Molecular changes in prostatic cancer

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Abstract

Prostate cancer is one of the most commonly diagnosed and potentially devastating cancers in men, throughout the world. However, the clinical manifestation of this disease varies greatly, from indolent tumours, requiring little or no treatment, to those aggressive cancers which require radical therapies. Prostate cancer, like all other cancers, develops and progresses as a consequence of an accumulation of genetic changes. While several putative genes have been isolated for the development of breast, ovarian and colon cancer, the aetiology and pathogenesis of prostate cancer remains poorly understood. In this review, we discuss important genetic markers in early, metastatic and hormone refractory prostate cancer which may, in the future, be used as markers for diagnosis and prognosis, as well as targets for therapeutic intervention.

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1. Introduction

The behaviour of every cell in the body is dependent on its DNA. Changes in this DNA affect the expression and function of critical genes. Altered genes result in altered mRNA expression and often, altered proteins. Many changes may occur; however, five to 10 specific alterations are required to convert a normal prostate epithelial cell into a malignant cell capable of invasion [1]. In a small minority of cases these alterations are inherited, in the majority however they are acquired or somatic mutations [2,3]. The problem for researchers is to identify which genetic changes are important in cancer progression, which can be used to predict prognosis and which can act as targets for therapeutic intervention.

2. Hereditary prostate cancer

In 1895, Dr Alfred Warthin's seamstress told him she was convinced she would die from cancer as so many of her family had. Indeed she died at a young age from endometrial cancer; this prompted Warthin to publish a description of her family, which included relatives dying prematurely from gastric, endometrial and colorectal cancers [4]. Progress was made in understanding familial cancers in the 1960s by Lynch [5], Li [6] and Knudson [7] who systematically examined at risk families. Knudson went on to predict the 'two-hit' hypothesis based on his study of a rare eye tumour in children—retinoblastoma. He postulated the presence of regulatory TSGs that are normally involved in cell-cycle, apoptosis and proliferation. Humans normally have two copies of these TSG, one from each parent. Where inherited mutations occur one copy is affected. The remaining TSG maintains sufficient control of its regulatory function, however, should a mutation occur in the other copy of the TSG in one cell a malignant potential is acquired. This hypothesis was confirmed 20 years later when germline mutations in the *RB* gene were detected in hereditary retinoblastoma [8].

The problem with studying familial prostate cancer is that as the incidence of the disease in the general population is so high there is a high rate of sporadic cases amongst the familial cluster. Furthermore, these families undergo PSA screening which will further

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enhance the detection rate. Despite these factors, a family history of prostate cancer still confers an increased risk of being diagnosed with prostate cancer and of developing the disease at an earlier age. A man with a first-degree relative with prostate cancer has a relative risk of 2.0 (1.2–3.3, 95%CI), with a second-degree relative risk of 1.7 (1.0–2.9) and with both a first and second degree relative an 8.8 (2.8–28.1) relative risk of developing prostate cancer [9].

Further evidence for a hereditary component and a link with another histologically similar malignancy is that prostate cancer is higher in men who have relatives with breast cancer [10,11]. Likewise, the risk of breast cancer is doubled in families with a history of prostate cancer [12,13].

Hereditary prostate cancer is usually defined by the pedigree as no associated genes have yet been firmly identified. This definition therefore includes nuclear families with three cases of prostate cancer, the presence of prostate cancer in each of three generations in the maternal or paternal lineage and a cluster of two relatives diagnosed with prostate cancer before the age of 55 years [14]. Using this definition 3–5% of patients with prostate cancer has hereditary disease [14]. This definition however excludes families with a hereditary susceptibility to prostate cancer (with mutations in autosomal dominant susceptibility genes), so the true proportion is likely to be 5–10% [14].

2.1. Candidate genes in hereditary prostate cancer

Prostate cancer susceptibility loci have been reported at 1q24–25, 1p36, 1q42, 20q13, Xq27–28 [15], 16q23 [16] and 17p [17] (Table 1). Several authors have reported a linkage (the inheritance of a recognised genetic marker with the disease) on the long arm of chromosome 1 [18–20]. The exact site of the susceptible genes on this chromosome region remains unclear; 1q24–25, named *HPC1* [18] and 1q42, termed *PCAP* [21] have been implicated. Two further studies confirmed linkage at the *HPC1* locus on 1q24–25 [19,20], other studies did not [21–23]. In a study from the CRC/BPG UK Familial Prostate Cancer Study Collaborators, the incidence of

allelic imbalance at *HPC1* was low in both sporadic tumours and small prostate cancer families [24]. The authors concluded *HPC1* is unlikely to be acting as a TSG in the development of familial or sporadic prostate cancer.

