Prostate cancer II

Pathological and molecular aspects of prostate cancer

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This review focuses on new findings and controversial issues in the 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.

This review, the second in *The Lancet* series in prostate cancer, follows on from the review on epidemiology and precedes the articles on clinical management and screening, but is relevant to all three.

Pathology

Relation between prostatic intraepithelial neoplasia and cancer

High-grade prostatic intraepithelial neoplasia consists of architecturally benign prostatic acini lined by cells that seem to be malignant.[1,2] Prostates with carcinoma have more of these foci than do those without carcinoma.[3] Prostate glands with extensive high-grade prostatic intraepithelial neoplasia also have more multifocal carcinomas. That carcinomas have zones of high-grade prostatic intraepithelial neoplasia from which glands of carcinoma seem to stem is further histological evidence that this neoplasia is a precursor to some prostate carcinomas.[4] High-grade prostatic intraepithelial neoplasia preferentially develops in the peripheral zone of the prostate, which is the site of origin for most adenocarcinomas.[3] Various biomarkers are either expressed in the same high quantities (compared with benign prostate tissue) in both high-grade prostatic intraepithelial neoplasia and carcinoma or are expressed at levels between those of benign prostate tissue and carcinoma.[3,5] High-grade intraepithelial neoplasia seems to be a precursor lesion to many peripheral intermediate to high-grade adenocarcinomas of the prostate, but the lesion is not a necessary precursor because many early cancers do not have adjacent high-grade prostatic intraepithelial neoplasia. Also, low-grade carcinomas, especially those within the transition zone, are not closely related to high-grade intraepithelial neoplasia.

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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%.[6] 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.[6] 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%.[7–9] 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.[9] The intraepithelial neoplasia itself can mimic prostate cancer on transrectal ultrasound, but it does not give rise to a raised PSA.[10–13] The presence of high-grade prostatic intraepithelial neoplasia indicates increased risk of cancer somewhere in the prostate, but not necessarily at the site where the neoplasia was found at biopsy.[14–16]

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.[17,18] 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[19] (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[20–25] (figure). cDNA microarrays have also identified markers specific for prostate cancer.[26–28] These markers, although improving the accuracy of diagnosis, have their limitations, and this technique should be used in conjunction with sections stained routinely.[29]

α-methylacyl-CoA racemase (AMACR) is a marker that is substantially upregulated in prostate cancer.[26–28] 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

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

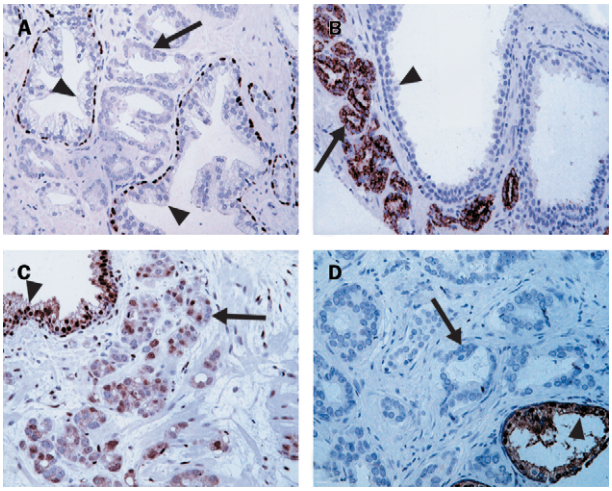
Panel 1: **Diagnostic features of adenocarcinoma of the prostate**

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
Amphophilic 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

Adjunctive findings seen with carcinoma
Intraluminal blue-tinged mucinous secretions seen on haemotoxylin and eosin sections
Intraluminal prostatic crystalloids
Eosinophilic amorphous intraluminal secretions
Features almost pathognomic 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 prostatic intraepithelial neoplasia cannot be ruled out



Differential immunohistochemical staining for molecular markers in prostate cancer

Magnification ×200. (A) Staining against the basal cell marker p63. Basal cells have strong nuclear staining in benign glands (arrowheads) and negative staining in carcinoma (arrow). (B) Staining against α-methylacyl-CoA racemase (AMACR). Note strong cytoplasmic staining in tumour cells (arrow) and negative staining in benign glands (arrowhead). (C) Staining against CDKN1B. Note strong nuclear staining in benign glands (arrowhead) and reduced staining in many of the tumour cell nuclei (arrow). (D) Staining against PTEN. Note strong staining in benign glands (arrowhead) and negative staining in tumour cells (arrow).

AMACR. AMACR also labels high-grade prostatic intraepithelial neoplasia glands and occasional benign glands.

Because diagnosis of prostate cancer is so difficult (eg, false positives, false negatives, and assessing the grade), a second opinion on prostate biopsy material is often important.[30,31] Atypical foci suspicious for cancer are seen in 3–5% of needle biopsy samples.[17] Patients with an atypical diagnosis on prostate biopsy have about a 50% risk that cancer will be identified on repeat biopsy, most commonly at or adjacent to the initial site of the atypical biopsy. Men with atypical diagnoses should have a further biopsy irrespective of serum PSA.

