

## REVIEW

## Molecular pathology of prostate cancer

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The molecular pathology of prostate cancer is complex; not only are multiple genes involved in its pathogenesis, but additional environmental factors such as diet and inflammation are also involved. The exhaustive research into prostate cancer to date has demonstrated a complex interaction of multiple genes and environmental factors, some of which may be more important in individual prostate cancer cases. This is an exciting era, with the emergence of new investigative tools such as DNA microarray technology and the application of the field of proteomics to the study of human cancers. Knowledge of genetic changes underlying the initiation, development, and progression of prostate cancer is accumulating rapidly. With increasing knowledge, it may be possible to distinguish indolent from aggressive prostate tumours by molecular fingerprinting. This review discusses the most consistently reported molecular pathological findings in hereditary and sporadic prostate cancer, together with new concepts and technologies.

largely reflects an increasing number of men being diagnosed with very early stage prostate cancer as a result of the widespread use of prostate specific antigen (PSA) testing. PSA is a protein produced by both normal and cancerous prostate cells and a high PSA value can be a sign of cancer. Most men diagnosed at a very early stage will die with prostate cancer but not from it; therefore, the survival rate has increased. The American Cancer Society recommends in its prostate cancer screening guidelines that men should be informed of what is known and what is uncertain about the benefits and limitations of early detection of prostate cancer, so that they can make an informed decision about testing. Therefore, the early diagnosis of prostate cancer through screening creates difficulties in predicting the outcome of individual patients. The difficulty is in distinguishing between clinically indolent prostate cancers, which will be asymptomatic, and aggressive prostate cancers with the potential to kill the patient. Gleason grading on histopathological examination is the best prognostic indicator to date in prostate cancer; however, interobserver variation can occur, grading on biopsies may not correlate with the prostatectomy specimen because of sampling problems, and cases of morphologically identical prostate cancer can behave differently.

Prostate cancer is the second leading cause of cancer deaths in men. It is not invariably lethal, however, and is a heterogeneous disease ranging from asymptomatic to a rapidly fatal systemic malignancy. The prevalence of prostate cancer is so high that it could be considered a normal age related phenomenon: in a Spanish study examining white Mediterranean men, 33% of men in their 8th decade had evidence of prostate cancer at necropsy and died with the disease, but not from it. Data from American postmortem studies show an even higher prostate cancer prevalence rate.<sup>1</sup> In recent years, there have been large increases in the five year survival rates for prostate cancer, with a five year relative age standardised survival rate of 65% in England and Wales for the years 1996–9. This was the third highest survival rate of all cancers over this time period, with only testicular cancer and melanoma having better survival rates, and was around 11% higher than that for patients diagnosed during 1991–5.<sup>2</sup>

This is an exciting era with the emergence of new investigative tools such as DNA microarray technology and the application of the field of proteomics to the study of human cancers. Knowledge of genetic changes underlying the initiation, development, and progression of prostate cancer is accumulating rapidly. With increasing knowledge it may be possible to distinguish indolent from aggressive prostate tumours by molecular fingerprinting. A clinical application of this knowledge would be that radical treatment and its associated morbidity could be avoided in prostate cancers that are unlikely to progress. Resources and radical treatment could be focused on prostate cancers with poor prognostic indicators. In this review, we shall discuss the most consistently reported molecular pathological findings in prostate cancer, together with new concepts and technologies.

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"The prevalence of prostate cancer is so high that it could be considered a normal age related phenomenon"

Unfortunately, this improvement does not reflect better treatment for prostate cancer. It

**Abbreviations:** AMACR,  $\alpha$ -methylacyl coenzyme A racemase; AR, androgen receptor; GSTP1, glutathione S-transferase; IFN, interferon; IL-6, interleukin 6; KLF, Kruppel-like factor; MAPK, mitogen activated protein kinase; P13K-Akt, phosphatidylinositol 3'-kinase-protein kinase B; PIN, prostate intraepithelial neoplasia; PSA, prostate specific antigen; PTEN, phosphatase and tensin homologue; Rb, retinoblastoma; STAT, signal transducer and activator of transcription; VDR, vitamin D receptor

## HEREDITARY PROSTATE CANCER

Prostate cancer can be divided epidemiologically into hereditary and sporadic forms,<sup>3</sup> but it is not possible to distinguish these two groups at a molecular level. Highly penetrant inherited genes conferring the prostate cancer phenotype have not been identified.

Linkage studies using genetic markers to search for chromosomal regions that show excessive sharing of inherited alleles in cancer affected families have been helpful in identifying important cancer susceptibility genes in other cancers. However, similar studies using families prone to prostate cancer have not yielded the same success.

*“The failure to identify highly penetrant genes in hereditary prostate cancer may result from the fact that multiple genes with a small to moderate effect are involved in hereditary prostate carcinogenesis”*

Although possible inherited prostate cancer susceptibility genes have been identified such as the ELAC2, RNASEL, MSR1, NSB1, and CHEK2 genes in some families (table 1), the proportion of cases of hereditary prostate cancer attributable to germline mutations in these loci is small. Many studies have not supported the role of these genes in hereditary prostate cancer. Mutations of these candidate genes have also been identified in sporadic prostate cancer. Because prostate cancer is a common cancer, it may be difficult to distinguish clustering of sporadic prostate cancer within families from true hereditary prostate cancer. This difficulty may have hindered research into hereditary prostate cancer to date. Alternatively, the failure to identify highly penetrant genes in hereditary prostate cancer may result from the fact that multiple genes with a small to moderate effect are involved in hereditary prostate carcinogenesis. The risk of disease in the presence of a susceptibility gene might be substantially increased only in the appropriate genetic, dietary, and environmental background.<sup>4</sup> We will briefly outline the most important hereditary prostate cancer susceptibility genes identified to date.