Other candidate genes include the breast cancer susceptibility genes *BRCA1* (17q21) and *BRCA2* (13q12.3) and these confer a relative risk of prostate cancer of 3.0 and 2.6–7.0, respectively [25]. The UK/Canadian/Texan Consortium found up to 30% of familial clusters may be linked to *BRAC1/2*, although the confidence intervals were wide and included zero [25]. Indeed the UK Familial Prostate Cancer Study was unable to find any mutations in the *BRAC1* gene but did find two germline mutations in *BRCA2*. They postulated the *BRCA2* mutations may be a coincidental finding or may play a role in modifying the expression of another cancer gene [25]. An association between breast and prostate cancer clearly exists, the molecular basis for this association is not yet fully understood.

Schaid et al., using segregation analysis, found prostate cancer was 1.5 times more common in brothers than in fathers of men with prostate cancer [26]. This may indicate that prostate cancer is inherited in some families in a recessive or an X-linked way. A study of 360 families in North America, Sweden and Finland strongly implicated a region on the long arm of the X-chromosome at Xq27–28, which the authors named *HPCX* [27]. This gene accounted for 15–16% of cases in North America and 41% of Finnish cases of hereditary prostate cancer.

Tavtigian et al., working on the Utah Population database, mapped an inherited predisposition to prostate cancer to the *ELAC2* gene on chromosome 17p in 33 families [17]. When the number of families studied increased to 127, the linkage was no longer significant as the prevalence of the mutation fell. The authors conclude this was due to the heterogeneity of the families involved. *ELAC2* codes for a protein involved in interstrand-cross link repair mechanisms, which is consistent with a possible role in prostate cancer.

Confirmatory studies showing weak or no linkage to these regions and a putative susceptibility gene has yet

Table 1
Chromosomal loci reported to contain hereditary prostate cancer genes

Locus	Putative gene	Number of families	Two-point lod score	Multipoint lod score
1q24–25	HPC1	91	3.7	5.4
1q42	PCAP	47	2.7	3.1
1p36	CAPB	12	3.7	2.2
17p	ELAC2	33	4.5	
20q13	HPC20	162	2.7	3.0
Xq27–28	HPCX	360	4.6	3.9

The lod score is a statistical estimate of whether two (two-point) or several (multipoint) loci are likely to lie near each other on a chromosome, and are therefore likely to be inherited together.

to be identified. This has led investigators to conclude that hereditary prostate cancer is a heterogeneous disease and no single gene is responsible for the high incidence in certain families. Alternatively, there may be no major susceptibility genes for prostate cancer as there is for say breast or colon cancer [1,15].

2.2. Low penetrance polymorphisms

Mutations in high penetrance susceptibility genes resulting in a significantly increased risk of prostate cancer are relatively uncommon in prostate cancer. On the other hand, polymorphisms (the occurrence of allelic variations) in low penetrance genes increase the risk of developing the disease only modestly, but occur with greater frequency in a population. Low penetrance polymorphisms may therefore have a greater impact on the frequency of prostate cancer in the population as a whole.

The polymorphic CAG (cytosine–adenosine–guanine) repeat in the androgen receptor (AR) gene has been studied extensively. This codes for a polyglutamine tract of varying lengths (dependent on the number of CAG repeats in the gene). Workers have shown an inverse relationship between the CAG repeat length and prostate cancer risk [28–30]. In normal healthy males there are 13–30 CAG repeats; however, African-American men have a higher prevalence of <22 CAG repeats in the AR gene [31], and Chinese men have a higher prevalence of longer CAG repeats [32]. The prevalence of prostate cancer is higher in African-Americans and lower in Chinese men [1]. Giovannucci et al. showed that if an individual had $\leqslant 18$ CAG repeats they had an increased relative risk of 1.52 for developing prostate cancer compared with men with $\geqslant 26$ CAG repeats [33].

Polymorphisms in the SRD5A2, the gene that codes for the enzyme 5 α-reductase type II, have been reported [1]. 5 α-reductase is responsible for the conversion of testosterone to its more active metabolite dihydrotestosterone in the prostate. Polymorphisms in the SRD5A2 gene result in increased catalytic activity of this enzyme and are associated with an increased risk of developing prostate cancer [34].

The vitamin D receptor (VDR) gene is also a polymorphic steroid hormone receptor gene implicated in prostate cancer. Vitamin D is antiproliferative to prostate cancer cell lines [35,36] and low serum vitamin D may be associated with an increased risk of prostate cancer [37,38]. Whether the VDR polymorphisms confer an increased susceptibility to prostate cancer remains contested [39–43]. However, a number of vitamin D analogues are currently undergoing clinical trials for prostate cancer treatment and prevention [43].

Other polymorphisms reported to influence the risk of developing prostate cancer include the cytochrome P450 family genes (CYP3A4 and CYP17). The ELAC2 gene

may be a low as well as possibly as a high penetrance gene [44–46]. Larger studies are required to corroborate these findings.

3. Somatic changes in prostate cancer

Some tumours may contain inherited mutations and all tumours contain acquired or somatic alterations. Advances in the techniques used to examine these changes resulted in the identification of chromosomal regions deleted or amplified that may play a role in tumourigenesis. Chromosomal changes have been identified in specific stages of disease progression with the average number of alterations significantly higher in distant metastases than primary tumours [47].