Gleason grading system

The Gleason score is the most frequently used grading system for prostate cancer.[32] 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[32] 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.[33] Gleason score 2–4 adenocarcinomas exist but they are usually seen on transuretheral 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.[34,35] 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 highest grade or an overall score averaging all cores' grades. Educational methods, such as the internet, can greatly improve pathologists' Gleason grading.[36]

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),[37] 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.[37]

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.[38–40] 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.[41] 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.[42] 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[43,44] 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,[45] 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.[46–57] 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.[53,58–63] However, when combined with low serum PSA concentrations, such a finding is usually associated with small, potentially unimportant cancers.[61] 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 prostates. Patients who were not classified correctly had larger cancers, yet still very favourable size, grade, and stage.[64] 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.[65,66]

In biopsy samples that have been graded accurately, DNA ploidy is not helpful in prediction of prostatectomy findings.[67] 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[68] 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,[69] 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 out 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,[70] are inherited susceptibility and diet. Dietary vitamin E, carotenoids, and selenium protect; whereas diets rich in fat and red meat exert a promotional effect.[71–74] 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.[75]

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.[76]

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.[77] 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,[77–80] 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.[81] Family history is a strong and consistent risk factor, and there is evidence for both autosomal dominant and X-linked inheritance in

Panel 3: **Selected genes proposed to be involved in prostate cancer initiation or progression, or in modifying the risk of prostate cancer development**

Gene	Proposed function
Mutations causing decreased activity	
MS	Anti-infectious, scavenger receptor
RNASEL	Anti-infectious, apoptosis
ELAC2	Metal-dependent hydrolase
Promoter hypermethylation resulting in gene silencing	
GSTP1	Carcinogen detoxification
Loss of heterozygosity and point mutation	
PTEN	Cell survival and proliferation
TP53 (also *P53*)	Cell survival and proliferation, genome stability
Loss of heterozygosity and haploinsufficiency	
NKX3-1	Cell differentiation and proliferation
CDKN1B (P27KIP1)	Cell proliferation
Point mutations	
COPEB (also *KLR6*)	Transcription regulator
AR	Cell proliferation, survival, and differentiation
Amplification	
AR	Cell proliferation, survival, and differentiation
Overexpressed at mRNA and protein level	
HTERT	Cell immortality
HPN	Transmembrane protease
FASN	Fatty-acid synthesis
AMACR	Fatty-acid metabolism, branched chain
EZH2	Transcription repressor, cell proliferation
MYC	Cell proliferation
BCL2	Cell survival
Polymorphisms affecting prostate cancer risks	
AR	Cell proliferation, survival, and differentiation
CYP17	Androgen metabolism
SRD5A2	Androgen metabolism

some families.[81] 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.[82] 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*,[83] *RNASEL*,[84] and *MSR1*.[85]

ELAC2 is a candidate for the hereditary prostate cancer 2 locus (*HPC2*).[83] 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,[86] more recent reports have provided little confirmatory evidence.[87–89]

RNASEL was suggested as a candidate for *HPC1*[76] 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,[90] 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,[91] *RNASEL* alleles carrying inactivating mutations were not associated with prostate cancer, but results of a case-control study[92] showed a positive association with an ezymatically 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.[93]

Mutations in *MSR1* have been implicated in a subset of families with hereditary prostate cancer and non-familial cases.[85] *MSR1*, 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* alleles (*Msr-A–/–*) accumulate fewer lipid-laden macrophages in cardiovascular lesions and are more susceptible to infection than are wild-type mice.[94]

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 inflamation have been advanced as potential causative agents in prostate cancer.[95,96]

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.[97] 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.[98] As with other solid tumours, the number of changes identified increases with the stage of disease,[98] 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[77] 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.[77] Loss of 8p seems to be an early event since high-grade prostatic intraepithelial neoplasia might lose heterozygosity at this location.[77] Several genes located on chromosome 8p have been examined as candidate tumour suppressors, with one of the most promising being *NKX3-1*.

NKX3-1 is expressed in normal prostate epithelium and is decreased in prostate tumour cells.[99,100] Further, mice that do not have either one or both *Nkx3-1* alleles develop abnormal prostate duct branching, prostatic hyperplasia, and lesions similar to human prostatic intraepithelial neoplasia.[100]

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.[77,101] Loss of PTEN in primary prostate cancer correlates with high Gleason score and advanced stage.[102] 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.[103] AKT can phosphorylate ODKN1B, resulting in cytoplasmic retention of this protein and lack of the cell cycle arrest that ODKN1B mediates.[104] Inactivation of ODKN1B cannot, however, be the only function of the PTEN pathway during prostate carcinogensis; in the mouse, *Pten* can cooperate with either *Nkx3-1* or *Cdk1b* (encoding ODKN1B) in increasing the frequency and extent of high-grade prostatic intraepithelial neoplasia lesions and perhaps early cancers.[105,106] Since this pathway is frequently changed in prostate cancer, inhibition of signalling through PI3K and AKT is a promising therapeutic strategy.[101]

In one study,[107] 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 *CDKN2A*, 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.[108] However, amplification of regions on chromosome 8q correlates with aggressiveness of tumours.[109] 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.[109] Abnormally high concentrations of MYC mRNA have been reported in prostate cancer.[80] Another region of gain, that is also accompanied by protein overexpression, contains *PSCA*.[110,111] Since *PSCA* 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 gene[112] and *EIF3S3*.[113] Other regions of gain include the androgen receptor gene itself (located on Xq12), where amplification occurs almost exclusively in the hormone refractory state.[114]

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 detoxify environmental electrophilic carcinogens and oxidants, might have a genome caretaker role by preventing oxidant and electrophilic DNA damage and resulting mutation.[115] *GSTP1* promoter methylation is being used in molecular diagnosis as a biomarker for prostate cancer in bodily fluids such as urine and semen.[115]

Other genes have also been shown to be selectively methylated in many prostate cancers in their 5′ promoter regions including: *EDNRB*,[116] encoding the endothelin B receptor; *CD44*,[117] a cell adhesion molecule encoding a gene with metastasis suppressor activity in rat prostate cancer (see below); *ER-α,* encoding the oestrogen receptor α;[118,119] and *ER-β,* encoding the oestrogen receptor β.[119]