### ELAC2

ELAC2 was the first possible hereditary prostate cancer gene to be identified. The function of ELAC2 is not definitively known and it has been proposed as a metal dependent hydrolase. An association of ELAC2 genotypes with familial prostate cancer has been reported.<sup>5</sup> However, multiple large subsequent studies have not provided confirmatory evidence of this association.<sup>6,7</sup>

Overall, it appears that if ELAC2 plays a role in prostate cancer it is a relatively minor role.

### Host response to infection genes

#### RNASEL

RNASEL is a ribonuclease that degrades viral and cellular RNA and can produce apoptosis on viral infection. Mutations

in the RNASEL gene have been identified in familial and sporadic prostate cancer in many studies,<sup>8-12</sup> although other studies have not supported these findings.<sup>13,14</sup>

Overall, there is strong support for the notion that RNASEL is the most important hereditary prostate cancer gene identified to date.

#### MSR1

MSR1 encodes a macrophage scavenger receptor responsible for cellular uptake of molecules, including bacterial cell wall products. The importance of MSR1 as a prostate cancer susceptibility gene in hereditary prostate cancer is controversial. Germline MSR1 mutations have been linked to prostate cancer in some families with prostate cancer and in sporadic prostate cancer.<sup>15,16</sup> However, a recent report, which investigated 163 families with familial prostate cancer, did not provide confirmatory evidence of the role of MSR1 in familial prostate cancer.<sup>17</sup>

Mutations of these host response to infection genes may increase the risk of prostate cancer by predisposing to chronic inflammation as a result of failure of viral RNA and bacterial degradation. There is accumulating knowledge supporting the role of inflammation in prostate cancer, which we will refer to again later in the article.

### Cell cycle checkpoint genes

#### NBS1

The rare human genetic disorder, Nijmegen breakage syndrome, is characterised by radiosensitivity, immunodeficiency, chromosomal instability, and an increased risk for cancer of the lymphatic system. The NBS1 gene, which is involved in this human genetic disorder, encodes a protein, nibrin, involved in the processing/repair of DNA double strand breaks and in cell cycle checkpoints.<sup>18</sup> Mutations in the gene for the Nijmegen breakage syndrome (NBS1) have been identified in both sporadic and familial cases of prostate cancer and are associated with a small increased risk of prostate cancer.<sup>19</sup>

#### CHEK2

The CHEK2 gene is an upstream regulator of p53 in the DNA damage signalling pathway. CHEK2 mutations have been identified in both sporadic and familial cases of prostate cancer and are associated with a small increased risk of prostate cancer.<sup>20,21</sup>

NBS1 and CHEK2 genes have only recently been identified as possible prostate cancer susceptibility genes. ELAC2 was the first hereditary prostate cancer susceptibility gene identified and subsequent studies have not provided confirmatory evidence of its role in prostate cancer. Therefore, it is not possible to comment on the importance of these two genes in hereditary prostate cancer until additional confirmatory studies have been performed.

The study of hereditary prostate cancer genes is in its infancy and the challenge for the future will be to detect genes with small to moderate effects. Advances in statistical

**Table 1** Hereditary prostate cancer genes

Gene	Chromosomal locus	Putative function	Status in prostate cancer
ELAC2	17p	Metal dependent hydrolase	Unknown
RNASEL	1q	Ribonuclease that degrades viral and cellular RNA and can produce apoptosis on viral infection	Deleted
MSR1	8p	Encodes a macrophage scavenger receptor responsible for cellular uptake of molecules, including bacterial cell wall products	Deleted
NBS1	5p	Encodes a protein, nibrin, involved in the processing/repair of DNA double strand breaks and in cell cycle checkpoints	Deleted
CHEK2	22q	Upstream regulator of p53 in the DNA damage signalling pathway	Deleted

methods to amplify signals from susceptibility genes in the presence of heterogeneous factors are required to decipher the genetics and molecular pathology of hereditary prostate cancer.

### SPORADIC PROSTATE CANCER

Most prostate cancers are sporadic. In our discussion of the molecular pathology of sporadic prostate cancer we will discuss the evidence to date under the following categories: polymorphisms associated with increased prostate cancer risk, somatic genetic changes, and factors involved in the progression of prostate cancer, such as the androgen receptor, growth factors, and invasion and metastasis genes. We will discuss separately recent findings of gene overexpression and underexpression by microarray technology. The application of the field of proteomics to the study of prostate cancer and current theories regarding the role of inflammation in prostate cancer will also be discussed.

### Polymorphisms associated with increased prostate cancer risk (table 2)

A polymorphism is a genetic variant that appears in at least 1% of the population. These common genetic polymorphisms probably have small relative risks, yet large population attributable risks because of their high frequencies.

#### TLR4

TLR4 encodes a receptor that is a central player in the signalling pathways of the innate immune response to infection by Gram negative bacteria. A TLR4 sequence polymorphism is associated with a small increased risk of prostate cancer.<sup>22</sup> This is in keeping with the current hypothesis of inflammation having a role in prostate carcinogenesis. We will discuss the current hypothesis of the role of inflammation in prostate cancer later in the article.

#### CDKN1B (p27)

The loss of cell cycle control is thought to be an important mechanism in the promotion of carcinogenesis. CDKN1B (p27) belongs to the Cip/Kip family and functions as an important cell cycle gatekeeper.

A recent study has revealed a significant association between a single nucleotide polymorphism of CDKN1B (p27) and prostate cancer.<sup>23</sup> There is also an association between CDKN1B (p27) and another Cip/Kip family member, CDKN1A (p21), and advanced prostate cancer.<sup>24</sup>

#### Androgen receptor (AR)

Growth of prostate cells depends on androgens. Genes that encode products that play a role in inducing androgen stimulation of the prostate gland are very important. The

androgen receptor (AR) is currently a therapeutic target for the treatment of prostate cancer. Other genes involved in androgen stimulation of the prostate such as SRD5A2 and CYP17 also hold potential as future therapeutic targets.

