCa in European and African populations. Two representative markers, a microsatellite repeat DG8S737 and a SNP rs1447295 showed the strongest association with PCa in three case-control series of European ancestry (Iceland & Sweden), as well as a cohort of US Caucasians [99, 103]. In an independent report, Freedman *et al.* (2006) [104] confirmed the association between rs 1447295 and PCa risk (overall $p<4.2 \times 10^{-9}$) by using four case-control study populations including Japanese Americans, Native Hawaiians, Latino Americans, and European Americans. Among African Americans, the association was statistically significant in men diagnosed with PCa at an early age [<55 years of age] ($p = 0.011$) and insignificant for those diagnosed at a later age ($p= 0.924$). More recently, studies by Gudmundsson *et al.* (2007) [101], Haiman *et al.* (2007) [105], and Yeager *et al.* (2007) [102] provided further evidence by demonstrating an extraordinarily strong association (adjusted p value = 4×10^{-29}) of rs1447295 SNP with PCa. Linkage scans and Genome- wide association studies have identified this genomic region also associated with risks of other cancers including CRC and breast [106, 107]. The 8q24 region appears to be a "gene-poor" region and is a common location for somatic gains for PCa. These findings are intriguing and suggest that the vast proportion of the non-coding repetitive regions of the genome may contain novel regulatory elements or even genes that we have yet to identify and understand. Finally, mechanism by which the nucleotide marker and the SNP contribute to an increased risk of prostate remains to be elucidated.

GENE FUSIONS AS NEW GENETIC MARKERS FOR PROSTATE CANCER

Gene rearrangements are associated with a number of cancers especially lymphomas, leukemias, and sarcomas. Recent studies have uncovered specific gene rearrangements implicated in a subset of prostate cancers. These novel rearrangements were discovered using "Oncomine", a collective database of gene expression profiling generated from various cancer studies across the globe [108]. One such rearrangement, identified using FISH and RTPCR, involves ETS transcription factor family- either ERG(21q22.2) and *ETV1*(7p21.2) or *ETV4 (17q21)* with TMPRSS2 (21q22.3) [109-113]. ERG and ETV1/4 are ETS transcription factor genes and TMPRSS2 is an androgen-regulated gene. While TMPRSS2: ERG fusions are the most predominant subtype of ETS gene fusions (50% of PCa), those involving ETV1 or ETV4 occur in ~ 1-10% of PCa cases [111, 114-118]. Although the fusion event seems to be cancer-specific (40-80% of cancers) and rare in BPH, it is detected in about 20% of preneoplasic (PIN) lesions [113,116]. These rearrangements occur *via* different processes involving either a deletion (due to loss of 3' TMPRSS2 signal) or a translocation (split of 5' and 3' TMPRSS2 signals), or alternatively involving both mechanisms in different tumor foci [119].

The TMPRSS2-ERG fusion is considered to be an early event in PCa development. The fusions were detected non-invasively in urine sample of patients with clinically localized PCa [120]. The test was developed using RNA amplification and quantitative PCR [120]. When urine was collected from 19 patients with clinically localized prostate cancer, 42% of the patients had the gene fusion detected, consistent with that found in tissue data analysis. The translocation of ERG and ETV1 may be redundant because only one or the other was found fused to the TMPRSS2 regulatory region in any given tumor.

Mosquera *et al.* (2007) [121] demonstrated a significant link between chromosomal fusion status in the prostate tumors and the tumor phenotype. They studied 253 prostate cancers for the presence of characteristic histopathological features using an ERG break-apart FISH assay. Five out of eight morphological features were significantly associated with the presence of TMPRSS2-ERG fusions in cancers ($p <0.05$). These were as follows: blue-tinged mucin, cribiform growth pattern, macronucleoli, intraductal tumor spread, and signet cell features. Only 24% of tumors without any of these features displayed the TMPRSS2-ERG fusion. Mucin positive carcinomas more often harbor such gene fusions compared to mucin-negative tumors ($p= 0.004$) [119]. It is likely that TMPRSS2-ERG over expression may initiate specific molecular pathways that favor a typical phenotype for the tumors. The fusion-specific phenotype may have prognostic implications, and merits further investigation using large series of prostate cancers with known fusion status. Emerging data suggest that gene fusion carrying prostate tumors exhibit a distinct clinical course and thus support it's use as a potential prognostic biomarker.

More recently, Helgeson *et al.* 2008 [109] identified additional 5' partners in *ETV1* fusions including TMPRSS2, SLC45A3, HERV-K_22q11.23, C150RF21, and HNRPA 2B1. These 5' partners are differentially regulated by androgen (androgen-induced, androgen-repressed and androgen insensitive) and they define distinct classes of ETS gene rearrangements. To date, the partners have only been identified in ETV1 fusions, it is not known if they fuse with ERG or additional ETS family members.

DNA METHYLATION AND PROSTATE CANCER

Hypermethylation of cytosine guanine dinucleotide islands (CGI) at promoter regions of tumor suppressor genes has been recognized for a number of tumors as an important event in tumorigenesis, including prostate cancer. CpG island hypermethylation causes gene silencing through transcriptional inactivation and thereby contributes to prostate cancer development and progression. Hypermethylation of candidate genes has been studied contribution of panel of some genes has been evaluated for potential role as biomarkers for diagnosis and/or prognosis of prostate cancer [26, 122-126]. GSTP1 is the most consistently hypermethylated marker in prostate cancer [127, 128]. GSTP1 hypermethylation was found to be highly tumor-specific, but also prevalent in HGPIN lesions, which makes it an attractive early

detection marker [127]. GSTP1 hypermethylation has been also studied in urine sediment as a non-interventional test for determining the need for prostate biopsies and as a biomarker for diagnosis [15, 129]. Emerging evidence suggests that a defined panel of methylated genes rather than a single gene is more likely responsible for prostate cancer progression. Candidate genes most commonly studied for methylation include APC, DAPK, ECDH1, GSTP1, MGMT, P14 [ARF], P16, RARβ2, RASSF1a, and TIMP3 [28, 130]. Hoque *et al.* (2005) [130] compared urine sediment from 52 prostate cancer patients undergoing radical prostatectomy with that of 91 age-matched controls. All 52 cancer patients had at least one hypermethylated gene, and 80% had 3 or more hypermethylated genes. The 4 most commonly methylated genes were GSTP1, p16, ARF, and MGMT. None of the controls showed hypermethylation of any gene. Recently, Roupret *et al.* (2007) [28] analyzed methylation patterns in 95 patients undergoing radical prostactomy and 38 age-matched controls with negative prostate biopsies. Eight of the loci had increased methylation in cancer patients compared to the controls ($p < 0.05$). Again, the methylated panel of genes consisted of GSTP1, APC, RASSF1a, and RARβ2. The sensitivity for prostate cancer detection was 86% and diagnostic accuracy was reported to be 89%. The potential for DNA methylation in the clinical arena including it's role as biomarker for early cancer detection, as prognostic indicator for PCa, as well as it's potential in designing novel therapeutic strategies for PCa has been reviewed [26].

CONCLUSION

The genetic basis of PCa is complex and includes both heritable and somatic genetic alterations. Traditional linkage studies and population-based genome-wide association studies have identified many candidate susceptibility loci, however the results from these studies have not been consistently replicated, possibly due to phenocopies in families and/or due to locus heterogeneity. Recent advances have provided significant insights into initiation and progression of prostate cancer, however much work is needed to elucidate how these alterations relate to each other. Future studies need to focus on these issues by building multifactorial inheritance models that account for interplay between genetic, epigenetic and environmental factors in prostate carcinogenesis.

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