Pathological and molecular aspects of prostate cancer

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This review focuses on new findings and controversial issues in the pathology and molecular biology of adenocarcinoma of the prostate. Since management of high-grade prostatic intraepithelial neoplasia on needle biopsy—the most common precursor lesion to prostate cancer—is the crucial issue with this lesion, we discuss the risk of cancer subsequent to this histological diagnosis and the issue of whether such neoplasia should be regarded as carcinoma-in-situ. We also look at prostate cancer itself, starting with its diagnosis, reporting on needle biopsy, and reviewing how the most frequently used grading system, the Gleason grading system, affects treatment. The molecular basis of prostate cancer includes inheritable and somatic genetic changes (tumour suppressor genes, loss of heterozygosity, gene targets and regions of chromosomal gain, CpG island promoter methylation, invasion and metastasis suppressor genes, telomere shortening, and genetic instability). Changed gene expression (eg, proliferation-related genes, changes in the androgen receptor, apoptosis and stress-response genes) have potential as biomarkers and therapeutic targets in prostate cancer.

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High-grade prostatic intraepithelial neoplasia on needle biopsy

The median frequency of high-grade prostatic intraepithelial neoplasia on biopsy is about 5%.1 In the early 1990s, men who were diagnosed with high-grade prostatic intraepithelial neoplasia on biopsy were reported as having a 50% risk of developing cancer.2 Thus, men with such neoplasia on their initial biopsy were advised to have their biopsy repeated. However, risk of cancer on subsequent biopsy after a diagnosis of high-grade prostatic intraepithelial neoplasia is 23–35%.3 If cancer is not identified at the first follow-up biopsy, it will probably not be found. Yet, because of sampling error, men with raised serum concentrations of prostate-specific antigen (PSA), are thought to have a 20% risk of cancer being identified on repeat biopsy after an initial benign biopsy. In view of this slightly higher risk of cancer in men with high-grade prostatic intraepithelial neoplasia compared with those with benign biopsy, whether men with high-grade prostatic intraepithelial neoplasia on biopsy need to have a repeat biopsy is controversial. Whether digital rectal examination, transrectal ultrasound findings, or serum PSA concentrations can improve prediction of which men with high-grade prostatic intraepithelial neoplasia will have carcinoma on repeat biopsy is also unclear.4 The intraepithelial neoplasia itself can mimic prostate cancer on transrectal ultrasound, but it does not give rise to a raised PSA.5–7 The presence of high-grade prostate intraepithelial neoplasia indicates increased risk of cancer somewhere in the prostate, but not necessarily at the site where the neoplasia was found at biopsy.8–11

Prostatic intraepithelial neoplasia versus carcinoma-in-situ

At present, it is not possible to determine whether a prostatic intraepithelial neoplasia focus identified at biopsy already has an infiltrating carcinoma at that site, or if

Search strategy and selection criteria

We searched PubMed for English-language papers from 1968 to November, 2002, using the term prostatic neoplasms. We also searched the reference list of relevant papers. Because of the reference limit for reviews, we were unable to include all relevant papers, and have instead used the most recent and relevant.
infiltrating carcinoma is subsequently discovered, whether it evolved in the immediate vicinity of the focus of the high-grade prostatic intraepithelial neoplasia. Because little is known about the natural history of high-grade prostatic intraepithelial neoplasia, the term carcinoma-in-situ is not a synonym for this disease. Carcinoma-in-situ implies that the prostate lesion will develop into infiltrating carcinoma often enough for some clinicians to treat the lesion radically, especially if asked to do so by an anxious patient.

**Adenocarcinoma of the prostate**

**Diagnosis**

Diagnosis of prostate cancer by biopsy is difficult. The preferred method of diagnosing early prostate cancer is needle biopsy. Modern needle biopsy techniques have low morbidity, result in fewer ambiguous diagnoses, and provide more specific information about the grade and extent of the tumour than does fine-needle aspiration. The difficulty with needle biopsy not only stems from the small amount of tissue available for histological examination, but also arises because biopsies often identify only a few malignant glands among many benign glands. Morphologically, prostate cancer is difficult to diagnose because the clues to malignant disease can be subtle, increasing the risk of underdiagnosis. Although a few histological findings are specific for prostate cancer—eg, perineural invasion, glomerulations, and collagenous micronodules—in general, diagnosis is made on the basis of architectural, cytological, and ancillary findings (panel 1). Many histological benign mimickers of cancer can also lead to misdiagnosis of cancer (panel 2). One distinguishing feature is that benign glands contain basal cells, which are absent in cancer, and pathologists have used immunohistological markers to label basal cells (figure). cDNA microarrays have also identified markers specific for prostate cancer. These markers, although improving the accuracy of diagnosis, have their limitations, and this technique should be used in conjunction with sections stained routinely.