The AR contains polymorphic polyglutamine (CAG)<sub>n</sub> trinucleotide repeats. It has been reported in the past that shortening of these repeats is associated with increased prostate cancer risk.<sup>25</sup>

Short CAG length has also been correlated with high grade, high stage, metastatic, and fatal prostate cancers. A hypothesis that has been proposed for the influence of the short CAG repeat on prostate carcinogenesis is that because of its role in AR function it causes an increase in activation of androgen dependent genes.<sup>26</sup>

Other groups have not identified CAG repeats as a risk factor for prostate cancer and a recent study and an epidemiological review article have shown that this risk factor is less important than thought previously.<sup>27 28</sup>

#### CYP17

CYP17 encodes cytochrome P-450c17 $\alpha$ , an enzyme responsible for the biosynthesis of testosterone. A variant CYP17 allele is associated with both hereditary and sporadic prostate cancer.<sup>29</sup> This allele is hypothesised to increase the rate of gene transcription, increase androgen production, and thereby increase the risk of prostate cancer.<sup>30</sup>

#### SRD5A2

SRD5A2 encodes the predominant isozyme of 5 $\alpha$ -reductase in the prostate, an enzyme that converts testosterone to the more potent dihydrotestosterone. The alleles that encode enzymes with increased activity have been associated with an increased risk of prostate cancer and with a poor prognosis for men with prostate cancer.<sup>31 32</sup>

### Polymorphisms associated with advanced sporadic prostate cancer (table 3)

#### Vitamin D receptor

Physiological concentrations of vitamin D promote the differentiation and growth arrest of prostate cancer cells in vitro.<sup>33</sup> The precise mechanism through which vitamin D mediates this effect is unknown, although it is probably through its effect on cell growth proteins. Allelic differences in the vitamin D receptor (VDR) gene result in variation in VDR activity.<sup>34</sup> VDR alleles have been significantly associated with prostate cancer and this association was stronger with advanced prostate cancers.<sup>35</sup> As discussed previously, polymorphisms in CDKN1A (p21<sup>cip</sup>) and CDKN1B (p27<sup>kip</sup>) are associated with advanced prostate cancer.<sup>24</sup> In addition, polymorphic variants of several other genes have been proposed as possible contributors to the risk of prostate cancer.<sup>36</sup>

**Table 2** Polymorphisms associated with increased prostate cancer risk

Gene	Chromosomal locus	Function
TLR4	9q	Encodes a receptor that is a central player in the signalling pathways of the innate immune response to infection by Gram negative bacteria
CDKN1B (p27)	12p	Belongs to the Cip/Kip family and functions as an important cell cycle gatekeeper
AR	Xq	May cause activation of androgen dependent genes
CYP17	10q	Enzyme responsible for the biosynthesis of testosterone.
SRD5A2	2p	Converts testosterone to the more potent dihydrotestosterone

AR, androgen receptor.

**Table 3** Polymorphisms associated with advanced prostate cancer risk

Gene	Chromosomal locus	Function
Vitamin D receptor	13q	Promotes the differentiation and growth arrest of prostate cancer cells in vitro
CDKN1A (p21)	6p	Belong to the Cip/Kip family and functions as an important cell cycle gatekeeper
CDKN1B (p27)	12p	

### Somatic genetic changes

The number of genetic loci involved in prostate carcinogenesis is large and the mechanisms are complex and not fully understood. Table 4 lists the most commonly reported chromosomal abnormalities in sporadic prostate cancer, together with the putative genes involved at these chromosomal sites.<sup>37 38</sup>

Although these are the most common areas of chromosomal loss and gain, prostate carcinogenesis is complex and multiple genes from other chromosomal loci are also thought to be involved.

### Tumour suppressor genes and loss of heterozygosity

Tumour suppressor genes are probably involved in the prostate carcinogenesis pathway. Loss of tumour suppressor genes was initially proposed to occur via loss of function of two alleles (the "two hit hypothesis") by mutation or deletion.<sup>48</sup> This model has been revised to include epigenetic modification by (a) inactivation of one or both alleles by DNA methylation of CpG sites in gene promoters, (b) function heritably downregulated, or (c) otherwise compromised in a clonal fashion.<sup>49</sup> The change can be by mutation, methylation of the promoter, or by some other modification of the protein product, and must be coupled with evidence that the normal (wild-type) gene does suppress growth of tumour cells.<sup>50</sup>

### Glutathione S-transferase gene

The glutathione S-transferase (GSTP1) gene is emerging as one of the most important tumour suppressor genes in prostate cancer. GSTP1 can detoxify environmental electrophilic carcinogens and oxidants and may play a genome caretaker role by preventing oxidant and electrophilic DNA damage.<sup>51</sup> GSTP1 has been shown to be inactivated by hypermethylation of the promoter region in prostate tumours.<sup>52 53</sup> Hypermethylation of GSTP1 is the most common (> 90%) reported epigenetic alteration in prostate cancer. It occurs early in cancer progression and is a promising marker for detecting organ confined disease. The quantitation of GSTP1 hypermethylation can accurately detect the presence of cancer even in small, limited tissue samples. It is a promising diagnostic marker that could possibly be used as an adjunct to tissue biopsy as part of prostate cancer screening.<sup>54</sup>

Aberrant DNA methylation patterns may be the earliest somatic genome changes in prostate cancer. A recent study found that CpG islands were hypermethylated in > 85% of prostate cancers and cancer cell lines but not in normal prostate cells and tissues. CpG island hypermethylation patterns in prostate cancer metastases were very similar to the primary prostate cancers and tended to show greater differences between cases than between anatomical sites of metastasis.<sup>55</sup>

### PTEN

PTEN (phosphatase and tensin homologue) is an important tumour suppressor gene in prostate cancer and influences the concentrations of CDKN1B (p27) another important tumour suppressor gene. The PTEN gene encodes a phosphatase active against both protein and lipid substrates and is a common target for somatic alteration during the progression of prostate cancer.<sup>56 57</sup> PTEN is present in normal epithelial cells and in cells in prostatic intraepithelial neoplasia (PIN). In prostate cancers, concentrations of PTEN are often reduced, particularly in cancers of high grade or stage. In prostate cancers that do contain PTEN, there is considerable heterogeneity in concentrations, with regions devoid of PTEN being described.<sup>44</sup> The mechanism by which PTEN might act as a tumour suppressor gene in the prostate may involve inhibition of the phosphatidylinositol 3'-kinase-protein kinase B (PI3K-Akt) signalling pathway, which is essential for cell cycle progression and cell survival.<sup>58</sup>