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.[78,120]

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.[121] Other cadherins and other members of the cadherin signalling pathway such as α-catenin, are also sometimes altered in prostate cancer.[78] 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.[122] 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 colleagues[123] 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.[120] Several candidate metastasis suppressor genes in addition to CD44 have been identified for prostate cancer—*KAI1*, *NME23*, mapsin, *BRMS1*, *KISS1*, and *MAP2K4*.[120]

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.[126] 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.[80] 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.[23] 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.[124] 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.[127,128] Loss of CDKN1B also correlates with poor prognosis in prostate cancer,[129] 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,[130] and androgen receptor seems to remain crucial for prostate cancer cell survival, even when tumours are hormone refractory to androgen deprivation.[131] Prostate cancer seems to act through several molecular mechanisms to achieve androgen receptor function in the absence of classic ligands. First, amplification of the androgen receptor gene, accompanied by overexpression of the receptor, might lead to androgen-independent prostate cancer cell growth by increasing the sensitivity of prostate cancer cells to low concentrations of circulating androgens. Second, mutations in the androgen receptor gene can occur that change the ligand specificity of androgen receptor with resultant activation by non-androgens or by antiandrogens. Third, androgen-independent prostate cancer might also progress, in the absence of mutations in the androgen receptor gene, via activation of ligand-independent androgen receptor signalling pathways. Finally, expression of androgen receptor coactivators might be changed to affect androgen receptor signalling in prostate cancer.

Apoptosis genes

Although prostate cancer has increased cell proliferation compared with healthy prostate tissue, as in other cancers, impairments in programmed cell death are also important. For example, the BCL2 protein, which is an antiapoptotic factor, is expressed mostly in prostate basal cells in healthy prostate tissue. However, this protein is upregulated at two stages of 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.[132] Thus, BCL2 might be a therapeutic target in advanced prostate cancer.[132] Other antiapoptotic genes are also overexpressed in prostate cancer.[133] TP53 is mutated in a small subset of prostate cancers with a higher proportion of mutations being found in metastatic lesions.[134] 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[135] 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.[136] However, our group assessed this mechanism with various techniques and did not find overexpression in primary prostate cancer.[136] 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.[136] 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.[137]

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).[138,139] FAS inhibitors might be selectively toxic to prostate cancer cells and have been proposed as therapeutic agents.[140] 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.[141] Another gene product overexpressed in prostate cancer, that was first identified by subtractive hybridisation and then by microarray analysis,[28] is *AMACR*. AMACR has a key role in β 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 regulatory gene that is a transcriptional repressor and is found in higher concentrations in metastatic prostate cancers than in primary tumours.[142]

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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References