α-methyl-CoA racemase (AMACR) is a marker that is substantially upregulated in prostate cancer. Because negative staining for basal cell markers, especially in a small focus of atypical glands, is not always diagnostic of prostate cancer, positive staining for AMACR can increase confidence in a diagnosis of malignant disease (figure). However, this procedure has some pitfalls in diagnostic use. Pseudohyperplastic, atrophic, and foamy gland adenocarcinoma of the prostate, variants that are especially difficult to diagnose, are positive for AMACR in only 62–77% of cases. Up to 20% of small foci of adenocarcinoma on needle biopsy can be negative for predicting carcinoma.

### Panel 1: Diagnostic features of adenocarcinoma of the prostate

**Architectural features suggestive of carcinoma**

- Small glands infiltrating in between larger benign glands
- Glands infiltrating haphazardly in different directions within the stroma
- Back-to-back glands that do not merge in with surrounding more recognisably benign glands
- Regions of increased cellularity that are not inflamed and might be high-grade cancer

**Cytological features suggestive of carcinoma**

- Nuclear enlargement with or without nucleoli when compared with surrounding more recognisably benign glands
- Nuclear hyperchromasia
- Mitotic figures
- Amorphophilic cytoplasm in glands suspicious for carcinoma by contrast with surrounding benign glands that have pale to clear cytoplasm
- Large glands which have a crisp even luminal surface without the ruffling and undulations seen in comparably sized benign glands

**Adjuvant findings seen with carcinoma**

- Intraluminal blue-tinged mucinous secretions seen on haematoxylin and eosin sections
- Intraluminal prostatic crystalloids
- Eosinophilic amorphous intraluminal secretions
- Features almost pathognomonic for prostate cancer
- Perineural invasion
- Collagenous micronodules (mucinous fibroplasia)
- Glomeruloid structures

**Features that should make doctors hesitate in diagnosing carcinoma**

- Acute or chronic inflammation where glandular nuclei may show reactive enlargement and visible nucleoli
- A densely cellular lesion suggestive of high-grade prostate carcinoma yet confounded by the presence of acute or chronic inflammation, which might be non-specific granulomatous prostatitis
- Atrophic glands despite an apparently infiltrative appearance
- Small glands with minimal atypia merging in with similar glands which seem more recognisably benign, which might be adenosis
- High-grade prostatic intraepithelial neoplasia with only a few adjacent atypical glands, where tangential sections or outpouchings off of the prostate intraepithelial neoplasia cannot be ruled out

### Panel 2: Benign mimickers of prostate adenocarcinoma

**Well to moderately differentiated**

- Adenosis (atypical adenomatous hyperplasia)
- Atrophy (complete and partial)
- Basal cell hyperplasia
- Cowper’s glands
- Mesonephric hyperplasia
- Nephrogenic adenoma
- Radiation atypia
- Seminal vesicles
- Verumontanum hyperplasia

**Moderately to poorly differentiated**

- Clear-cell cribriform hyperplasia
- Non-specific granulomatous prostatitis
- Paraganglia
- Prostatic infarcts
- Sclerosing adenosis
- Signet ring cell lymphocytes
- Xanthoma
Gleason grading system

The Gleason score is the most frequently used grading system for prostate cancer. Unusually, the overall grade is not based on the highest grade within the tumour. In 1974, Gleason, Mellinger, and the Veterans’ Administration Cooperative Study team showed that prognosis of prostate cancer was intermediate between that of the most predominant pattern of cancer and that of the second most predominant pattern. These predominant and second most prevalent patterns are identified and each is graded 1 (most differentiated) to 5 (least differentiated) and the two grades are added.

If a tumour had only one histological pattern, the primary and secondary scores are the same. The combined Gleason grade, sometimes called the Gleason sum or score, thus ranges from 2 (for tumours uniformly of pattern 1), to 10 (for undifferentiated tumours). A tumour that is mostly Gleason 3 with a lesser amount of pattern 4 scores 7, as does a tumour that is mostly pattern 4 with a lesser amount of Gleason 3. Most cases with divergent patterns, especially on needle biopsy, do not differ by more than one pattern.