### CDKN1B (p27)

CDKN1B (p27) is an important tumour suppressor gene in prostate cancer. Reduced concentrations of p27, a cyclin dependent kinase inhibitor encoded by the CDKN1B gene, are common in prostate cancers, particularly in those with a poor prognosis.<sup>59 60</sup> The somatic loss of DNA sequences at 12p12-3, encompassing CDKN1B, has been described in 23% of localised prostate cancers, 30% of prostate cancer metastases in regional lymph nodes, and 47% of distant prostate cancer metastases.<sup>61</sup> Concentrations of p27 are suppressed by the PI3K-Akt signalling pathway.<sup>58</sup> By inhibiting PI3K-Akt, PTEN can increase the concentration of CDKN1B mRNA and p27 protein.<sup>62</sup> For this reason, low p27 concentrations may be as much a result of the loss of PTEN function as of CDKN1B alterations.

### NKX3.1

Loss of 8p appears to be an early event in prostate cancer and the most promising candidate tumour suppressor gene at this site is NKX3.1, which encodes a prostate specific homeobox gene that is probably essential for normal prostate development. NKX3.1 binds to DNA and represses expression of the PSA gene.<sup>63</sup> Loss of NKX3.1 expression appears to be related to the progression of prostate cancer. One study found that NKX3.1 was absent in 20% of PIN lesions, 6% of low stage prostate cancers, 22% of high stage prostate cancers, 34% of androgen independent prostate cancers, and 78% of prostate cancer metastases.<sup>42</sup> The loss of this gene is of particular interest because when present it represses expression of the PSA gene, and the loss of NKX3.1 may be involved in the increasing concentrations of PSA seen with prostate cancer progression.

**Table 4** The most commonly described areas of chromosomal loss and gain in prostate cancer

Chromosome locus	Putative genes	Normal function of gene	Status of gene in prostate cancer	Ref
7p	EGFR	Growth factor	Amplified	39
7q	CAV 1	Structural protein of caveolae membranes in fibroblasts and endothelia	Amplified	40
8p	MSR	Encodes a macrophage scavenger receptor responsible for cellular uptake of molecules including bacterial cell wall products	Deleted	41
8p	NKX3-1	Tumour suppressor gene	Deleted	42
8q	c-myc	Transcriptional activator	Amplified	43
10q	PTEN	Tumour suppressor gene	Mutated	44
13q	Rb	Tumour suppressor gene	Deleted	45
16q	E-CAD	Adhesion molecule	Deleted	46
Xq	AR	Androgen receptor	Amplified	47

EGFR, epidermal growth factor receptor; PTEN, phosphatase and tensin homologue; Rb, retinoblastoma.

## KLF6

Kruppel-like factors (KLFs) comprise a group of transcription factors that appear to be involved in different biological processes including carcinogenesis. Important genetic alterations of KLF6 have been reported, including deletions and loss of expression in a minority of high grade prostate cancers.<sup>64 65</sup>

KLF6 and NKX3.1 have not been reported as frequently as the tumour suppressor genes previously discussed and have been identified because of the fact that they are within areas of frequent allelic loss in prostate tumours.

## Retinoblastoma

Retinoblastoma (Rb) has been reported to be an important tumour suppressor gene in many human cancers, and prostate cancer is no exception. The disruption of the normal Rb regulatory pathway is associated with the pathogenesis of many human cancers. The Rb gene plays an important role in the G1 phase of the cell cycle. The Rb protein binds tightly to the E2F family of transcription factors. When phosphorylated, the Rb protein releases the E2F proteins, causing transcriptional activation of a variety of genes involved in cell growth.<sup>45 66 67</sup> Inactivation of Rb appears to be important in neoplastic transformation, because expression of wild-type Rb in Rb negative prostate cancer lines results in loss of tumorigenicity.<sup>68</sup> The predominant mechanism of Rb inactivation involves allelic loss or mutation, but decreased transcription of Rb has also been reported.<sup>69 70</sup>

## p53

Mutations in p53 are common in human neoplasms, but there is only a low frequency of mutation of this gene in prostate cancer. However p53 has an important role in prostate cancer progression because abnormal p53 expression is associated with bone metastases and the development of androgen independent disease. Abnormal p53 expression correlates with high histological grade, high stage, and clinical disease progression.

The p53 tumour suppressor gene product restricts entry into the synthetic phase of the cell cycle and promotes apoptosis in cells that are disorganised or have damaged DNA. Loss of normal p53 function results in uncontrolled cell growth.<sup>71</sup>

The analysis of p53 expression can be difficult. The mutated p53 gene product has a longer half life, thus rendering it detectable by immunohistochemistry. However, sensitive immunohistochemical techniques may detect over-expressed normal p53. Therefore, it is more reliable to detect mutations in p53 by molecular techniques. Abnormal p53 expression is correlated with reduced survival after radical prostatectomy.<sup>72</sup>

## Oncogenes

c-myc and bcl-2 are well known and important oncogenes not only in prostate cancer but in many human cancers.

### c-myc

Several studies have demonstrated increased myc expression in prostate cancer and a significant correlation of myc overexpression with Gleason grade. The myc oncogenes are members of the basic helix-loop-helix-leucine zipper (bHLHZ) family of transcription factors. Myc proteins act as transcriptional activators or repressors through dimerisation or with other bHLHZ family members.<sup>43 70 73-75</sup>

### bcl-2

The bcl-2 family of genes are commonly expressed in primary and metastatic prostate cancers. bcl-2 is not expressed in the normal prostate. Proteins of the bcl-2 family play a crucial role in the regulation of apoptosis and the bcl-2 gene inhibits

programmed cell death.<sup>76-78</sup> bcl-2 has also been implicated in the development of androgen independent prostate cancer because of its increased expression in androgen independent cancers in the advanced stages of disease.<sup>79 80</sup>

Other oncogenes have only recently been recognised, but may emerge as novel targets for molecular genetic intervention, or through modified expression may allow accurate prediction of the way in which a tumour is likely to progress.