One of the most widely reported techniques for examining chromosomal alterations is comparative genomic hybridisation (CGH). This allows the detection of DNA sequence copy number changes throughout the genome and can therefore identify regions where deleted TSG or amplified oncogenes may be harboured. Using CGH, researchers have shown that locally recurrent hormone refractory prostate cancers contain almost four times as many alterations as untreated primary tumours [48]. The early development of prostate cancer is associated with inactivation of TSG, whereas later progression, including the development of hormone refractory disease, is associated with activation of oncogenes.

CGH and AI (allelic imbalance) studies have demonstrated the most common chromosomal aberrations in prostate cancer are deletions in chromosome regions 3p, 6q, 7q, 8p, 9p, 10q, 13q, 16q, 17q and 18q and gains in 7p, 7q, 8q and Xq [1,47,49–51]. Furthermore, the frequency of AI at several loci increases with higher grade [52,53] and higher stage disease [53,54].

4. Early genetic changes

4.1. GSTP1

Hypermethylation of the GSTP1 gene is seen in 70% of cases of high-grade prostatic intraepithelial-neoplasia (HGPIN) and over 90% of prostate cancers, but is a rare event in benign prostate tissue [55,56]. The GSTP1 gene on 11q13, encodes for glutathione S-transferase which conjugates electrophilic and hydrophobic environmental carcinogens with glutathione, thus protecting the cell [57]. Hypermethylation of the CpG islands of the promoter region prevents the transcription of the gene, thus removing the cell's in-built protection mechanism against potential environmental carcinogens, including dietary factors [43]. As GSTP1 inactivation occurs in the vast majority of cases of HGPIN and prostate cancer

early in the disease course, it may be used as a molecular marker [55]. Indeed studies have already reported measurement of *GSTP1* methylation in cells in the urine of men with prostate cancer [58,59]. Cairns et al. detected *GSTP1* methylation in the urine of 27% of patients with *GSTP1* methylation in their primary tumour [58]. Goessl et al. improved the sensitivity of this technique to 73% by looking at *GSTP1* methylation in the urine sediment after prostatic massage. They found *GSTP1* methylation in one (2%) of 45 patients with BPH, two (29%) of seven patients with HGPIN, 15 (68%) of 22 patients with localised prostate cancer and 14 (78%) of 18 with locally advanced or disseminated disease [59]. Augmentation of GST activity, using pharmaceutical GST inducers, may have a role in prostate cancer prevention [60].

4.2. 8p

Two of the most common deletions found using AI and CGH are on 8p and 13q [61]. Laboratory techniques including fluorescence in situ hybridisation (FISH) and AI have found that losses in these regions are frequently found in HGPIN [62,63]. It would appear that inactivation of one or more as yet unidentified TSG in 8p and 13q is an early event in prostate cancer pathogenesis.

At least two regions of loss on 8p have been identified (8p12–21, 8p22) [61,62,64–66]. Loss of 8p12–21 is an early event in prostate carcinogenesis, occurring in HGPIN and early invasive disease, whereas loss of 8p22 is a late event, found more commonly in advanced cancers [47,67]. The *NKX3.1* gene [68] at 8p12–21, and *FEZ1* [69] at 8p22 have been implicated. *FEZ1* codes for a leucine zipper protein and alterations in this gene have been reported in oesophageal and breast as well as prostate cancer [69]. In mice with a disrupted *NKX3.1* gene, defects in prostate duct morphology and secretory function occur [70]. Furthermore *NKX3.1* mutant mice develop lesions that closely resemble HGPIN [70]. Yet mutations in the coding region of *NKX3.1* gene have not been identified [71] nor is NKX3.1 mRNA lost in prostate cancer [72]. Knock-out of a single NKX3.1 allele may be sufficient to lead to the development of HGPIN, perhaps haploinsufficiency at 8p12–21 accounts for the lack of mutations found in this region.

NKX3.1 expression is found only in adult prostate [70], whereas deletions of 8p are common in other malignancies including lung and colorectal tumours [73] suggesting the existence of other TSG in the 8p22 region.

4.3. 13q

Loss of chromosome 13q occurs in at least 50% of prostate tumours [49,74]. TSGs on the long arm of chromosome 13 include the retinoblastoma gene *RB1* (13q14), *DBM* (13q14) and the breast cancer susceptibility gene *BRCA2* (13q12–13). Mutations in the *RB* gene and loss of Rb protein has been reported in localised and advanced prostatic carcinomas [75]. *RB* is thought to regulate apoptosis in prostatic cells, particularly in response to androgens [76,77]. Several studies have however failed to demonstrate mutations in the *RB* gene [49,74,78], so an alternative gene on 13q may be a significant gene in prostatic carcinogenesis.