Should a Gleason score of 2–4 (low grade) be assigned to cancers on needle biopsy? Most of such tumours are graded 5 or more when reviewed by experts in urological pathology, and such grading has poor reproducibility, even by experts. Furthermore, clinicians sometimes assume that low-grade cancers on needle biopsy do not need definitive therapy, despite the substantial risk of aggression. Gleason score 2–4 adenocarcinomas exist but they are usually seen on transurethral resection. Low-grade cancers are rarely seen on needle biopsy because they are mostly located anteriorly in the prostate within the transition zone and tend to be small.

As with any grading system, the Gleason method has difficulties with interobserver reproducibility. Other difficulties include grading of cribriform patterns, grading of small foci of cancer at biopsy, borderline histology between grades, how to account for a tertiary pattern, how to assess cases with multiple cores having different grades, and whether the overall grade of the tumour should be the score of the core with the high grade or an overall score averaging all cores’ grades. Educational methods, such as the internet, can greatly improve pathologists’ Gleason grading.

A group from Stanford, CA, USA, has been a strong proponent of using the proportion of high-grade tumour (Gleason 4 and 5) to grade prostate cancer. However, this method is only predictive for progression at the extremes (greater than 70% or less than 20% pattern 4 or 5), and the Stanford group later showed that the proportion Gleason pattern 4 or 5 on needle biopsy did not correlate well with the corresponding radical prostatectomy sample.

Gleason score, prognosis, and treatment

The Gleason score is a powerful prognostic indicator. It correlates with all important pathological variables seen in the radical prostatectomy sample, with prognosis after radical prostatectomy, and with outcome after radiotherapy. The major prognostic shift is between 6 and 7. Gleason score 7 tumours behave much worse than tumours scoring 5 or 6 and should not be combined as intermediate-grade carcinoma. In predictive terms, the following combinations are helpful: score 2–4 (well differentiated); 5–6 (moderately differentiated); 7 (moderately to poorly differentiated); and 8–10 (poorly differentiated). Score 7 tumours can further be subclassified into 3+4 or 4+3 and the worse prognosis associated with 4+3 can affect decisions on surgery or radiotherapy.

The Gleason grade does influence treatment. Whereas some younger men with limited amounts of Gleason score 5–6 on needle biopsy and low PSA concentrations can simply be followed up (wait-and-see), a score of 7 almost always indicates active management. Clinicians also use the grade in nomograms to predict the probability of tumour extension out of the prostate. A man with a Gleason score 6 tumour could be a candidate for interstitial radiotherapy (brachytherapy) alone, but if he had a tumour scoring 7, with a greater probability of extension of the tumour outside the prostate, he would probably be given external beam radiotherapy alone or with brachytherapy, since seed therapy is not effective for extraprostatic disease. A surgeon could also be influenced by tumour grade and extent on biopsy in deciding whether to resect the neurovascular bundle or bundles, which will affect potency. Accuracy in diagnosing Gleason scores of 8 and above is also crucial because a man with a Gleason score of 8–10 might not be offered surgery, depending on the extent of tumour and other clinical factors; whereas the same man with a Gleason score of 7 would be offered radical prostatectomy.

Other nomograms predict the probability of lymph-node metastasis. A man with a Gleason score of 6, a
normal digital rectal examination, and a serum PSA concentration of less than 10 μg/L has such a low probability of lymph-node metastases that some urologists might not remove the lymph nodes at the time of prostatectomy.

Although high-grade cancer produces less PSA per cell than does a low-grade tumour, overall, tumours that are poorly differentiated are associated with higher PSA concentrations because they tend to be larger and more advanced. However, some such cancers are so poorly differentiated that serum PSA concentrations are disproportionately low, whereas some subtypes of prostate cancer are associated with lower PSA concentrations than those seen in typical acinar prostate cancer (eg, small-cell carcinoma and ductal adenocarcinoma).

**Change over time**

Research on changes in grade of prostate cancer has been limited. In two studies of men who had had two transurethral resections, both containing cancer, the second resection tended to have higher grade, suggesting that grade had worsened over time. However, the second resection had been done because the tumour had progressed, so most men whose cancer did not progress and whose grade may not have changed would not have had a second resection and could have been excluded. In our study, all men undergoing watchful waiting for cancer detected on needle biopsy had a repeat biopsy as part of the protocol. Over 2–3 years, the grade of cancer did not tend to change, suggesting that, over the short term, men do not need to fear dedifferentiation of their cancer if they defer treatment. Whether the grade will change with longer follow-up remains to be seen.

