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Review

Molecular markers in prostate cancer. Part I: predicting lethality

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Abstract

Assessing the lethality of 'early,' potentially organ-confined prostate cancer (PCa) is one of the central controversies in modern-day urological clinical practice. Such cases are often considered for radical 'curative' treatment, although active surveillance may be equally appropriate for many men. Moreover, the balance between judicious intervention and over-treatment can be difficult to judge. The patient's age, comorbidities, family history and philosophy of self-health care can be weighed against clinical features such as the palpability of disease, the number and percentage of biopsy cores involved with the disease, histological grade, presenting prostate-specific antigen (PSA) and possible previous PSA kinetics. For many years, scientists and physicians have sought additional molecular factors that may be predictive for disease stage, progression and lethality. Usually, claims for a 'new' unique marker fall short of true clinical value. More often than not, such molecular markers are useful only in multivariate models. This review summarizes relevant molecular markers and models reported up to and including 2008.

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1 Introduction

Current ideological frameworks for the pathogenesis of prostate cancer (Pca) emphasize the roles of precursor lesions such as prostatic intraepithelial neoplasia (PIN), proliferative inflammatory atrophy (PIA) and tissue hypoxia. Alternative theories suggest that prostate stem cells function as reservoirs of disease. Research has focused on identifying potential molecular and genetic markers, which may help to clarify and predict the natural history of PCa: thus helping to identify those suitable for active surveillance [1]. A number of potential diagnostic, prognostic and surveillance markers have been identified [2–46] (Table 1).

Developments in molecular technology have helped to identify and alter the expression of these genes and the related markers. Techniques such as fluorescence-activated cell sorting, fluorescence *in situ* hybridization, reverse transcription polymerase chain reaction and proteomics have improved sensitivity, facilitating the detection of smaller groups of prostate cells and markers. The identification of many markers still relies on tissue sampling, which is invasive and susceptible to sampling error. Urine and serum samples are safer, more acceptable alternatives and have been analysed using deoxyribonucleic acid (DNA)/ribonucleic acid (RNA)-based assays. However, urine and serum samples may have lower sensitivities because early well-differentiated PCa is less likely to be present [21]. In metastatic PCa, > 5 cells per 7.5 mL of blood are detectable, setting a minimum target for molecular assays [20]. The role of seminal plasma as a rich source of markers has also been reviewed, but limited studies exist because of difficulties in sampling and tolerance [21]. Difficulties in obtaining sufficient amounts of high-quality ribonucleic acid for microarrays have also

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Table 1. Summary of molecular markers in prostate cancer (Pca): mechanisms of action and potential roles.