## c-Kit/tyrosine kinase receptor

c-Kit (tr-Kit) is a strong activator of the Src family tyrosine kinases. In a recent study it has been reported that human tr-Kit mRNA and protein are expressed in prostatic cancer cells. This study also describes for the first time the existence of a truncated c-Kit protein in primary tumours and shows a correlation between tr-Kit expression and activation of the Src pathway in the advanced stages of the disease.<sup>81</sup>

## STAT5

A signal transducer and activator of transcription, 5 (STAT5) has been identified as a crucial survival factor for prostate cancer cells.<sup>82</sup> Activation of STAT5 is also associated with high histological grade of prostate cancer.<sup>83</sup>

## Telomerase and telomere shortening

Telomere length has been found to be strikingly shorter in prostate cancer, including PIN, than in normal prostate. Telomeres stabilise and protect the ends of chromosomes, but shorten because of cell division and/or oxidative damage. Critically short telomeres, in the setting of abrogated DNA damage checkpoints, have been shown to cause chromosomal instability, leading to an increase in cancer incidence as a result of chromosome fusions, subsequent breakage, and rearrangement. In normal cells, successive cycles of cellular replication result in progressive loss of telomeric sequences. Normal cells sense very short telomeres as evidence of DNA damage and exit the cell cycle.<sup>84</sup> Telomerase is an enzyme that maintains telomere length by adding repetitive telomeric sequences to chromosome ends. Telomerase activity is present in most prostate cancers and not in normal prostate epithelium.<sup>85 86</sup>

## Androgen receptor (AR)

The AR plays a crucial role in prostate cancer. AR blockade can delay the progression of prostate cancer and is used to treat patients unsuitable for radical surgery or with cancer that has spread beyond the prostate. It has been studied extensively in prostate cancer because androgens are required for the development of both the normal prostate and prostate cancer. Initially, most prostate cancers are sensitive to androgen deprivation.

However, in patients with advanced disease, most tumours progress to an androgen independent state with proliferation of cells that do not require androgens for growth.

The mechanism of acquired androgen insensitivity is unknown and has been the subject of much research, because androgen insensitive prostate cancers can no longer be treated with endocrine therapy.

Mutations, amplifications, and deletions of the AR gene and structural change in the AR protein have been postulated to cause androgen insensitivity.<sup>47 87-91</sup>

*"In patients with advanced disease, most tumours progress to an androgen independent state with proliferation of cells that do not require androgens for growth"*

In an analysis of 44 mutant ARs from prostate cancers, seven had loss of function, three maintained wild-type

function, 14 demonstrated partial function, and 20 displayed a gain in function.<sup>92</sup>

However, structural change of the AR has only been identified in a minority of androgen insensitive prostate cancers, so that other factors must also be involved in this phenomenon.

Growth factor stimulation may sensitise the AR transcriptional complex to subphysiological concentrations of androgen.<sup>93</sup> We will refer to this topic once again when we discuss the role of growth factors in prostate cancer.

### Growth factors

Growth factors are important in the normal regulation of prostate development and growth. However, the inappropriate expression of members of the growth factor families has been associated with prostate cancer progression.<sup>94</sup>

#### Interleukin 6

Interleukin 6 (IL-6) modulates cell growth and apoptosis. It is a multifunctional cytokine that activates the STAT and/or mitogen activated protein kinase (MAPK) signalling pathways. IL-6 values are raised in tissues and sera from patients with prostate cancer and IL-6 receptor expression has been detected in prostate cancer cell lines and clinical specimens.

Chronic exposure of prostate cancer cells to IL-6 has been found to facilitate tumour growth in vivo by abolishing growth control by the Rb protein and activation of the MAPK signalling pathway.<sup>95</sup> IL-6 has also been shown to play a role in the interaction between epithelial and stromal cells in prostate cancer.<sup>96</sup>

#### Epidermal, transforming, vascular endothelial, and insulin growth factors

Many of these growth factor receptors engage the Ras–MAPK pathway as part of their signalling activities. These growth factors have been shown to be associated with invasion and metastasis of prostate cancer.<sup>93 97–99</sup> Transforming growth factor  $\beta$  and vascular endothelial growth factor can also cause prostate cancer progression by acting as angiogenic factors increasing microvessel density around the cancer.<sup>100 101</sup> There is evidence that chronic activation of endogenous c-Ras by autocrine and paracrine growth factor stimulation sensitises the AR transcriptional complex to subphysiological concentrations of androgen. Progression to hormone refractory disease is often correlated with overexpression of growth factors and receptors capable of establishing autocrine and/or paracrine growth stimulatory loops.<sup>93</sup> Chemotherapy with the aim of interrupting these loops may be a possibility for the treatment of prostate cancer in the future.

### Growth factor receptors

Growth factor receptors have been recognised as important oncogenes in many cancers, particularly the growth factor c-erb 2 (Her-2 neu).

#### C-erb 2 (Her-2 neu)

There is some controversy over the role of c-erb 2 (Her-2 neu) in prostate cancer.