**Reporting cancer on needle biopsy**

The number of positive cores has been correlated with pathological stage, tumour volume, risk of positive surgical margins, and progression of the cancer after prostatectomy. The other widely used method to quantify the amount of cancer on needle biopsy is measurement of the proportion of each biopsy core containing cancer. Involvement of multiple biopsy cores on systematic prostate biopsy does predict adverse pathological findings at radical prostatectomy, but the converse is not true. Very restricted cancer on needle biopsy, by itself, does not always predict insubstantial amounts of tumour in the entire prostate. However, when combined with low serum PSA concentrations, such a finding is usually associated with small, potentially unimportant cancers.

In a study of men with very small intermediate-grade cancers on needle biopsy and low PSA concentrations, we were able to predict with 83% accuracy that they had potentially unimportant tumours in their prostate. Patients who were not classified correctly had larger cancers, yet still very favourable size, grade, and stage. Perineural invasion extensive enough to be sampled on needle biopsy signals increased risk of extraprostatic extension and is associated with a higher probability of unsuccessful radiotherapy.

In biopsy samples that have been graded accurately, DNA ploidy is not helpful in prediction of prostatectomy findings. However, ploidy correlates with prostatectomy stage and grade, and could be of value if the accuracy of Gleason grading is a concern. Results of a large study have shown that proliferation of cancer on biopsy (as measured by proliferation marker ki67) adds to grade as a predictive marker. New methods applied to needle biopsy could improve prediction of prognosis for prostate cancer, but these techniques are not yet ready for routine clinical practice.

**Prognosis after radical prostatectomy**

In multivariate analyses, the features in the radical prostatectomy sample that contribute to prediction of progression are lymph-node metastases, seminal-vesicle invasion, Gleason grade, surgical margin status, and presence and degree of extraprostatic extension. Lymph-node metastases indicate systemic disease and prostatectomy has failed in all men with this complication 5 years after surgery. Seminal vesicle invasion carries a dire prognosis, with more than 85% of such tumours progressing at 5 years after surgery. About 50% of tumours with positive margins progress. Extraprostatic extension should be stratified into those showing focal spread outside of the gland and those with more extensive spread. Although tumour volume correlates with progression, once the Gleason score of the prostatectomy sample and pathological stage the status of the surgical margins are known, tumour volume probably has little prognostic value.

**Molecular changes associated with prostate cancer**

Molecular knowledge of prostate cancer can improve prediction of prognosis, but has not yet yielded information that is ready to be incorporated into clinical practice. The molecular basis of prostate cancer is reviewed here in the hope that it might one day be useful for pathologists and clinicians.

Among the risk factors for prostate cancer, reviewed in the first article of this Lancet series, are inherited susceptibility and diet. Dietary vitamin E, carotenoids, and selenium protect; whereas diets rich in fat and red meat exert a promotional effect. All the dietary factors that seem protective are potent antioxidants, so oxidative stress (which can directly damage DNA) could contribute to prostate carcinogenesis. Potential sources of oxidant stress are endogenous metabolism, inflammation, and diet. Circulating concentrations of insulin-like growth factor I (IGF-I), which can be affected by diet or genetics, have been implicated in development of aggressive prostate cancer.

At the cellular and molecular level, genetic aberrations drive the formation and aggressiveness of prostate cancer. Every carcinoma focus is presumed to arise from a single cell that accumulates genome changes affecting regulatory genes resulting in a growth or survival advantage. Additional changes lead to local invasion and metastasis. Since the yearly incidence of prostate cancer greatly exceeds the death rate, and since clinically apparent prostate cancers can have a widely variable course, finding genes that control aggressiveness is of particular interest.

Mutations in classic oncogenes or tumour suppressor genes are uncommon in primary prostate cancer, and mutations specific for prostate cancer (eg, prostate gatekeeper genes) have not been identified. However, several molecular or genetic changes have been found. Although none of them is unequivocally linked to prostate cancer initiation or progression, some are directly involved in prostatic carcinogenesis (panel 3). The molecular genetics of prostate cancer has been reviewed in specialist texts, so here we summarise selected findings, with emphasis on very recent progress.