1 McNeal JE. Origin and development of carcinoma in the prostate. *Cancer* 1969; **23:** 24–34.
2 McNeal JE, Bostwick DG. Intraductal dysplasia: a pre-malignant lesion of the prostate. *Hum Pathol* 1986; **17:** 64–71.
3 Haggman MJ, Macoska JA, Wojno KJ, Oesterling JE. The relationship between prostatic intraepithelial neoplasia and prostate cancer: critical issues. *J Urol* 1997; **158:** 12–22.
4 McNeal JE, Villers A, Redwine EA, Freiha FS, Stamey TA. Microcarcinoma in the prostate: its association with duct-acinar dysplasia. *Hum Pathol* 1991; **22:** 644–52.
5 Bostwick DG, Pacelli A, Lopez-Beltran A. Molecular biology of prostatic intraepithelial neoplasia. *Prostate* 1996; **29:** 117–34.
6 Wills ML, Hamper UM, Partin AW, Epstein JI. Incidence of high-grade prostatic intraepithelial neoplasia in sextant needle biopsy specimens. *Urology* 1997; **49:** 367–73.
7 Davidson D, Bostwick D, Qian J, et al. Prostatic intraepithelial neoplasia is a risk factor for adenocarcinoma: Predictive accuracy in needle biopsies. *J Urol* 1993; **154:** 1295–99.
8 O'Dowd GJ, Miller MC, Orozco R, Veltri RW. Analysis of results within 1 year after a noncancer diagnosis. *Urology* 2000; **55:** 553–59.
9 Kronz JD, Allan CH, Shaikh AA, Epstein JI. Predicting cancer following a diagnosis of high grade PIN (HGPIN) on needle biopsy. *Am J Surg Pathol* 2001; **25:** 1079–85.
10 Hamper UM, Sheth S, Walsh PC, Holtz PM, Epstein JI. Stage B adenocarcinoma of the prostate: transrectal US and pathologic peripheral zone lesions. *Radiology* 1991; **44:** 101–04.
11 Ronnette BM, CarMichael MJ, Carter HB, Epstein JI. Does prostatic intraepithelial neoplasia result in elevated serum prostate specific antigen levels? *J Urol* 1993; **150:** 386–89.
12 Alexander EE, Qian J, Wollan PC, Myers RP, Bostwick DG. Prostatic intraepithelial neoplasia does not appear to raise serum prostate-specific antigen concentration. *Urology* 1997; **47:** 693–98.
13 Ramos CG, Carvahal GF, Mager DE, Haberer B, Catalona WJ. The effect of high grade prostatic intraepithelial neoplasia on serum total and percentage of free prostate specific antigen levels. *J Urol* 1999; **162:** 1587–90.
14 Shepherd D, Keetch DW, Humphrey PA, Smith DS, Stahl D. Repeat biopsy strategy in men with isolated prostatic intraepithelial neoplasia on prostate needle biopsy. *J Urol* 1996; **156:** 460–63.
15 Langer JE, Rover ES, Coleman BG, et al. Strategy for repeat biopsy of patients with prostatic intraepithelial neoplasia detected by prostate needle biopsy. *J Urol* 1996; **155:** 228–31.
16 Kamoi K, Troncoso P, Babaian RJ. Strategy for repeat biopsy in patients with high grade prostatic intraepithelial neoplasia. *J Urol* 2000; **163:** 819–23.
17 Epstein JI. Interpretation of prostate biopsies. 3rd edn. Philadelphia, PA: Lippincott Williams and Wilkins, 2002:
18 Epstein JI. Diagnostic criteria of limited adenocarcinoma of the prostate on needle biopsy. *Hum Pathol* 1995; **26:** 233–39.
19 Baisden BL, Kahane H, Epstein JI. Perineural invasion, mucinous fibroplasia and glomerulations: diagnostic features of limited cancer on prostate needle biopsy. *Am J Surg Pathol* 1999; **23:** 918–24.
20 Hedrick L, Epstein JI. Use of keratin 903 as an adjunct in the diagnosis of prostate carcinoma. *Am J Surg Pathol* 1989; **13:** 389–96.
21 Wojno KJ, Epstein JI. The utility of basal cell specific anti-cytokeratin antibody (34 beta E12) in the diagnosis of prostate cancer: a review of 228 cases. *Am J Surg Pathol* 1995; **19:** 251–60.
22 O'Malley FP, Grignon DJ, Shum DT. Usefulness of immunoperoxidase staining with high-molecular-weight cytokeratin in the differential diagnosis of small-acinar lesions of the prostate gland. *Virchows Archiv A Pathol Anat Histopathol* 1990; **417:** 191–96.
23 Shah IA, Schlageter MO, Stinnett P, Lechago J. Cytokeratin immunohistochemistry as a diagnostic tool for distinguishing malignant from benign epithelial lesions of the prostate. *Mod Pathol* 1991; **4:** 220–24.
24 Brawer MK, Peehl DM, Stamey TA, Bostwick DG. Keratin immunoreactivity in the benign and neoplastic human prostate. *Cancer Res* 1985; **45:** 3663–67.
25 Nagle RB, Ahmann FR, McDaniel KM, Paquin ML, Clark VA, Celniker A. Cytokeratin characterization of human prostatic carcinoma and its derived cell lines. *Cancer Res* 1987; **47:** 281–86.
26 Jiang Z, Woda BA, Rock KL, et al. P504S: A new molecular marker for the detection of prostate carcinoma. *Am J Surg Pathol* 2001; **25:** 1397–404.
27 Rubin MA, Zhou M, Dhanasekaran SM, Varambally S, Barrette TR, Sanda MG. α-methylacyl coenzyme A racemase as a tissue biomarker for prostate cancer. *JAMA* 2002; **287:** 1662–70.
28 Luo J, Zha S, Gage WR, et al. Alpha-methyl CoA racemase: a new molecular marker for prostate cancer. *Cancer Res* 2002; **62:** 2220–26.
29 Goldstein NS, Underhill J, Roszka J, Neill JS. Cytokeratin 34 beta E-12 immunoreactivity in benign prostatic acini: quantitation, pattern assessment, and electron microscopic study. *Am J Clin Pathol* 1999; **112:** 69–74.
30 Epstein JI, Walsh PC, Sanfilippo F. Clinical and cost impact of second opinion pathology review of prostate needle biopsies prior to radical prostatectomy. *Am J Surg Pathol* 1996; **20:** 851–57.
31 Chan TY, Magi-Galluzzi C, Leclercq N, Epstein JI. Patient and urologist driven second opinion of prostate needle biopsies. *Mod Pathol* 2002; **15:** 157A.
32 Gleason DF, Mellinger GT, and the Veterans Administration Cooperative Urological Research Group. Prediction of prognosis for prostatic adenocarcinoma by combined histologic grading and clinical staging. *J Urol* 1974; **111:** 58–64.
33 Steinberg DM, Sauvageot J, Piantadosi S, Epstein JI. Correlation of prostate needle biopsy and radical prostatectomy Gleason grade in academic and community settings. *Am J Surg Pathol* 1997; **21:** 566–76.
34 Allsbrook WC Jr, Mangold KA, Johnson MH, et al. Interobserver reproducibility of Gleason grading of prostatic carcinoma. I. Urologic pathologists. *Hum Pathol* 2001; **32:** 74–80.
35 Allsbrook WC Jr, Mangold KA, Johnson MH, Lane RB, Lane CG, Epstein JI. Interobserver reproducibility of Gleason grading of prostatic carcinoma. *II Gen Pathol Hum Pathol* 2001; **32:** 81–88.
36 Kronz JD, Silberman MA, Allsbrook WC Jr, Epstein JI. A web-based tutorial improves practicing pathologists' Gleason grading of prostate cancer on needle biopsies: validation of a new medical education paradigm. *Cancer* 2000; **89:** 1818–23.
37 Noguchi M, Stamey TA, McNeal JE, Yemoto CM. Relationship between systematic biopsies and histological features of 222 radical prostatectomy specimens: lack of prediction of tumour significance for men with nonpalpable prostate cancer. *J Urol* 2001; **16:** 104–10.
38 McNeal JE, Villers AA, Redwine EA, Freiha FS, Stamey TA. Histologic differentiation, cancer volume, and pelvic lymph node metastasis in adenocarcinoma of the prostate. *Cancer* 1990; **66:** 1225–33.
39 Epstein JI, Partin AW, Sauvageot J, Walsh PC. Prediction of progression following radical prostatectomy: a multivariate analysis of 721 men with long-term follow-up. *Am J Surg Pathol* 1996; **20:** 286–92.
40 Green GA, Hanlon AL, Al-Saleem T, Hanks GE. A Gleason score of 7 predicts a worse outcome for prostate carcinoma patients treated with radiotherapy. *Cancer* 1998; **83:** 971–76.
41 Partin AW, Kattan MW, Subong EN, et al. Combination of prostate-specific antigen, clinical stage, and Gleason score to predict pathological stage of localized prostate cancer: a multi-institutional update. *JAMA* 1997; **277:** 1445–51.
42 Partin AW, Carter HB, Chan DW, et al. Prostate-specific antigen in the staging of localized prostate cancer: Influence of tumor differentiation, tumor volume, and benign hyperplasia. *J Urol* 1990; **143:** 747–52.
43 Brawn PN. The dedifferentiation of prostate carcinoma. *Cancer* 1983; **52:** 246–51.
44 Cumming JA, Ritchies AW, Goodman CM, McIntyre MA, Chisholm GD. De-differentiation with time in prostate cancer and the influence of treatment on the course of the disease. *Br J Urol* 1990; **65:** 271–74.
45 Epstein JI, Carter HB. Is there dedifferentiation of prostate cancer grade change over time in men followed expectantly for stage T1C disease? *J Urol* 2001; **166:** 1688–91.
46 Badalament RA, Miller MC, Peller PA, et al. An algorithm for predicting nonorgan confined prostate cancer using the results obtained from sextant core biopsies with prostate specific antigen level. *J Urol* 1996; **156:** 1375–80.