C-erb 2 belongs to the epidermal growth factor receptor family. Some fluorescence in situ hybridisation studies of primary prostate cancer specimens have suggested that c-erb 2 gene amplification and neu overexpression are significantly correlated with DNA content, advanced grade, and advanced stage.<sup>102–104</sup> However, large studies using fluorescence in situ hybridisation (339 cases) and comparative in situ hybridisation (126 cases) showed that c-erb 2 is not amplified in prostate cancer.<sup>105 106</sup>

“There is some controversy over the role of c-erb 2 (Her-2 neu) in prostate cancer”

Immunohistochemical studies have given rise to conflicting results because of the use of different methodologies and different antibodies. Some studies report c-erb 2 (Her-2 neu) overexpression in prostate cancer and some suggest that expression increases as prostate cancer progresses to androgen independence.<sup>107 108</sup> Other studies have not identified Her-2 neu amplification or overexpression in prostate cancer.<sup>109–111</sup> In summary, when evaluated scientifically the research to date shows that the c-erb 2 (Her-2 neu) gene is not amplified in prostate cancer. Whether c-erb 2 (Her-2 neu) is overexpressed in prostate cancer remains controversial, but studies with immunostaining and with quantitative reverse transcription polymerase chain reaction have shown that the expression of c-erb 2 is much lower than in—for example, breast carcinomas, in which c-erb 2 (Her-2 neu) amplification and overexpression is common.<sup>112</sup> Chemotherapy currently targeted towards c-erb 2 overexpression in breast cancer is unlikely to have similar clinical application in prostate cancer.

#### Fas/Fas ligand

Fas is a type I membrane receptor of the tumour necrosis factor/nerve growth factor family. On binding to Fas ligand, a type II transmembrane protein, the Fas–Fas ligand complex induces apoptosis in target cells. Dysregulation of Fas and Fas ligand mediated apoptosis is thought to be involved in prostate tumorigenesis. The Fas–Fas ligand complex has been found to be raised in prostatic intraepithelial neoplasia and prostatic adenocarcinoma.<sup>113</sup>

#### c-met

Hepatocyte growth factor and its receptor, the c-met protooncogene product (c-met), have been implicated in embryogenesis, tissue reorganisation, and tumour progression.

The c-met protein has been detected in a substantial number of prostate cancers and has been found more often in metastatic growths of prostate carcinoma and in androgen insensitive prostate cancer cell lines. There is also evidence that c-met (hepatocyte growth factor) enhances the invasive potential of prostate cancer cells.<sup>114</sup> High c-met receptor expression has also been identified in prostate cancer metastasis to bone.<sup>115 116</sup>

#### Invasion and metastasis suppressing genes

For cancer cells to spread to distant sites they must invade the stroma, penetrate the vasculature, implant at distant sites, and be able to survive there. Changes of adhesion to the substratum are crucial for tumour cell invasion and distant metastasis. Several genes encoding proteins involved in invasion and metastasis in prostate cancer have been identified.

#### E-cadherins

The cadherins are membrane glycoproteins that play an important role in cellular differentiation by mediating cell–cell recognition and adhesion. Reduction of E-cadherin expression is a common occurrence in prostate cancer, and has been reported to correlate with tumour grade, stage, and survival.<sup>46 117–119</sup> However, the degree of E-cadherin expression in prostate cancer remains controversial. Normal expression of E-cadherin was found in most prostate carcinoma cases examined in an immunohistochemical study that systematically evaluated E-cadherin expression in a broad range of formalin fixed prostate tissues.<sup>120</sup>

#### Integrins

Normal basal epithelial cells in the human prostate express integrins but their expression is abnormal or absent in most prostate cancers.<sup>121–123</sup>

### C-CAM

The C-CAM cell adhesion molecule is expressed on the surface of normal prostate epithelium but is absent in most prostate cancers.<sup>124</sup> Loss of C-CAM1 expression occurs early in the development of prostate cancer, suggesting that C-CAM1 may help maintain the differentiated state of the prostate epithelium. Reintroduction of C-CAM1 into cancer cells can reverse their cancerous growth.<sup>125</sup>

### KAI1/CD82

Metastasis suppressor genes are defined as genes that do not affect the growth of primary tumour cells but can inhibit development of distant metastases.<sup>126</sup> The cancer metastasis suppressor KAI1/CD82 belongs to the tetraspanin superfamily and inversely correlates with the metastatic potential of a variety of cancers, including prostate cancers. CD82 expression is reduced or absent in most primary prostate cancers and in more than 90% of metastatic prostate cancers.<sup>127 128</sup> It is thought that the mechanism of KAI1/CD82 mediated metastasis suppression involves a cell surface protein physically associated with KAI1/CD82, named KASP.<sup>129</sup>

### CD44

CD44 is another metastasis suppressor gene for prostatic cancer and CD44 expression is inversely correlated with histological grade, ploidy, and distant metastases.<sup>130 131</sup>

### Other metastasis suppressor genes

Additional candidate metastasis suppressor genes that have been identified for prostate cancer are NME23, mapsin, BRMS1, KISS1, and MAP2K4.<sup>126</sup>

### Clinical implications

The identification of invasion and metastasis suppression genes has potential clinical applications. Prostate cancers with loss of these genes may have a potentially metastatic phenotype and may require more aggressive treatment in contrast to cancers that have retained expression.

*“The identification of invasion and metastasis suppression genes has potential clinical applications”*

In our opinion, the most promising genes that could be used as specific targets for the detection, diagnosis, and treatment of prostate cancer include the tumour suppressor genes GSTP1, NKX3.1, PTEN, and p27. NKX3.1, PTEN, and p27 also involve growth factor signalling pathways, which have potential for molecular genetic intervention. Genes that play a role in inducing androgen stimulation of the prostate gland such as AR, SRD5A2, and CYP17 are also potential targets for gene therapy in the future.

### GENE OVEREXPRESSION AND UNDEREXPRESSION IN PROSTATE CANCER DETECTED BY CDNA MICROARRAYS

Given the difficulty in finding inherited susceptibility genes, the evidence that prostate cancer is probably caused by multiple genes with complex interactions continues to grow. Many studies have concentrated on one gene at a time. Future studies may need to consider the simultaneous effects of multiple genes. This will involve larger sample sizes, particularly if the effect of each gene is small, and microarray technology is suited to examining multiple genes simultaneously. Sophisticated statistical models using DNA and cDNA microarrays allow the analysis of large numbers of genes and gene expression at a high resolution. We will discuss those genes that, in our opinion, are the most important overexpressed and underexpressed genes in

prostate cancer, and which have been identified by microarrays.