4.4. Cell cycle regulatory genes

In the normal prostatic epithelium the relatively low rate of cell proliferation is matched by the low rate of apoptosis. In HGPIN and early invasive disease the cell proliferation rate increases by 7–10 fold. In advanced and metastatic prostate cancer the apoptotic rate is reduced by 60% [67]. Changes in the genes that code for the cell cycle regulatory proteins may be critical early events in the development and progression of prostate cancer. The CDK4 inhibitor p27^{kip1} is a cell cycle regulatory gene whose function is frequently lost in advanced prostate cancer [79]. Loss of p27^{kip1} expression, which is mapped to 12p12–13.1, is associated with increasing tumour grade [80,81]. This gene is rarely mutated, instead lack of expression is due to aberrant phosphorylation and/or ubiquitinylation [82–84].

Another cell cycle regulatory gene is the *CDKN2* (p16) gene located on 9p21. *CDKN2* codes a cyclin-dependent kinase inhibitory protein, which controls passage through the G1 phase of the cell cycle [85]. Inactivation of *CDKN2* gene may facilitate progression through the cell cycle. Mutations have been identified in advanced and metastatic prostate cancer but not in primary tumours [86–88]. In primary prostate cancer it has been suggested that inactivation may occur by point deletion of one p16 allele and promoter methylation of the remaining allele [87]. Jarrard et al. reported three (13%) of 24 primary and one (8%) of 12 metastatic tumour samples had hypermethylation in the promoter region [87]. Deletions near the *CDKN2* gene were detected in 12 (20%) of 60 primary tumours and in 13 (46%) of metastatic lesions. Gu et al. looked at *CDKN2* in early prostate cancers and could find no evidence of methylation, although deletions close to the gene were identified [88]. Surprisingly, up-regulation of the p16 protein has been reported as a prognostic marker of disease recurrence in prostate cancer [89,90]. Similarly, up-regulation of *p16* has been reported in ovarian cancer and is associated with disease progression and unfavourable prognosis in this tumour [91]. The reason for this anomaly is not clear, it may be that the up-regulated p16 protein is abnormal and this confers a growth advantage, but there is no evidence for this theory.

Other cell cycle regulatory genes include *cyclin D1*, this gene is over-expressed in metastatic prostate cancer [92] but not primary tumours [93]. In addition, a somatic mutation has been reported in $p21^{WAF1/CIP1}$ in human prostate cancer [94] and subsequently it has been proposed that p21$^{WAF1/CIP1}$ protein expression may predict tumour recurrence [95,96].

4.5. 10q

Up to 50% of prostate cancers have been found to harbour deletions on 10q detected by FISH, CGH, AI and cytogenetics [61,63,97–100]. Two commonly deleted regions have been identified, 10q23.1 and 10q24–5. Putative TSG in these regions include *PTEN/MMAC1* (Phosphatase and Tensin homologue/Mutated in Multiple Advanced Cancers) at 10q23.23 [101] and *MXI1* on 10q25 [102,103], though their respective roles in prostate carcinogenesis is not fully established.

Germ line mutations are found in the autosomal dominant *PTEN* in cancer syndromes, such as Cowden's disease (breast and thyroid cancer) [104] and Bannayan–Zonana syndrome (multiple lipomas and haemangiomas) [105]. *PTEN* codes a lipid phosphatase, which induces p27^{kip1} expression. Loss of *PTEN* activates PKB/AKT kinase resulting in enhanced cell proliferation, decreased apoptosis, and increased angiogenesis [106].

Mice deficient in one *PTEN* allele and both *Cdkn1b* alleles (which codes for p27^{kip1}) all develop prostate cancer within 3 months of birth [107]. *PTEN* mutations have also been found in 5–27% of early and 30–58% of metastatic prostate cancers [47,101,108–110]. Inactivation of one of the two *PTEN* genes may be sufficient to allow the progression of tumour—haploinsufficiency. To demonstrate this *PTEN* knock-out, mice were bred with TRAMP mice (Transgenic Adenocarcinoma of Mouse Prostate) [111]. All TRAMP mice develop prostate cancer, mice with one *PTEN* gene knocked out developed larger more aggressive tumours and had a significantly reduced survival compared with mice with both *PTEN* genes activated. Mice with both *PTEN* genes knocked out had the same survival rate and had similar sized tumours to those with one *PTEN* gene knocked out. Rapamycin, an immunosuppressive agent that acts on FRAP/mTOR protein kinase, a downstream effector protein of *PTEN*, reduces the volume of prostate cancer xenografts in *PTEN* knock-out mice. Clinical trials are currently underway to see whether rapamycin has a similar affect in patients with prostate cancer that do not express *PTEN* [112].

MXI1 is implicated as a TSG as it codes for a *MYC*-binding protein [103,113]. The *MYC* gene has been implicated in a number of cancers, it is also mapped on 8q, a region frequently amplified in prostate cancer [114]. Some studies have found a high mutation rate in MXI1 [113,115], others have failed to detect mutations in primary prostatic tumours [116].

4.6. Metastatic prostate cancer

Metastasis requires a complex interrelated series of events including vascularisation, invasion, survival in blood or lymph, adhesion, extravasation and proliferation at a distant site [117]. This requires several critical genetic changes and these changes may act as molecular markers and targets for therapeutic intervention.