47 Huland H, Hammerer P, Henke RP, Huland E. Preoperative prediction of tumor heterogeneity and recurrence after radical prostatectomy for localized prostatic carcinoma with digital rectal examination, prostate specific antigen and the results of 6 systematic biopsies. *J Urol* 1996; **155:** 1344–47.

48 Peller PA, Young DC, Marmaduke DP, Marsh WL, Badalament RA. Sextant prostate biopsies: a histopathologic correlation with radical prostatectomy specimens. *Cancer* 1995; **75:** 530–38.

49 Ravery V, Boccon-Gibod LA, Dauge-Geffroy MC, et al. Systematic biopsies accurately predict extracapsular extension of prostate cancer and persistent/recurrent detectable PSA after radical prostatectomy. *Urology* 1994; **44:** 371–76.

50 Wills ML, Sauvageot J, Partin AW, Gurganus R, Epstein JI. Ability of sextant biopsies to predict radical prostatectomy stage. *Urology* 1998; **51:** 759–64.

51 Sebo TJ, Bock BJ, Cheville JC, Lohse CM, Wollan P, Zincke H. The percent of cores positive for cancer in prostate needle biopsy specimens is strongly predictive of tumor stage and volume at radical prostatectomy. *J Urol* 2000; **163:** 174–78.

52 Terris MK, Haney DJ, Johnstone IM, McNeal JE, Stamey TA. Prediction of prostate cancer volume using prostate-specific antigen levels, transrectal ultrasound, and systemic sextant biopsies. *Urology* 1995; **45:** 75–80.

53 Conrad S, Graefen M, Pichlmeier U, Henke RP, Hamerer PG, Huland H. Systematic sextant biopsies improve preoperative prediction of pelvic lymph node metastases in patients with clinically localized prostatic carcinoma. *J Urol* 1998; **159:** 2023–29.

54 Humphrey PA, Baty J, Keetch D. Relationship between serum prostate specific antigen, needle biopsy findings, and histopathologic features of prostatic carcinoma in radical prostatectomy tissues. *Cancer* 1995; **75:** 1842–49.

55 Ackerman DA, Barry JM, Wicklund RA, Olson N, Lowe BA. Analysis of risk factors associated with prostate cancer extension to the surgical margin and pelvic node metastasis at radical prostatectomy. *J Urol* 1993; **159:** 1845–50.

56 Tigrani VS, Bhargava V, Shinohara K, Presti JC Jr. Number of positive systematic sextant biopsies predicts surgical margin status at radical prostatectomy. *Urology* 1999; **54:** 689–703.

57 Presti JC Jr, Shinohara K, Bacchetti P, Tigrani V, Bhargava V. Positive fraction of systematic biopsies predicts risk of relapse after radical prostatectomy. *Urology* 1998; **52:** 1079–84.

58 Cupp MR, Bostwick DG, Myers RP, Oesterling JE. The volume of prostate cancer in the biopsy specimen cannot reliably predict the quantity of cancer in the radical prostatectomy specimen on an individual basis. *J Urol* 1995; **53:** 1543–48.

59 Bruce RG, Rankin WR, Cibull ML, Rayens MK, Banks ER, Wood DP Jr. Single focus of adenocarcinoma in the prostate biopsy specimen is not predictive of the pathologic stage of disease. *Urology* 1996; **48:** 75–79.

60 Dietrick DD, McNeal JE, Stamey TA. Core cancer length in ultrasound-guided systematic sextant biopsies: a preoperative evaluation of prostate cancer volume. *Urology* 1995; **45:** 987–92.

61 Epstein JI, Walsh PC, CarMichael M, Brendler CB. Pathological and clinical findings to predict tumor extent of non-palpable (stage T1c) prostate cancer. *JAMA* 1994; **271:** 368–74.

62 Wang X, Brannigan RE, Rademaker AW, McVary KT, Oyasu R. One core positive prostate biopsy is a poor predictor of cancer volume in the radical prostatectomy specimen. *J Urol* 1997; **158:** 1431–35.