**Inherited genetic changes**

No known cancer syndrome includes prostate cancer. However, concordance of prostate cancer in monozygotic twins is greater than that in dizygotic twins. Family history is a strong and consistent risk factor, and there is evidence for both autosomal dominant and X-linked inheritance in
Panel 2: Selected genes proposed to be involved in prostate cancer initiation or progression, or in modifying the risk of prostate cancer development

<table>
<thead>
<tr>
<th>Gene</th>
<th>Proposed function</th>
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<tbody>
<tr>
<td>MS</td>
<td>Anti-infectious, scavenger receptor</td>
</tr>
<tr>
<td>RNASEL</td>
<td>Anti-infectious, apoptosis</td>
</tr>
<tr>
<td>ELAC2</td>
<td>Metal-dependent hydrolase</td>
</tr>
<tr>
<td>Promoter hypermethylation resulting in gene silencing</td>
<td>Carcinogen detoxification</td>
</tr>
<tr>
<td>GSTP1</td>
<td>Anti-infectious, apoptosis</td>
</tr>
<tr>
<td>Loss of heterozygosity and point mutation</td>
<td>Cell survival and proliferation</td>
</tr>
<tr>
<td>PTEN</td>
<td>Cell survival and proliferation</td>
</tr>
<tr>
<td>TPS3 (also PS3)</td>
<td>Cell survival and proliferation, genome stability</td>
</tr>
<tr>
<td>Loss of heterozygosity and haploinsufficiency</td>
<td>Cell differentiation and proliferation</td>
</tr>
<tr>
<td>NKX3-1</td>
<td>Cell proliferation and differentiation</td>
</tr>
<tr>
<td>CDKN1B (P27KIP1)</td>
<td>Cell proliferation</td>
</tr>
<tr>
<td>Point mutations</td>
<td>Cell proliferation</td>
</tr>
<tr>
<td>COPEB (also KLR6)</td>
<td>Cell proliferation survival, and differentiation</td>
</tr>
<tr>
<td>Amplification</td>
<td>Cell proliferation survival, and differentiation</td>
</tr>
<tr>
<td>Overexpressed at mRNA and protein level</td>
<td>Cell proliferation survival, and differentiation</td>
</tr>
<tr>
<td>H1RT</td>
<td>Cell immortalani</td>
</tr>
<tr>
<td>HPA4</td>
<td>Transmembrane protease</td>
</tr>
<tr>
<td>FASN</td>
<td>Fatty-acid synthesis</td>
</tr>
<tr>
<td>AMACR</td>
<td>Fatty-acid metabolism, branched chain</td>
</tr>
<tr>
<td>EZH2</td>
<td>Transcription repressor, cell proliferation</td>
</tr>
<tr>
<td>MYC</td>
<td>Cell proliferation</td>
</tr>
<tr>
<td>BCL2</td>
<td>Cell survival</td>
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<tr>
<td>Polymorphisms affecting prostate cancer risks</td>
<td>Cell proliferation survival, and differentiation</td>
</tr>
<tr>
<td>AR</td>
<td>Cell proliferation survival, and differentiation</td>
</tr>
<tr>
<td>CYP17</td>
<td>Androgen metabolism</td>
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<tr>
<td>SRD5A2</td>
<td>Androgen metabolism</td>
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</table>

some families. As with other hereditary cancer types (eg, colorectal and breast cancer), the hunt is on for rare highly penetrant alleles in genes associated with hereditary prostate cancer. In the first reported genome-wide screen of polymorphic markers, seven regions of linkage (logarithm of the odds ratio >1) were identified. The 1q24–25 region showed strong linkage to prostate cancer and was designated the HPC1 gene locus, but the candidacy of other regions with genes related to familial prostate cancer on this chromosome complicates the position. Up to now, three candidate genes have been identified. They are HPC2/ELAC2, RNASEL, and MSR1.

ELAC2 is a candidate for the hereditary prostate cancer 2 locus (HPC2). Although an initial attempt to confirm these findings was promising in that there was an increased risk of prostate cancer in men with the same variant alleles, more recent reports have provided little confirmatory evidence.

RNASEL was suggested as a candidate for HPC1 when it was found that mutated alleles segregated with the disease in several families with hereditary prostate cancer. RNASEL is a ribonuclease that degrades viral and cellular RNA and can produce apoptosis on viral infection. Mice deficient in RNASEL have an increased susceptibility to infection by some bacteria. In one study in Europe, Finnish families with hereditary prostate cancer had a significantly higher frequency of an RNASEL-truncating mutation than did controls, although the mutation did not strictly segregate with the disease in those families. In a second study, RNASEL alleles carrying inactivating mutations were not associated with prostate cancer, but results of a case-control study showed a positive association with an non-inferentially compromised RNASEL allele, with an attributable risk of about 13%. A fourth study reported a specific mutant RNASEL allele in 7% of Ashkenazi men with prostate cancer compared with 3% of Ashkenazi men without prostate cancer.