4.7. 16q

CGH and AI studies have shown losses of 16q in late stage prostate disease [50,51,118] and loss of 16q23 and q24 are associated with metastatic potential [119,120]. A candidate gene, *CDH1* coding for the E-cadherin protein, is located on 16q22.1. Decreased expression of this gene is associated with high-grade prostate cancer [50,108,109,119–122]; in addition, decreased expression is associated with metastatic potential in the primary tumour [123]. Although no mutations have been described, hypermethylation of the CpG island has been reported in cell lines. E-cadherin is calcium dependent and is responsible for cell-to-cell recognition and adhesion [124]. α and β-catenins form part of the same adhesion mechanism and decreased expression of α-catenin has been demonstrated in prostate cancers which have normal E-cadherin expression [122]. About 5% of prostate cancers contain mutations in β-catenin [125,126]. β-catenin has two known roles, firstly in cell adhesion with α-catenin and secondly as part of the *Wnt* signalling pathway.

4.8. 17p

Mutations in the TSG *p53* have been described in the majority of cancers including breast, colon and lung [127]. Located at 17p13.1, p53 codes for a nuclear phosphoprotein that has a negative effect on cell growth. In the event of DNA damage, the cell cycle is arrested or apoptosis is induced. If *p53* is inactivated and loses its function, damaged DNA may be transcribed [128]. The frequency of *p53* mutations in prostatic cancer ranges from 1% to 42% [129–134], but a consistent finding is its association with high-grade and high-stage disease coupled with disease recurrence and poor prognosis [132–136]. The frequency of *p53* mutations is relatively low in prostate compared with other cancers [67]. Prostate cancer occurs only rarely in the Li–Fraumeni cancer syndrome where patients carry a germline mutation in *p53* [137], although this may be because patients die from other malignancies before they acquire prostate cancer.

The low mutation rate, particularly in early stage disease, precludes *p53* from being an effective target for curative intervention in prostate cancer [130,138]. *p53* may however play a role as a molecular marker in radiotherapy resistance along with *BCL*, *MYC* and *RAS*.

Radiotherapy which induces apoptosis may be less effective in patients who have defective apoptotic pathways as a result of mutations in these genes [139]. Likewise the *p53* gene mutation predicts poorer overall survival for patients treated with antiandrogen therapy in locally advanced cases of prostate cancer after radiotherapy [140]. The presence of mutations in the *p53* gene may therefore be used before radiotherapy and antiandrogen therapy to predict the likelihood of treatment failure [139,141].

The breast cancer susceptibility gene *BRCA1* is also mapped to 17p. This gene is not deleted however in prostate cancer [130].

4.9. EZH2

The *EZH2* gene, situated on 7q35-36, encodes the EZH2 protein, and EZH2 protein levels are significantly increased in prostate cancer compared with BPH tissue. *EZH2* increases histone deacetylation, which is a repressor of transcription in prostate cancer [142]. Elevated levels of *EZH2* mRNA and protein in clinically localised prostate cancer predicts poor prognosis. In addition, repression of EZH2 by small interfering RNA (RNAi) decreases the rate of cell proliferation [142]. This gene represents an exciting development both as a prognostic marker and also as a therapeutic target.

4.10. ERBB2

Mutations in the *ERBB2* gene have been widely reported in a number of tumours including breast and ovarian. *ERBB2* or *HER-2*/*neu* codes for a transmembrane tyrosine kinase growth factor, which is involved in interleukin 6 (IL6) signalling through the MAP kinase pathway. IL6 induces phosphorylation in the ErbB2 and ErbB3 receptors in prostate cancer cell lines [143]. Phosphorylation inactivates the receptors resulting in inhibition of the MAP kinase pathway [143]. The recent development of a monoclonal antibody to the epidermal growth factor (EGF) receptor, anti-ERBB2 (Herceptin, Genetech Inc., South San Francisco, CA) for use in advanced breast cancer [144] has led researchers to re-focus on the role of this oncogene in prostate cancer. Trials on the use of Herceptin in prostate cancer are ongoing [145], but it is clear that high-level *ERBB2* amplification does not occur in this disease [3]. However, Herceptin does inhibit growth in prostate cancer cell lines, including LNCaP [146] and *ERBB2*

enhances AR signalling in the presence of low androgen levels [147].

4.11. KAI1

The *KAI1* gene (Kang Ai, Chinese for anticancer), found on 11p11.2-13, encodes a 2.4 kb mRNA for a membrane glycoprotein belonging to the TM4 super-family, suggesting a role in cell-to-cell interaction or cell–extracellular matrix binding [148]. *KAI-1* has been shown to suppress metastatic potential in highly metastatic Dunning rat models [149]. In addition, expression of this gene is reduced in metastatic prostate cancer cell lines [150], but no mutation in this gene has yet been found, suggesting epigenetic silencing may be the mechanism of down-regulation.