Gene/Protein	Action	Selected literature	Potential use
AKT and PTEN	Prostate-specific phosphatase and tensin homologue (PTEN) loss of function induces AKT (protein kinase) inhibiting apoptosis and may cause tubule regeneration with prostatic intraepithelial neoplasia (PIN) [2].	PTEN null mice develop high-grade PCa and metastasis [3]. Genetic PTEN alteration seen in 10% primary PCa, >30% metastases, PTEN loss induces p53 senescence [4]. Possible use as gene therapy vector [5].	Prognostic and therapeutic
AMACR	Alpha methlyacyl coenzyme A racemase protein voided in urine. Involved in fatty acid β-oxidation. Androgen-independent function as promoter of PCa [6].	100% sensitive, 58% specific. Small study [7]. Histopathological biomarker [6].	Diagnostic
AR	Androgen receptor, nuclear transcription factor mediates steroid hormones and stromal cell growth. AR activation in luminal cells suppresses growth [8].	Stimulates early PCa growth, gene amplification in 30% AI tumours [4]. AR mutations rare in untreated PCa [9]. Vorinostat (histone deacetylase inhibitor) may reduce AR expression acting synergistically with bicaltamide (AR antagonist) to inhibit PCa [10].	Prognostic and therapeutic
Bcl-2	B-cell CLL/lymphoma 2, antiapoptotic protein found in basal cells and stem cells [2]. Loss of expression linked to PIN, progression and androgen independence [11].	Antisense oligonucleotides (Oblimersen) against Bcl-2 delay progression, improves chemo-sensitivity. Docetaxel combination trial underway (NCT00085228) [12, 13].	Prognostic and therapeutic
BRCA2	Breast cancer type 2 susceptibility protein, tumour suppressor gene, predisposes to Pca, chromosome 13q.	< 5% Familial/young-onset cases diagnosed < 55 years old [14].	Prevention
CgA	Chorionic gonadotropin alpha, neuroendocrine prehormone peptide. Unclear mechanism of action.	Limited use and reproducibility. Correlation with time to androgen independence and adverse outcome [6].	Prognostic
Cyclin D1	Role in cell cycle from G1- to S-phase.	Upregulation may be associated with androgen independence and poor prognosis [8].	Prognostic
E-cadherin	Cell adhesion molecule. Downregulation/loss associated with invasion and metastasis.	Close univarate T and M stage and survival significance [8].	Prognostic
EGFR (Erb B1) Her-2/Neu (Erb 2)	Activation associated with proliferation, malignant transformation, relapse, progression and androgen independence [4]. Epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her2).	Higher levels in PCa than BPH [15]. Monoclonal antibodies directed against specific binding domains, anti-EGFR, e.g. cetuximab, anti-HER2, e.g. trastuzumab [16]. Lack of significant role in PCa [12].	Prognostic and therapeutic
EN-2	Mouse engrailed-2 gene. Homoeobox-containing transcription factor/candidate oncogene, overexpressed in aggressive HRPC/PCa. May be positively modulated by PAX2 [17].	En-2 downregulation by siRNA decreases PAX2 and decreases PCa cell proliferation [17]. PAX2 has been postulated as essential for PCa cell survival [18].	Diagnostic and prognostic

(To be continued)

Table 1. Summary of molecular markers in prostate cancer (Pca): mechanisms of action and potential roles (Continued).

Gene/Protein	Action	Selected literature	Potential use
EPCA 1 and 2	Early prostate cell antigen, nuclear matrix protein. Associated with proliferative inflammatory atrophy (PIA), PIN and PCa. Generally not-detected in non-cancer cases.	No link to Gleeson score. Reported positive in BPH pts who subsequently developed PCa. EPCA 1 and 2, 92% sensitive, 94% specific. EPCA2 distinguishes localized *vs.* extracapsular disease ($P < 0.0001$) [cited in [6]. Awaiting larger trials [19].	Diagnostic and prognostic
ER	Oestrogen receptor located in stromal. Role unclear [8].	Close univarate M stage and survival significance [8].	Prognostic
EZH2	Enhancer of Zeste homolog, histone methyl transferase upregulation.	Linked to high-grade PCa development [20].	Prognostic
GSTP-1	Glutathione-S-transferase P1 protects DNA from free radicals (caretaker gene). Loss of gene expression due to hypermethylation associated with PCa [21, 22].	GSTP-1 hypermethylation: > 90% PCa, > 70% PIN, rare in benign disease. Urinary marker, may need prostatic massage. Small study. Diagnostic performance improved as a part of multiple gene panel [6].	Diagnostic
IL-6	Interleukin-6: cytokine immunomodulator. Linked to AR cells and suppression of androgen-dependent cells [23, 24].	Elevated levels related to advanced disease but not prognostic. Possible role as combination markers [6].	Prognostic
KLK3/PSA	Encodes PSA, a kallikrein-related peptidase (serine protease subgroup) on chromosome 19 [12].	Combination rV-PSA and fowl pox primed rF-PSA vaccines trialled. Phase II studies: median time to PSA progression increased (9.2–18.2 months) (study ECOG7897). Phase III PARADIGM trial underway [12].	Prognostic and therapeutic
KLK2/hK2	Encodes hK2, a kalikrein-related peptidase (serine protease subgroup) on chromosome 19. Serum levels 1% of PSA and undetectable in healthy males [25, 26].	hK2 with free and total PSA significantly improved diagnostic sensitivity/specificity . Predicts prostate biopsy outcome ($P < 0.001$). Role in predicting recurrence/progression [6 , 25].	Diagnostic and prognostic
MIB-1	Mindbomb homolog 1, monoclonal antibody and cell proliferation marker by Ki-67 antigen recognition.	Close univarate T, M stage and survival significance [8].	Prognostic
MSMB	Microseminoprotein, beta-, encodes PSP94, immunoglobulin-binding factor synthesized in prostatic epithelial cells [25].	Loss of PSP94 expression associated with recurrence after radical prostatectomy [27].	Prognostic
NKX3.1	NK3 transcription factor related, locus 1, homoeobox tumour suppressor gene, exclusive to prostate, undergoes epigenic inactivation [4] Ch 8p12–21 [28].	Increasing loss of protein expression in hormone-refractory and metastatic disease [29].	Prognostic
NSE	Neuron-specific enolase, neuroendocrine cell product.	Unknown paracrine/autocrine function [6].	Prognostic