Mutations in MSRI have been implicated in a subset of families with hereditary prostate cancer and non-familial cases. MSRI, on chromosome 8p, encodes a trimeric macrophage scavenger receptor responsible for cellular uptake of several charged molecules including bacterial cell wall products. Mice that do not have functional Msr1 (Msr-A−/−) accumulate fewer lipid-laden macrophages in cardiovascular lesions and are more susceptible to infection than are wild-type mice.

Although it is not clear how RNASEL and MSR1 are involved in the pathogenesis of prostate cancer, both genes take part in the host response to infectious agents, so mutations might reduce the ability to eradicate certain infectious agents within the prostate, resulting in a chronic inflammatory reaction. Infection and chronic inflammation have been advanced as potential causative agents in prostate cancer.

Other genes that have common sequence variant alleles in the population might also be important in determining or modifying risk of prostate cancer, especially those involved in androgen signalling. Examples are polymorphic polyglutamine and polyglycine repeats in the short androgen-receptor genes affecting the metabolism of sex steroids such as SRD5A2 (encodes the predominant isozyme of 5α-reductase in the prostate). Genetic variation in candidate genes in other pathways implicated in prostate carcinogenesis (eg, inflammation, carcinogen metabolism) are also being investigated.

No gene has been identified in prostate cancer that has the same high penetrance as adenomatous polyposis coli gene (APC) in familial colon cancer so even if RNASEL, ELAC2, and MSR1 are prostate cancer susceptibility genes, the proportion of cases of hereditary prostate cancer attributable to germline mutations in these loci will be small. The risk of disease in the presence of a specific risk factor allele might be substantially increased only in the appropriate genetic, dietary, and environmental background.

Somatic genetic changes

Prostate cancers often contain genetic changes at the chromosomal or subchromosomal level. The most common chromosomal abnormalities are gains at 7p, 7q, 8q, and Xq, and losses at 8p, 10q, 13q, and 16q. As with other solid tumours, the number of changes identified increases with the stage of disease, suggesting that disease progresses as a result of an accumulation of clonal genetic changes.

Tumour suppressor genes and loss of heterozygosity

Classic tumour suppressors such as RB1 generally show biallelic inactivation, usually by a point mutation in one allele coupled with deletion or rearrangement of the other. However, an expanded view of a tumour suppressor gene is emerging such that a reasonable definition is a gene whose function when heritably downregulated or otherwise compromised, in a clonal fashion, promotes cancer development or progression. The change can be by mutation, methylation of the promoter, or by some other modification to the protein product and must be coupled
with evidence that the normal (wild-type) gene does suppress growth of tumour cells. Regions of frequent allelic loss in tumours might contain tumour suppressor genes. Two separate sites on chromosome 8 (8p23 and 8p12–22) have shown allelic loss or chromosomal deletions most frequently in prostate cancer.107 Loss of 8p seems to be an early event since high-grade prostatic intraepithelial neoplasia might lose heterozygosity at this location.108 Several genes located on chromosome 8p have been examined as candidate tumour suppressors, with one of the most promising being NKPX3-1.

NKPX3-1 is expressed in normal prostate epithelium and is decreased in prostate tumour cells.96,109 Further, mice that do not have either one or both Nkpx3-1 alleles develop abnormal prostate duct branching, prostatic hyperplasia, and lesions similar to human prostatic intraepithelial neoplasia.110

PTEN on 10q23 is mutated in up to a third of hormone-refractory prostate cancers,77 and homozygous deletions and mutations have been identified in a subset of primary prostate cancers.78,103 Loss of PTEN in primary prostate cancer correlates with high Gleason score and advanced stage.111 PTEN is responsible for dephosphorylation and inactivation of phosphatidylinositol-3,4,5-trisphosphate (PIP3), a second messenger that is produced after activation of PIP3 kinase in response to ligation of several growth factor receptors, including IGF-I. PIP3 activates the protein kinase AKT. AKT signalling leads to inhibition of apoptosis and to increased cell proliferation.105 AKT can phosphorylate ODKN1B, resulting in cytoplasmic retention of this protein and lack of the cell cycle arrest that ODKN1B mediates.112 Inactivation of ODKN1B cannot, however, be the only function of the PTEN pathway during prostate carcinogenesis; in the mouse, Pten can cooperate with either Nkpx3-1 or Cdk4b (encoding ODKN1B) in increasing the frequency and extent of high-grade prostatic intraepithelial neoplasia lesions and perhaps early cancers.104,113 Since this pathway is frequently changed in prostate cancer, inhibition of signalling through PI3K and AKT is a promising therapeutic strategy.114

In one study,115 77% of primary prostate tumours showed loss of heterozygosity for Kruppel-like factor 6, also known officially as core promoter element binding protein (COPEB), and the retained COPEB allele had mutations in 71% of these tumours. This discovery has yet to be replicated.