### Gene overexpression

#### Hepsin

The hepsin gene product is a membrane bound serine protease present in most tissues but at its highest concentrations in liver tissue. This protein is thought to have an important role in cell growth. The hepsin gene product was found to be overexpressed in PIN and in prostate cancer using cDNA expression arrays. Using both microarrays of cDNA and tissue microarrays, the degree of hepsin expression distinguished prostate neoplasms of clinically stratified prostate cancer.<sup>132</sup> Expression of the hepsin protein in prostate cancer correlated inversely with patient prognosis.<sup>133</sup>

Identification of features that can accurately predict the behaviour of prostate cancer within a specific patient is a major challenge. Gleason grading is based on morphological features and is a powerful prognostic indicator, although there can be difficulties with interobserver reproducibility. In addition, prostate carcinomas that are morphologically indistinguishable and discovered incidentally can behave in a clinically indolent fashion or aggressively. The identification of genes by the new microarray technology that correlate with patient prognosis is an exciting development with potential clinical application.

#### $\alpha$ -Methylacyl coenzyme A racemase

The  $\alpha$ -methylacyl coenzyme A racemase (AMACR) gene is involved in the  $\beta$  oxidation of branched chain fatty acids and fatty acid derivatives.<sup>134</sup> The enzyme encoded by the AMACR gene plays a crucial role in peroxisomal  $\beta$  oxidation of branched chain fatty acid molecules. AMACR positivity in prostate cancer could have important epidemiological and preventive implications, because the main sources of branched chain fatty acids are dairy products and beef, the consumption of which has been associated with an increased risk for prostate cancer in multiple studies.<sup>135</sup> Both untreated metastases and hormone refractory prostate cancers have been found to be strongly positive for AMACR.<sup>136</sup> AMACR expression has also been found to be a marker of tumour differentiation.<sup>137</sup> In diagnostic histopathology, the AMACR marker has the ability to support a diagnosis of malignancy in prostate needle biopsies. Although it has limitations with respect to sensitivity and specificity, AMACR will no doubt become a standard adjunctive stain used by pathologists seeking to reach a definitive diagnosis in prostate biopsies considered to be atypical but not diagnostic of malignancy on haematoxylin and eosin stained sections alone.<sup>138</sup>

#### PIM1

PIM1 encodes a protein kinase upregulated in prostate cancer. The PIM1 gene product was also found to be overexpressed in PIN and in prostate cancer using cDNA expression arrays. The degree of PIM1 expression distinguished clinically stratified prostate neoplasms using microarrays of cDNA and tissue microarrays. Decreased expression of PIM1 kinase in prostate cancer correlated significantly with measures of poor patient outcome, to an even greater extent than hepsin.<sup>133</sup>

A remarkably similar cotranscriptional regulation or gene amplification of PIM1 and the oncogene c-myc (previously discussed) has been identified, possibly mediating a synergistic oncogenic effect in prostate cancer.

#### MTA1

Expression of the metastasis associated protein 1 (MTA1) has previously been found to be associated with progression to the metastatic state in various cancers. A recent study identified an association of MTA1 expression and prostate

cancer progression. Metastatic prostate cancer demonstrated significantly higher mean MTA1 protein expression intensity compared with clinically localised prostate cancer or benign prostate tissue.<sup>139</sup>

## EZH2

EZH2 is a developmental regulatory gene that is a transcriptional repressor and is found in higher concentrations in metastatic prostate cancers than in primary tumours.<sup>140</sup> Other genes such as CSEIL, ZNF217, MYBL2, and STK15 have also been found to be overexpressed in prostate cancer.<sup>141 142</sup>

## Gene underexpression

Gene expression profiling using microarray technology and other techniques has also demonstrated genes that are downregulated or underexpressed in prostate cancer. In our opinion, two of the most important groups of genes that are down regulated are the interferons and annexins.

## Interferons and interferon inducible molecules

In a study that compared gene expression profiles of tumorigenic versus non-tumorigenic human prostatic epithelium a large proportion of the downregulated genes encoded interferon (IFN) inducible molecules. IFN was also shown to inhibit tumorigenic human prostatic epithelium cell proliferation and colony formation in vitro and inhibit tumour growth in xenografts in vivo.<sup>143</sup>

IFNs are mainly thought to play an indirect immunosurveillance role that is not specific for prostate cancer. IFNs are thought to have an antiproliferative effect and can affect the expression of CDKN1A (p21), which belongs to the Cip/Kip family. This finding has possible clinical applications and suggests IFN inducible molecules as potential therapeutic targets for the treatment of prostate cancer.

## Annexins

Annexins play important roles in maintaining calcium homeostasis and regulating the cytoskeleton and cell motility. Downregulation of annexins has been identified in prostate cancers using cDNA microarrays. Annexins have also been found to be significantly downregulated in prostate cancer cell lines, suggesting that loss of expression may contribute to prostate cancer development and progression.<sup>144 145</sup>

## EPHB2

EPHB2 is a receptor tyrosine kinase gene and is thought to have an essential role in cell migration and maintenance of normal tissue architecture. It has been reported to be overexpressed in gastric cancer; however, in a study using combined nonsense mediated RNA decay microarrays and array based comparative genomic hybridisation, mutational inactivation of EPHB2 was identified in a small fraction of prostate carcinomas. The identification of this possible tumour suppressor gene in prostate cancer is an example of how microarray technology is a powerful new molecular pathology tool.<sup>146</sup>

## Other genes

Decreased expression of the tumour suppressor gene PTEN and the adhesion gene (E-cadherin) have also been identified by cDNA microarrays.<sup>133</sup>

## PROTEOMICS

The system wide study of proteins presents an exciting challenge in this information rich age of whole genome biology. Although traditional investigations have yielded abundant information about individual proteins, they have been less successful at providing us with an integrated

understanding of biological systems. The promise of proteomics is that, by studying many components simultaneously, we will learn how proteins interact with each other and with non-proteinaceous molecules, to control complex processes in cells, tissues, and even whole organisms.