The *KAI1* gene is in close proximity to *CD44* (11p13), this latter gene codes for a membrane glycoprotein, which is also present on lymphocytes and participates in cell-to-cell interaction. Dong et al. transfected the *CD44* gene into metastatic rat prostate cells and demonstrated suppression of metastatic potential, without suppression in vivo of growth rate or tumorigenicity [151]. Moreover down-regulation of *CD44* at a protein and mRNA level correlated with metastatic potential in the Dunning rat model [151]. Studies in patients have found an inverse correlation between prostate cancer histological grade and stage with CD44 expression [152]. Immunohisto-chemical studies have shown CD44 expression is either negligible or non-existent in prostate cancer lymph node metastases.

4.12. 18q

Finally, losses of 18q have been reported to be associated with metastatic prostate cancer [63]. Two TSGs implicated in cancer progression are located in this region, *DCC* (Deleted in Colon Cancer) and *DPC4* (Deleted in Pancreatic Cancer); however, no mutation in these genes have been found in prostate cancer [153,154].

5. Hormone refractory prostate cancer

5.1. The androgen receptor

During embryological development and puberty, androgens play a key role in prostate development. Testosterone is converted to its more potent metabolite dihydrotestosterone by the enzyme 5 α-reductase. There are no reported cases of prostate cancer in men who have a congenital deficiency of 5 α-reductase. This enzyme can be blocked pharmacologically by finasteride (Proscar®) and this drug is currently undergoing a phase III trial in over 18,000 men to evaluate a possible

role in prostate cancer chemoprevention [155]. Early reports suggest a reduction in the incidence of prostate cancer in the treated arm, disturbingly, there appears to be an increased risk of high-grade tumours in men receiving finasteride. Whether this is a genuine difference or a result of the difficulties in interpreting the glandular architecture in men receiving finasteride remains to be seen [156].

Dihydrotestosterone and testosterone bind to the AR, which initiates phosphorylation and dimerisation of the receptor. The AR binds to specific DNA sequences, the androgen responsive elements, located in the promoter region of androgen responsive genes. The AR complex, in collaboration with cofactor proteins, up- or down-regulates the transcriptional activity of the androgen response genes (Fig. 1).

The removal of androgens by surgical castration or therapeutic blockade initiates apoptosis in prostate cancer cells resulting in a clinical response in up to 95% of men for a median 18 months [157]. However, some prostate cancer cells have the ability to grow even in low or absent androgen levels. These cells have a selective growth advantage over the androgen-dependent cells following androgen withdrawal. Androgen-independent cells are able to proliferate and typically have a highly aggressive phenotype.

The AR is amplified in 30% of hormone refractory prostate cancers, but is rarely amplified in untreated cases [1,158]. This suggests *AR* gene amplification is selected following androgen withdrawal. Amplification is associated with over-expression of the gene in hormone refractory disease [159,160]. Linja et al. reported some hormone refractory tumours express *AR* highly even in the absence of *AR* gene amplification [158]. Over-expression allows even very low levels of

androgen to promote androgen-dependent growth, and patients with AR amplification may respond better to therapeutic maximum androgen blockade [159].

However, Kinoshita et al. reported 10–15% of hormone refractory metastatic lesions express only low AR levels as a result of methylation in the promoter CpG islands [161]. The authors' disparity was the Linja group looked at only 13 locally recurrent cancers rather than metastases. In this small cohort they may not have detected a small subgroup with low AR levels.

AR gene amplification may enable low levels of androgen (such as adrenal androgen levels after surgical or medical castration) to stimulate androgen-dependent functions. There is also evidence that growth factors including insulin-like growth factor 1 (IGF-1), keratinocyte growth factor (KGF) and EGF may be able to activate the AR through a ligand-independent pathway; for example, the cAMP, protein kinase A and protein kinase C pathway may be able to activate the AR [162].

As well as amplification of the *AR* gene several hundred *AR* gene mutations have been reported (www.mcgill.ca), although not all these reported mutations are associated with prostate cancer. These mutations may alter the AR function and ligand specificity allowing other steroid hormones to bind, overcoming the need for androgens to stimulate growth. Mutations are uncommon in untreated prostate cancer and in cases treated with surgical castration, they are found in patients treated with the testosterone antagonist flutamide [163,164]. Approximately one-third of patients treated with flutamide have specific mutations, which result in AR activation [22]. This mutation, T887A, found only in patients treated with flutamide, changes the AR response from an antagonist to an agonist. This may explain why a minority of patients paradoxically show a temporary clinical improvement when flutamide therapy is withdrawn.

As stated, the AR complex exerts its affect on the target gene in conjunction with various cofactor proteins. Several of these proteins have been cloned including SNURF, ARIP3, ARA54, 55, 70, 160 and ANPK. Their role in cancer progression in vivo remains unknown at present.