Other sites of loss or deletion in prostate cancer occur mainly in the late stages of cancer progression. Genetic inactivation of the classic tumour suppressor genes TP53, RB1, and ODKN2A, are seen rarely in primary cancers, but occur at higher frequencies in metastatic and hormone refractory lesions,77 suggesting that these genes might be involved in progression of prostate cancer.

Gene targets in regions of chromosomal gain

High-level amplification of ERBB2 does not take place in prostate cancer to any great extent.116 However, amplification of regions on chromosome 8q correlates with aggressiveness of tumours.117 One candidate for amplification on 8q is the MYC gene, since it is amplified in several cases, and amplification of MYC correlates with a worse prognosis in prostate cancer.106 Abnormally high concentrations of MYC mRNA have been reported in prostate cancer.117 Another region of gain, that is also accompanied by protein overexpression, contains PSA.118 Since PSA is a cell surface marker, it is being investigated as a therapeutic target. Other genes on chromosome 8q have also been implicated as potential targets of amplification, including the elongin c gene119 and EIF3S3.120 Other regions of gain include the androgen receptor gene itself (located on Xq12), where amplification occurs almost exclusively in the hormone refractory state.104

CpG island promoter methylation

Silencing of the gene encoding the pi class of glutathione-S transferase (GSTP1) by hypermethylation of the promoter region is linked to prostate carcinogenesis. This DNA change takes place in 90–95% of cancer lesions and in 70% of high-grade prostatic intraepithelial neoplasia lesions.115 GSTP1, which can detect xenobiotic environmental electrophilic carcinogens and oxidants, might have a genome caretaker role by preventing oxidant and electrophilic DNA damage and resulting mutation.121,116 GSTP1 promoter methylation is being used in molecular diagnosis as a biomarker for prostate cancer in bodily fluids such as urine and semen.122,123 Other genes have also been shown to be selectively methylated in many prostate cancers in their 5′ promoter regions including: EDNRRB,117 encoding the endothelin B receptor; CD44,124 a cell adhesion molecule encoding a gene with metastasis suppressor activity in rat prostate cancer (see below); ER-α, encoding the oestrogen receptor α;125,126 and ER-β, encoding the oestrogen receptor β.127

Invasion and metastasis suppressor genes

For cancer cells to spread to distant sites they must invade the stroma, penetrate the vasculature, and 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.78,105

The cadherins are a class of cell adhesion molecule that govern epithelial morphogenesis in the embryo and maintain adult epithelial tissue differentiation and structural integrity. Abnormal or reduced expression of E-cadherin is associated with advanced stage and poor clinical outcome in human prostate cancer.105 Other cadherins and other members of the cadherin signalling pathway such as α-catenin, are also sometimes altered in prostate cancer.77 The CD44 gene product is downregulated in high-grade prostate cancer and metastases and in a rat model of prostate cancer behaves as a metastasis suppressor gene.128 The mechanism of downregulation of the CD44 protein might be via hypermethylation of CpG island promoter.117 Other cell adhesion systems are changed in prostate cancer. For example, Nagle and colleagues129 have shown loss of laminin 5, collagen VII, and β4-integrin protein expression in prostate cancer.

Metastasis suppressor genes are defined as genes that do not affect cell growth of primary tumour cells, but can inhibit development of distant metastases.107 Several candidate metastasis suppressor genes in addition to CD44 have been identified for prostate cancer—KAH1, NME23, mps3, BRMS1, KISS1, and MAP2K4.130

Telomere shortening

Another somatic DNA change that occurs with high frequency in prostate cancer, and not in benign prostate tissue, is telomere length shortening.124 Using an in-situ method we found that telomere shortening occurs frequently in high-grade prostatic intraepithelial neoplasia.125

Genetic instability

Genetic instability is a hallmark of adult epithelial and many haemopoietic malignant diseases, and it has been proposed as a necessary component for initiation and progression of the malignant phenotype.106 Two main types of instability occur. The least common form,
especially in prostate cancer, is microsatellite instability, which is related to defects in genes for DNA mismatch repair. The other type involves numerical and complex structural changes to whole chromosomes, and is much more common in solid tumours such as prostate cancer.