Proteomics presents a new horizon for biomarker discovery and uses protein profiling technologies that can simultaneously resolve and analyse multiple proteins. The identification of proteomic patterns in serum has been used to distinguish neoplastic from non-neoplastic disease within the prostate.

Study cohorts of healthy controls, benign prostate neoplasia, and prostate cancer could be separated based on the overexpression or underexpression of nine protein masses. This study required only the mass values of the proteins using a protein biochip mass spectrometry approach, coupled with an artificial intelligence learning algorithm. Knowing the protein identities was not required for the purposes of differential diagnosis.

*“Proteomics presents a new horizon for biomarker discovery and uses protein profiling technologies that can simultaneously resolve and analyse multiple proteins”*

Efforts are under way to identify and characterise these peptide/protein biomarkers because this knowledge will be important in understanding what biological role they play in prostate cancer oncogenesis.<sup>147 148</sup> A protein known as growth differentiation factor 15 has been identified as a proteomic alteration in a recent study using laser capture microdissection, and may be involved in early prostate carcinogenesis.<sup>149</sup> Downregulation of IFNs has also been identified by proteomic analysis.<sup>150</sup> Proteomics is a very exciting molecular tool and studies to date have shown a higher specificity for prostate cancer than PSA screening.

## THE ROLE OF INFLAMMATION IN PROSTATE CANCER

Inflammation has a role in many cancers.<sup>151</sup> Symptomatic prostatitis occurs in at least 9% of men > 40 years of age, many suffering from multiple episodes.<sup>152</sup> The prevalence of asymptomatic prostatitis is not known.<sup>153</sup> Inflammatory cells produce numerous oxidants with potential to cause cellular or genomic damage in the prostate.<sup>154</sup> There is accumulating knowledge supporting the role of inflammation in prostate cancer, including epidemiological studies showing that there is a decreased risk of prostate cancer associated with the intake of antioxidants or non-steroidal anti-inflammatory drugs.<sup>155 156</sup>

In addition, epidemiological studies have shown an increased risk of prostate cancer associated with sexually transmitted infections, regardless of the particular infection, suggesting that it is the associated inflammation rather than a particular causal infection that is involved in prostate cancer.<sup>157 158</sup>

Molecular pathology studies also support the hypothesis that inflammation is important in the aetiology of prostate cancer. Two of the prostate cancer susceptibility genes identified thus far, RNASEL and MSRI, encode proteins with crucial functions in host responses to infections. In addition, a polymorphism of TLR4 is associated with an increased risk of prostate cancer. TLR4 encodes a receptor that is a central player in the signalling pathways of the innate immune response to infection by Gram negative bacteria.<sup>8 15 22</sup>

Diagnostic histopathologists have also proposed that a prostatic lesion called proliferative inflammatory atrophy is a precursor of PIN and prostate cancer.

The term proliferative inflammatory atrophy applies to focal atrophic lesions associated with chronic inflammation and often adjacent to foci of PIN or prostate cancer.

These lesions are thought to arise as a consequence of the regenerative proliferation of prostate epithelial cells in response to injury caused by inflammatory oxidants.<sup>159</sup> Somatic genomic abnormalities similar to those in PIN and prostate cancer have been found in foci of proliferative inflammatory atrophy.<sup>160</sup> Epithelial cells in lesions of proliferative inflammatory atrophy also show many molecular signs of stress, such as high concentrations of GSTP1, glutathione S-transferase A1, and cyclooxygenase 2.<sup>159 161 162</sup>

“The evidence for a role of inflammation in prostate cancer is convincing with support from epidemiological, molecular, and histopathological studies”

Loss of GSTP1, probably as a result of hypermethylation of the CpG island sequences of GSTP1, may define the transition between proliferative inflammatory atrophy and PIN or prostate cancer.<sup>163–165</sup>

Phenotypically intermediate cells between basal and secretory cells have been identified in normal prostate epithelium and these cells are increased in proliferative inflammatory atrophy lesions. The finding of a large number of highly proliferating intermediate cells in proliferative inflammatory atrophy indicates that these cells may serve as preferred target cells in prostate carcinogenesis.<sup>166</sup>

The evidence for a role of inflammation in prostate cancer is convincing with support from epidemiological, molecular, and histopathological studies. In the near future it may be common practice for the prescription of anti-inflammatory drugs to decrease the risk of development of prostate cancer, just as the prescription of aspirin is common practice now to decrease the risk of stroke.

### Take home messages

- The molecular pathology of prostate cancer is complex: many genes are involved in its pathogenesis and additional environmental factors such as diet and inflammation also play a role
- Epidemiologically, prostate cancer can be divided into hereditary and sporadic forms, but they cannot be distinguished molecularly and, unlike in many other cancers, highly penetrant inherited genes conferring the prostate cancer phenotype have not been identified
- Several polymorphisms have been associated both with increased risk of prostate cancer and with increased risk of progression
- Many somatic mutations and chromosomal abnormalities have been identified in prostate cancer, including overexpression of oncogenes, such as bcl-2, and underexpression of tumour suppressor genes, such as GSTP1, and changes in the expression of growth factors and their receptors
- The application of new investigative tools such as DNA microarray technology and proteomics to the study of prostate cancer should improve knowledge of the genetic changes underlying the initiation, development, and progression of this disease and hopefully help distinguish indolent from aggressive prostate tumours by molecular fingerprinting

## CONCLUSIONS

Powerful new molecular pathology tools such as DNA microarrays are providing information that is already being incorporated into diagnostic pathology such as AMACR staining of prostate cancer cells. The molecular pathology of prostate cancer is complex; not only are multiple genes involved in its pathogenesis, but additional environmental factors such as diet and inflammation also play a role.

In other cancers such as colon cancer there are gatekeeper genes and multistep models of carcinogenesis. No such genes have been identified in prostate cancer despite exhaustive research. There is a complex interaction of multiple genes and environmental factors, some of which may be more important in individual patients with prostate cancer. This may explain why the molecular pathology findings in prostate cancer have not been useful in clinical practice to date; however, this looks likely to change.

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