63 Weldon W, Tavel FR, Neuwirth H, Cohen R. Failure of focal prostate cancer on biopsy to predict focal prostate cancer: the importance of prevalence. *J Urol* 1995; **154:** 1074–77.

64 Allan RW, Sanderson H, Epstein JI. correlation of minute (< 1mm) focus of adenocarcinoma of the prostate on needle biopsy with radical prostatectomy specimen: role of PSA density. *J Urol* (in press).

65 Bastacky SI, Walsh PC, Epstein JI. Relationship between perineural tumor invasion on needle biopsy and radical prostatectomy capsular penetration in clinical stage B adenocarcinoma of the prostate. *Am J Surg Pathol* 1993; **17:** 336–41.

66 Anderson PR, Hanlon AL, Patchefsky A, Al-Saleem T, Hanks GE. Perineural invasion and Gleason 7-10 tumors predict increased failure in prostate cancer patients with pretreatment PSA <10 ng/mL treated with conformal external beam radiation therapy. *Int J Radiat Oncol Biol Phys* 1998; **41:** 1087–92.

67 Brinker DA, Ross JS, Tran TA, Jones DM, Epstein JI. Can ploidy of prostate carcinoma diagnosed on needle biopsy predict radical prostatectomy stage and grade? *J Urol* 1999; **162:** 2036–39.

68 Sebo TJ, Cheville JC, Riehle DL, et al. Perineural invasion and MIB-1 positivity in addition to Gleason score are significant predictors of progression after radical retropubic prostatectomy. *Am J Surg Pathol* 2002; **26:** 431–39.

69 Epstein JI. Pathological assessment of the surgical specimen. *Urol Clin North Am* 2001; **28:** 567–94.

70 Grönberg H. Prostate cancer epidemiology. *Lancet* 2003; **361:** 859–64.

71 Hsing AW. Hormones and prostate cancer: what's next? *Epidemiol Rev* 2001; **23:** 42–58.

72 Platz EA, Helzlsouer KJ. Selenium, zinc, and prostate cancer. *Epidemiol Rev* 2001; **23:** 93–101.

73 Kolonel LN. Fat, meat, and prostate cancer. *Epidemiol Rev* 2001; **23:** 72–81.

74 Chan JM, Giovannucci EL. Vegetables, fruits, associated micronutrients, and risk of prostate cancer. *Epidemiol Rev* 2001; **23:** 82–86.

75 Pollak M. Insulin-like growth factors and prostate cancer. *Epidemiol Rev* 2001; **23:** 59–66.

76 Grossfeld GD, Carroll PR. Natural history. In: Carroll PR, Grossfeld GD, eds. Prostate cancer. Hamilton, Ontario: B C Decker, 2001: 149–63.

77 Bookstein R. Tumor suppressor genes in prostate cancer. In: Chung LW, Isaacs WB, Simons JW, eds. Prostate cancer: biology, genetics, and the new theraputics. Totowa, NJ: Humana Press, 2001: 61–93.

78 Gao AC, Isaacs JT. Molecular basis of prostate carcinogenesis. In: Coleman WB, Tsongalis GJ, eds. The molecular basis of human cancer. Totowa, NJ: Humana Press, 2002: 365–79.

79 Chung LWK, Isaacs WB, Simons JW. Prostate cancer: biology, genetics, and the new therapeutics. Totowa, NJ: Humana Press, 2001.

80 Meng MV, Dahiya R. Molecular genetics. In: Carroll PR, Grossfeld GD, eds. Prostate cancer. Hamilton, Ontario: B C Decker, 2002: 42–59.

81 Isaacs WB, Xu J, Walsh PC. Hereditary prostate cancer. In: Chung LW, Isaacs WB, Simons JW, eds. Prostate cancer: biology, genetics, and the new theraputics. Totowa, NJ: Humana Press, 2001: 13–37.

82 Smith JR, Freije D, Carpten JD, et al. Major susceptibility locus for prostate cancer on chromosome 1 suggested by a genome-wide search. *Science* 1996; **274:** 1371–74.

83 Tavtigian SV, Simard J, Teng DH, et al. A candidate prostate cancer susceptibility gene at chromosome 17p. *Nat Genet* 2001; **27:** 172–80.

84 Carpten J, Nupponen N, Isaacs S, et al. Germline mutations in the ribonuclease L gene in families showing linkage with HPC1. *Nat Genet* 2002; **30:** 181–84.

85 Xu J, Zheng SL, Komiya A, et al. Germline mutations and sequence variants of the macrophage scavenger receptor 1 gene are associated with prostate cancer risk. *Nat Genet* 2002; **32:** 321–25.

86 Rebbeck TR, Walker AH, Zeigler-Johnson C, et al. Association of HPC2/ELAC2 genotypes and prostate cancer. *Am J Hum Genet* 2000; **67:** 1014–19.

87 Wang L, McDonnell SK, Elkins DA, et al. Role of HPC2/ELAC2 in hereditary prostate cancer. *Cancer Res* 2001; **61:** 6494–99.

88 Rokman A, Ikonen T, Mononen N, et al. ELAC2/HPC2 involvement in hereditary and sporadic prostate cancer. *Cancer Res* 2001; **61:** 6038–41.

89 Suarez BK, Gerhard DS, Lin J, et al. Polymorphisms in the prostate cancer susceptibility gene HPC2/ELAC2 in multiplex families and healthy controls. *Cancer Res* 2001; **61:** 4982–84.