Genetic inactivation of genes that control chromosome number and structure in prostate cancer has not yet been identified. Since telomere shortening, and resultant dysfunction, can lead to numerical and complex chromosome changes, investigators have proposed that telomere shortening during prostate carcinogenesis and progression might lead to the genetic instability seen in this disease. Proliferating cells need a mechanism to stabilise their telomeres so that they can prevent massive chromosomal instability and cell death, and this is achieved in most cases of prostate cancer by activating telomerase. Since telomerase is active in prostate cancer but generally not in benign prostatic hyperplasia or healthy prostate tissue, it is under investigation as a potential diagnostic and therapeutic target in prostate cancer.

Changes in gene expression

**Proliferation-related genes**

The CDKN1B protein is expressed at high concentrations in healthy prostate epithelium and is downregulated in most high-grade prostatic intraepithelial neoplasia and prostate cancer lesions. Loss of CDKN1B also correlates with poor prognosis in prostate cancer, and, as mentioned above, inactivation of Cdk1b (encoding CdK1b) in mice can cooperate with loss of Pten and Nkx3-1 in mouse prostate carcinogenesis. In human beings, the mechanism of CDKN1B downregulation does not seem to be genetic, but might be at the protein level.

**Androgen receptors**

Most prostate cancers express androgen receptors and will regress on withdrawal of androgens. Most metastatic prostate cancer is treated with hormonal therapy aimed at androgen suppression, blockade of the androgen receptor, or a combination of these. Despite an initial beneficial response to such treatment, prostate cancers inevitably stop responding as they progress to an androgen-independent state. Therefore, much research has focused on androgen signalling in prostate cancer and how cells that are initially hormone dependent become hormone independent for growth. Surprisingly, even androgen-independent prostate cancers retain functional expression of androgen receptors in most cases, and, as a consequence, might lead to androgen-independent prostate cancer progression. BCL2 is overexpressed within the luminal epithelium in a subset of high-grade prostatic intraepithelial neoplasia lesions, is absent in most low to intermediate grade carcinomas, and accumulates in many androgen independent prostate cancers. Thus, BCL2 might be a therapeutic target in advanced prostate cancer. Other antiandrogenic genes are also overexpressed in prostate cancer. TP53 is mutated in a small subset of prostate cancer with a higher proportion of mutations being found in metastatic lesions. TP53 has potent antiapoptotic activity and could be responsible for bypass of cell-cycle checkpoints allowing continued proliferation and the accumulation of additional genetic changes.

**Stress-response genes**

Results of studies suggest that aspirin and other non-steroidal anti-inflammatory drugs might inhibit prostate carcinogenesis. However, the mechanisms of action by which these agents prevent cancer remain unclear. Several investigators have indicated that prostate cancers overexpress one of the targets of treatment with non-steroidal anti-inflammatory drugs, the COX2 enzyme, at high frequency. However, our group assessed this mechanism with various techniques and did not find overexpression in primary prostate cancer. We did find, however, frequent expression of COX2 in regions of proliferating atrophic epithelium associated with chronic inflammation (proliferative inflammatory atrophy), and at times in macrophages in regions of inflammation. This finding raised the possibility that COX inhibitors prevent prostate cancer by inhibiting the inflammatory response in the prostate, or by non-COX2 mediated effects that have been shown in cells without COX2 alleles.

**Other overexpressed genes with clinical potential**

One gene product that has been consistently found to be overexpressed in prostate cancer is fatty acid synthetase (FAS). FAS inhibitors might be selectively toxic to prostate cancer cells and have been proposed as therapeutic agents. Recently, techniques for comprehensive profiling of gene expression have uncovered several genes that could be important new targets in prostate cancer. Hepsin, a proposed trypsin-like transmembrane serine protease, has been implicated as being overexpressed in prostate cancer. Another gene product overexpressed in prostate cancer, that was first identified by subtractive hybridisation and then by microarray analysis, is AMACR. AMACR has a key role in α oxidation of dietary branched-chain fatty acids and is overexpressed at both the RNA and protein level in prostate cancer. The ability of antibodies against AMACR to bind to prostate cancer cells, as opposed to benign epithelial cells, in clinical prostate tissue samples could be exploited as a potential diagnostic marker in prostate needle biopsies (see above).

mRNA profiling might also reveal new prognostic markers, or groups of markers, that can aid in determination of tumour aggressiveness. One of these genes, EZH2, is a developmental regulator gene that is a transcriptional repressor and is found in higher concentrations in metastatic prostate cancers than in primary tumours.
Together with the emerging technologies for proteomics, the potential for discovery of genes or pathways driving the aggressiveness of prostate cancers is accelerating—a worthy goal in the quest to decrease the burden of this disease.

Conflict of interest statement

WB Nelson and WG Isaacs have a patent titled Genetic Diagnosis of Prostate Cancer.

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