(To be continued)

Table 1. Summary of molecular markers in prostate cancer (Pca): mechanisms of action and potential roles. (Continued)

Gene/Protein	Action	Selected literature	Potential use
p27Kip1	Cell cycle inhibitor found in basal compartment. Chromosome 12p12–13.1 [11].	Functional loss linked to progression and AI [4, 30]. PIA association [31]. Gene therapy target with recombinant adenovirus [11].	Prognostic and therapeutic
PAP	Prostate acid phosphatase, glycoprotein more specific to prostatic tissue than PSA [12].	APC8015 autologous vaccine. Phase I/II trials in HRPC. 31 patients, 38% developed immune response, three had PSA reduction > 50%. Overall survival increased 4.5 months ($P = 0.01$). Phase III IMPACT trial ongoing [12].	Diagnostic and therapeutic
PCA3 (DD3)	Prostate cancer antigen 3. Chromosome 9, messenger RNA overexpressed in > 95% PCa and metastases. Detected by reverse transcriptase on urine sediment [32, 33]. PROGENSA™ PCA3 Assay available in Europe.	First biopsy, PCA3 sensitivity 50%, specificity 77% [34]. Repeat biopsy, PCA3 score > 35, sensitivity 57% and specificity 73% [35]. Predictive of extracapsular extension and tumour volume, 94% specific, 80% positive predictive value [36].	Diagnostic
PSMA	Prostate-specific membrane antigen. Androgen-independent prostatic epithelium transmembrane protein found in PCa/lymph node metastasis [37].	DCVax-prostate vaccine target. Phase I/II trial underway [12].	Prognostic and therapeutic
PSCA	Prostate stem cell antigen. Membrane glycoprotein. Normal late–intermediate prostate cell marker upregulated in PCa [6].	Correlated to Gleason score, advanced stage, metastasis and AI (cited in [6]]). High independent prognostic value and specificity [38].	Prognostic
p53	Tumour suppressor gene allows DNA repair/cell apoptosis in cellular stress conditions [8].	Less significant in PCa, uncommon mutation in early PCa [39]. Independently prognostic in late-stage PCa [40]. Concomitant homozygous PTEN–p53 inactivation lead to PCa lethality in mice [41].	Prognostic & Therapeutic
RNASEL	Ribonuclease L (2',5'-oligoisoadenylate synthetase-dependent). Candidate tumour suppressor gene product [42] implicated in viral defence, regulates cell proliferation and apoptosis via an interferon pathway Ch 1q23–25 [43].	RNasel variant Arg462Gln significantly associated with PCa ($P = 0.007$). Mutated allele found in 60% of the men in the study group. 50% greater risk in heterozygous carriers [44].	Diagnostic
TGF- β1	Transforming growth factor. pleiotrophic growth factor known to promote stem cell quiescence [45].	Does not accurately distinguish PCa and benign disease. May have a role in progression and metastasis [6].	Prognostic
TMPRSS2:ETS	Transmembrane protease, serine 2 fusion gene (Ch 21), upregulates ETS target genes controlling cell proliferation, differentiation, apoptosis and transformation [2, 4] .	May be an early marker, as seen in 20% of PIN lesions [46].	Prognostic and prevention
Sex hormones and binding globulin	Testosterone is essential for prostatic development and maintenance. Oestrogens are associated with low risk of PCa [6].	High testosterone levels=lower PCa risk (non-Gleason > 7, stage 4, N+, M+) ($P = 0.003$). Serum testosterone < 300 ng per 100 mL predictor of PSA failure after radical prostatectomy. High levels of SHBG predict extracapsular extension ($P = 0.006$) [6].	Prognostic and prevention
u-PA/u-PAR	Urokinase plasminogen activator/cell surface receptor. Role in basement membrane/extracellular matrix degradation and metastases [6].	u-PA/u-PAR correlated to tumour stage and grade and inversely to androgen receptor status [6].	Prognostic