90 Rokman A, Ikonen T, Seppala EH, et al. Germline alterations of the RNASEL gene, a candidate HPC1 gene at 1q25, in patients and families with prostate cancer. *Am J Hum Genet* 2002; **70:** 1299–304.

91 Wang L, McDonnell SK, Elkins DA, et al. Analysis of the RNASEL gene in familial and sporadic prostate cancer. *Am J Hum Genet* 2002; **71:** 116–23.

92 Casey G, Neville PJ, Plummer SJ, et al. RNASEL Arg462Gln variant is implicated in up to 13% of prostate cancer cases. *Nat Genet* 2002; **32:** 581–83.

93 Rennert H, Bercovich D, Hubert A, et al. A novel founder mutation in the RNASEL gene, 471delAAAG, is associated with prostate cancer in Ashkenazi Jews. *Am J Hum Genet* 2002; **71:** 981–84.

94 Ishiguro T, Naito M, Yamamoto T, et al. Role of macrophage scavenger receptors in response to Listeria monocytogenes infection in mice. *Am J Pathol* 2001; **158:** 179–88.

95 Smith CJ, Gardner WA Jr. Inflammation-proliferation: possible relationships in the prostate. *Prog Clin Biol Res* 1987; **239:** 317–25.

96 De Marzo AM, Marchi VL, Epstein JI, Nelson WG. Proliferative inflammatory atrophy of the prostate: implications for prostatic carcinogenesis. *Am J Pathol* 1999; **155:** 1985–92.

97 Makridakis NM, Reichardt JK. Molecular epidemiology of hormone-metabolic loci in prostate cancer. *Epidemiol Rev* 2001; **23:** 24–29.

98 Elo JP, Visakorpi T. Molecular genetics of prostate cancer. *Ann Med* 2001; **33:** 130–41.

99 Bhatia-Gaur R, Donjacour AA, Sciavolino PJ, et al. Roles for Nkx3.1 in prostate development and cancer. *Genes Dev* 1999; **13:** 966–77.