5.2. BCL2

Bcl2 over-expression is a characteristic of advanced and hormone refractory prostate cancer and it may account for the dramatic reduction in apoptosis that occurs at this late stage [165]. Like *p53*, *Bcl2* expression maybe used as a molecular marker that correlates with disease outcome [166]. Over-expression in prostate cell lines confers a resistance to chemotherapeutic agents [167] and in a clinical setting several studies are testing whether Bcl2 inactivation can be used to prevent tumour recurrence [168,169].
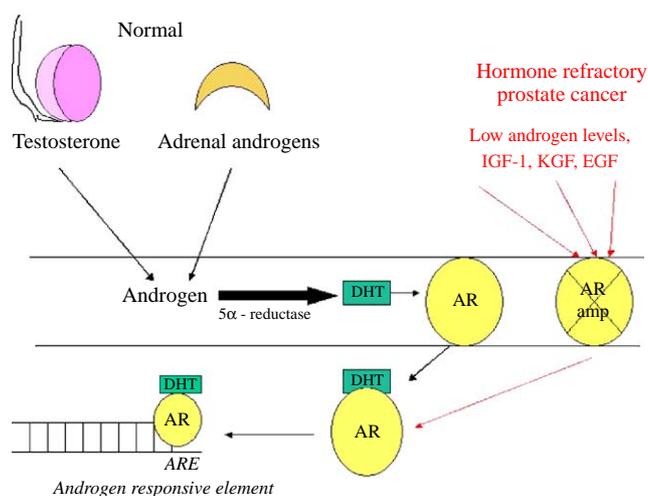

Fig. 1. In hormone refractory prostate cancer, the Androgen receptor (AR) complex, in collaboration with cofactor proteins, up- or down-regulates the transcriptional activity of the androgen response genes.

5.3. 8q

Several studies using CGH have shown that gain in 8q is a characteristic of hormone refractory disease [61,65]. Almost 90% of samples of hormone refractory prostate cancer and distant metastases compared with 5% of untreated primary tumours contained gains of the whole of 8q. Alers et al. found the number of individuals with 8q gains increases with advancing tumour stage [47]. Furthermore, gain in 8q, as well as loss of 6q, is significantly less frequently encountered in regional lymph node metastases than in distant metastases [47]. This implies differential genetic changes for haematogenous versus lymphatic spread [47].

Several sites within 8q have been suggested as containing critical oncogenes, 8q21 [65] and 8q23–q24 [170]. The oncogene *MYC* is located at 8q24.1 and amplification of this oncogene has been found in 8% of primary and 11–30% of hormone refractory or advanced prostate cancers [114,170,171]. Furthermore, amplification of this gene is associated with poorer prognosis in patients with locally advanced disease [171]. *CMYC* codes for a nuclear phosphoprotein whose function is in the promotion of DNA replication, regulation of the cell cycle and control over cell differentiation [76].

Other sites of amplification on 8q include 8q23 and 8q24.2, which harbours the putative oncogenes *EIF3S3* and *PSCA* [159,172]. Using FISH, Nupponen et al. found *EIF3S3* in 30% of cases of hormone refractory prostate cancers. Moreover, *EIFS3S* was frequently, but not exclusively, co-amplified with *MYC* [159]. *PSCA* is often co-amplified with *MYC*, and over-expression of *PSCA* has been reported in high-grade and metastatic prostate cancer [172].

6. Microarray technology

Gene expression profiling using microarrays allows a large-scale analysis of gene expression and cancer characterisation. DNA microarrays consist of thousands of spots of DNA oligonucleotides on a 'chip' each coding for part of a known or unidentified gene. Radio or fluorescently labelled DNA or RNA is applied to the chip and hybridises where there is a complementary oligonucleotide sequence. The site on the chip and intensity of the radioactive or fluorescent signal is proportional to the amount of a particular DNA/RNA. Rather than studying individual genes this technique allows examination of as many as 25,000 genes at one time.

These chips have been used in young women with node negative breast cancer to identify a gene expression signature that predicts poor prognosis [173]. In this study, 5000 genes were either significantly up- or down-regulated, a group of 70 genes could be used to accurately predict disease outcome in 83% of 78 patients. This had important clinical implications as patients with the good prognosis genetic signature could safely avoid adjuvant chemotherapy, which they would otherwise have received, saving unnecessary side effects and cost.

The signature derived included genes involved in regulating the cell cycle, invasion, metastases, cell signalling and angiogenesis (e.g. cyclin *E2*, *MCM6*, *VEGF receptor FL1*). In breast cancer, like prostate cancer, numerous studies have shown associations between individual gene expression and patient outcome (*cyclin D2*, *ER-*, *HER2/neu* and *c-myc*) [174]. In the RNA microarray analysis, none of these genes was included in the 70 marker genes. The authors concluded cancer occurs as a result of a coordinated action of several genes, so genes looked at in isolation have only limited predictive power.