100 Bowen C, Bubendorf L, Voeller HJ, et al. Loss of NKX3.1

expression in human prostate cancers correlates with tumor progression. *Cancer Res* 2000; **60:** 6111–15.
101 Wang SI, Parsons R, Ittmann M. Homozygous deletion of the PTEN tumor suppressor gene in a subset of prostate adenocarcinomas. *Clin Cancer Res* 1998; **4:** 811–15.
102 McMenamin ME, Soung P, Perera S, Kaplan I, Loda M, Sellers WR. Loss of PTEN expression in paraffin-embedded primary prostate cancer correlates with high Gleason score and advanced stage. *Cancer Res* 1999; **59:** 4291–96.
103 Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase AKT pathway in human cancer. *Nat Rev Cancer* 2002; **2:** 489–501.
104 Fujita N, Sato S, Katayama K, Tsuruo T. Akt-dependent phosphorylation of p27Kip1 promotes binding to 14-3-3 and cytoplasmic localization. *J Biol Chem* 2002; **277:** 28706–13.
105 Di Cristofano A, De Acetis M, Koff A, Cordon-Cardo C, Pandolfi PP. Pten and p27KIP1 cooperate in prostate cancer tumor suppression in the mouse. *Nat Genet* 2001; **27:** 222–24.
106 Kim MJ, Cardiff RD, Desai N, et al. Cooperativity of Nkx3.1 and Pten loss of function in a mouse model of prostate carcinogenesis. *Proc Natl Acad Sci USA* 2002; **99:** 2884–89.
107 Narla G, Heath KE, Reeves HL, et al. KLF6, a candidate tumor suppressor gene mutated in prostate cancer. *Science* 2001; **294:** 2563–66.
108 Savinainen KJ, Saramaki OR, Linja MJ, et al. Expression and gene copy number analysis of ERBB2 oncogene in prostate cancer. *Am J Pathol* 2002; **160:** 339–45.
109 Sato K, Qian J, Slezak JM, et al. Clinical significance of alterations of chromosome 8 in high-grade, advanced, nonmetastatic prostate carcinoma. *J Natl Cancer Inst* 1999; **91:** 1574–80.
110 Reiter RE, Sato I, Thomas G, et al. Coamplification of prostate stem cell antigen (PSCA) and MYC in locally advanced prostate cancer. *Genes Chromosomes Cancer* 2000; **27:** 95–103.
111 Gu Z, Thomas G, Yamashiro J, et al. Prostate stem cell antigen (PSCA) expression increases with high gleason score, advanced stage and bone metastasis in prostate cancer. *Oncogene* 2000; **19:** 1288–96.
112 Porkka K, Saramaki O, Tanner M, Visakorpi T. Amplification and overexpression of Elongin C gene discovered in prostate cancer by cDNA microarrays. *Lab Invest* 2002; **82:** 629–37.
113 Saramaki O, Willi N, Bratt O, et al. Amplification of EIF3S3 gene is associated with advanced stage in prostate cancer. *Am J Pathol* 2001; **159:** 2089–94.
114 Visakorpi T. AR gene alterations in prostate cancer progression. In: Chung LW, Isaacs WB, Simons JW, eds. Prostate cancer: biology, genetics, and the new therapeutics. Totowa, NJ: Humana Press, 2001: 29–37.
115 Nelson WG, De Marzo AM, Deweese TL, et al. Preneoplastic prostate lesions: an opportunity for prostate cancer prevention. *Ann NY Acad Sci* 2001; **952:** 135–44.
116 Nelson JB, Lee WH, Nguyen SH, et al. Methylation of the 5′ CpG island of the endothelin B receptor gene is common in human prostate cancer. *Cancer Res* 1997; **57:** 35–37.
117 Lou W, Krill D, Dhir R, et al. Methylation of the CD44 metastasis suppressor gene in human prostate cancer. *Cancer Res* 1999; **59:** 2329–31.
118 Li LC, Chui R, Nakajima K, et al. Frequent methylation of estrogen receptor in prostate cancer: correlation with tumor progression. *Cancer Res* 2000; **60:** 702–06.
119 Sasaki M, Tanaka Y, Perinchery G, et al. Methylation and inactivation of estrogen, progesterone, and androgen receptors in prostate cancer. *J Natl Cancer Inst* 2002; **94:** 384–90.
120 Jaeger EB, Samant RS, Rinker-Schaeffer CW. Metastasis suppression in prostate cancer. *Cancer Metastasis Rev* 2001; **20:** 279–86.
121 Umbas R, Isaacs WB, Bringuier PP, et al. Decreased E-cadherin expression is associated with poor prognosis in patients with prostate cancer. *Cancer Res* 1994; **54:** 3929–33.
122 Gao AC, Lou W, Dong JT, Isaacs JT. CD44 is a metastasis suppressor gene for prostatic cancer located on human chromosome 11p13. *Cancer Res* 1997; **57:** 846–49.
123 Nagle RB, Hao J, Knox JD, Dalkin BL, Clark V, Cress AE. Expression of hemidesmosomal and extracellular matrix proteins by normal and malignant human prostate tissue. *Am J Pathol* 1995; **146:** 1498–507.
124 Sommerfeld HJ, Meeker AK, Piatyszek MA, Bova GS, Shay JW, Coffey DS. Telomerase activity: a prevalent marker of malignant human prostate tissue. *Cancer Res* 1996; **56:** 218–22.
125 Meeker AK, Hicks JL, Platz EA, et al. Telomere shortening is an early somatic DNA alteration in human prostate tumorigenesis. *Cancer Res* 2002; **62:** 6405–09.
126 Loeb LA. Mutator phenotype may be required for multistage carcinogenesis. *Cancer Res* 1991; **51:** 3075–79.
127 Guo YP, Sklar GN, Borkowski A, Kyprianou N. Loss of the cyclin-dependent kinase inhibitor P27(Kip1) protein in human prostate cancer correlates with tumor grade. *Clin Cancer Res* 1997; **3:** 2269–74.
128 De Marzo AM, Meeker AK, Epstein JI, Coffey DS. Prostate stem cell compartments: expression of the cell cycle inhibitor p27Kip1 in normal, hyperplastic, and neoplastic cells. *Am J Pathol* 1998; **153:** 911–19.
129 Cote RJ, Shi Y, Groshen S, et al. Association of p27Kip1 levels with recurrence and survival in patients with stage C prostate carcinoma. *J Natl Cancer Inst* 1998; **90:** 916–20.
130 Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. *Nat Rev Cancer* 2001; **1:** 34–45.
131 Zegarra-Moro OL, Schmidt LJ, Huang H, Tindall DJ. Disruption of androgen receptor function inhibits proliferation of androgen-refractory prostate cancer cells. *Cancer Res* 2002; **62:** 1008–13.
132 DiPaola RS, Patel J, Rafi MM. Targeting apoptosis in prostate cancer. *Hematol Oncol Clin North Am* 2001; **15:** 509–24.
133 Krajewska M, Krajewski S, Epstein JI, et al. Immunohistochemical analysis of bcl-2, bax, bcl-X, and mcl-1 expression in prostate cancers. *Am J Pathol* 1996; **148:** 1567–76.
134 Navone NM, Troncoso P, Pisters LL, et al. p53 protein accumulation and gene mutation in the progression of human prostate carcinoma. *J Natl Cancer Inst* 1993; **85:** 1657–69.
135 Roberts RO, Jacobson DJ, Girman CJ, Rhodes T, Lieber MM, Jacobsen SJ. A population-based study of daily nonsteroidal anti-inflammatory drug use and prostate cancer. *Mayo Clin Proc* 2002; **77:** 219–25.
136 Zha S, Gage WR, Sauvageot J, et al. Cyclooxygenase-2 is up-regulated in proliferative inflammatory atrophy of the prostate, but not in prostate carcinoma. *Cancer Res* 2001; **61:** 8617–23.
137 Waskewich C, Blumenthal RD, Li H, Stein R, Goldenberg DM, Burton J. Celecoxib exhibits the greatest potency amongst cyclooxygenase (COX) inhibitors for growth inhibition of COX-2-negative hematopoietic and epithelial cell lines. *Cancer Res* 2002; **62:** 2029–33.
138 Shurbaji MS, Kuhajda FP, Pasternack GR, Thurmond TS. Expression of oncogenic antigen 519 (OA-519) in prostate cancer is a potential prognostic indicator. *Am J Clin Pathol* 1992; **97:** 686–91.
139 Swinnen JV, Roskams T, Joniau S, et al. Overexpression of fatty acid synthase is an early and common event in the development of prostate cancer. *Int J Cancer* 2002; **98:** 19–22.
140 Pizer ES, Pflug BR, Bova GS, Han WF, Udan MS, Nelson JB. Increased fatty acid synthase as a therapeutic target in androgen-independent prostate cancer progression. *Prostate* 2001; **47:** 102–10.
141 Rhodes DR, Barrette TR, Rubin MA, Ghosh D, Chinnaiyan AM. Meta-analysis of microarrays: interstudy validation of gene expression profiles reveals pathway dysregulation in prostate cancer. *Cancer Res* 2002; **62:** 4427–33.
142 Varambally S, Dhanasekaran SM, Zhou M, et al. The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature* 2002; **419:** 624–29.