Dhanasekaran et al. were one of the first groups to report gene expression profiling in prostate cancer. Using a selection of normal tissue prostate, benign prostatic pathology, primary cancer, metastatic cancer and cell lines, they identified a number of genes with altered expression patterns depending on the stage of disease. A hierarchical clustering algorithm was used to group genes based on the similarities of genes expressed. They found benign prostatic conditions, including BPH, clustered separately from malignant tissue or cell lines. Within the cancer cluster, metastatic and clinically localised cancers clustered into two succinct groups. Several genes already implicated in prostate cancer carcinogenesis had altered expression levels. *PTEN* and *CDH1* (E-cadherin) were down-regulated and *MYC* and the fatty acid synthase (*FSAN*) gene were up-regulated. In addition, numerous genes not previously implicated in prostate cancer were identified. Notably, hepsin, a cell-surface serine protease, was up-regulated by 4.3 fold on microarray analysis and this was confirmed on Northern Blot studies. Immunohistochemistry showed hepsin was preferentially expressed by neoplastic and HGPIN samples over benign prostate. In 78 men with clinically localised disease, absent or low hepsin expression was significantly associated with PSA recurrence after radical prostatectomy ($p = 0.03$). In multivariate analysis, the association of weak or absent hepsin protein expression was similar to having high-grade disease in terms of PSA outcome (hazard ratios 2.9 ($p = 0.0004$) and 1.65 ($p = 0.037$), respectively).

Micoarrays have also been used to predict tissue identity and pathological outcome after radical prostatectomy [175]. This study also examined gene expression in relation to clinical outcome after radical prostatectomy, albeit involving small patient numbers. Using RNA extracted from tumours and normal tissue of fresh radical prostatectomy specimens, the authors were able

to identify 317 genes highly expressed in the tumours and 139 genes more highly expressed in normal prostatic tissue. Using a 4- or 16-gene model they were able to predict tumour from normal tissue with an accuracy of 77% and 86%, respectively. When the gene expression patterns were compared with the clinico-pathological prognostic indicators only the Gleason score correlated with gene expression, not pathological stage, positive surgical margins or perineural invasion. Fifteen genes had expression positively correlated with Gleason score with 14 genes having expression negatively correlated with Gleason score. Most high-grade cancers expressed the positively correlated genes. Interestingly, a subset of intermediate-grade tumours also expressed these genes, possibly identifying a subset of phenotypically intermediate grade tumours with a more aggressive genotype.

Singh et al. also examined the relationship between gene expression and tumour recurrence in 21 unmatched patients, eight having relapsed and 13 having remained PSA free for at least 4 years. No single gene was statistically associated with recurrence, when a 5-gene model was used (including chromogranin A, platelet-derived growth factor receptor β, HOXC6, inositol triphosphate receptor 3 (ITPR3) and siayltransferase-1), 90% accuracy could be achieved in predicting recurrence. The Gleason score of tissue adjacent to the sample tissue was $\geqslant 8$ in 4/8 relapse patients compared with 0/13 in the non-relapse group. In this small sample of unmatched patients it is not possible to draw meaningful conclusion about the gene expression patterns and clinical outcome. This study does however demonstrate that gene expression patterns can be used to distinguish benign from malignant prostatic tissue with 86–92% accuracy.

Welsh et al. performed microarray analysis on the cRNA of 24 patients with clinically localised prostate cancer [176]. They identified a large number of genes with expression specific to malignant cells including hepsin and *FASN*. The gene expression of malignant cells could be divided into two groups by the expression patterns of a group of ribosomal genes and patients with higher Gleason score disease had significantly lower ribosomal gene expression ($p<0.01$). The authors emphasised the potential for therapeutic targeting of differentially expressed genes including *FASN*, hepsin and MIC-1, though no new evidence was presented in this study to support this [176].

The results of gene expression profiling across the cancer spectrum are promising, and classification of cancers according to their genetic expression are now possible [177–179]. Tailoring cancer treatments according to gene expression patterns is already underway and therapeutic targets are being tested. Prostate cancer poses several difficulties. The heterogeneous nature of the disease means that while the expression pattern of one area of tumour may accurately predict prognosis for that tumour the other foci with different genetic make-up may behave differently to the index lesion. For the same reason, therapeutic modulation of genes with altered expression patterns may select out those tumours which do not have altered expression of the particular gene, in the same way that antiandrogen treatment selects androgen-independent prostate cancer cells. In prostate cancer, the results of prospective studies to examine gene expression and patient outcome take many years to mature. The studies to date are retrospective and have involved small sample sizes, larger-scale trials are required to demonstrate the validity and reproducibility of these results before these experimental techniques are used in the prostate cancer clinic.

Advances in the molecular biology laboratory will have a fundamental affect on our understanding, diagnosis and treatment of many clinical conditions, including prostate cancer. During the last 10 years, our knowledge of genetic alterations in prostate cancer has significantly increased and it appears that due to the heterogeneity of prostate cancer, multiple genes may be involved in the neoplastic process. However, these genetic aberrations are natural targets for new treatment strategies and identification of specific molecular genetic changes in prostate carcinomas may become clinically useful diagnostic and prognostic tools.

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