been overcome [16, 47]. Notably, multiple marker arrays may also improve detection; Glutathione-S-transferase P1 (GSTP-1) has been used in a four-gene panel (p16/ARF/MGMT/GSTP-1) in 52 PCa patients, resulting in 87% sensitivity and 100% specificity. These data need to be reproduced in large-scale randomized studies [48].

2 Genetics

Familial and epidemiological studies have supported the concept of a genetic predisposition to PCa and have helped to clarify susceptible loci [14, 28, 49, 50]. Some studies have focused on specific groups such as Ashkenazi Jews, but to date no single gene has reproducibly been found to be responsible for PCa, reflecting the multifocal and heterogenic nature of PCa [4, 51]. Familial studies have shown the relative risks of PCa to be 2.0 and 1.7 in first- and second-degree relatives, respectively, with the risk increasing to 8.8 when first- and second-degree relatives are both afflicted [49, 52]. A recent genome-wide association study identified several loci (chromosomes 3, 6, 7, 10, 11, 19 and X) associated with PCa [25]. The previously known 8q24 and17q loci were also confirmed, along with the identification of three new candidate susceptibility genes (MSMB, LMTK2, KLK3) [25]. Several groups have identified links to other loci; however, reproducibility between data sets has been limited. Nonetheless, common loci have been found on chromosomes 1, 8p12–22, 17, 19 and 22 [4, 50]. Genetic changes in PIN have also been identified and implicated in PCa pathogenesis, with 8p12–22 loss of heterozygosity seen in 63% of PIN lesions [53]. Viral DNA expression upon genomic screening of PCa specimens has also been reported [54].

3 Proteomics

Current research has focused on the mass identification of proteins. Proteomics has resulted in the ability to rapidly process large numbers of clinical samples, potentially deeming it to be a highly sensitive diagnostic, surveillance and prognostic tool [55]. Proteomics uses surface-enhanced laser desorption/ionization time-of-flight mass spectrometry with selective primed surface arrays [55]. Notably, proteomic pattern analysis has correctly identified up to 95% of PCa and 78% of benign samples [56]. Prostate biopsies could thus be avoided in men with PSA ranges from 2.5 to 15 ng mL^{-1} without missing any instances of PCa [57]. Nonetheless, several problems need to be overcome, including artefacts, sample errors, storage and collection techniques; proteomic profiles may vary at different stages of PCa, resulting in inconsistency [55].

4 Hypoxia, PIA and PIN

One proposed mechanism for prostate carcinogenesis is that genomic DNA may undergo genotoxic stress such as occurs in hypoxia or inflammation. This may occur under normal cellular conditions or secondary to external insults, resulting in activation of cellular checkpoint cascades capable of repair, cell-cycle arrest or apoptosis [4]. Abnormalities in this control mechanism have been implicated in progression to PCa, including defects in the p53 response and lack of DNA damage checkpoint enforcement by Wee1A (a G_2/M regulator) [58, 59].

Tissue hypoxia is detectable in 30%–90% of PCa samples, and its role in PCa microenvironments has been reviewed [60]. Hypoxia may arise when tumour growth surpasses the vascular supply provided by angiogenic growth, and it has been implicated as a prognostic factor linked to androgen independence and chemo/radiotherapy resistance. Hypoxia may alter cell-cycle checkpoints and DNA repair (leading to genetic instability), up-regulate VEGF/HIF-1 expression (promoting angiogenesis and metastasis) and reduce apoptosis through p53-mutated cells [60]. Studies have also shown that antiandrogens improve tissue oxygenation by reducing angiogenesis [60].

PIN and PIA are found in PCa specimens and are thought to be precursor lesions, with PIA found to merge with areas of PIN [61]. PIA is postulated as a link between prostatitis and PIN and is common in the peripheral zone of the prostate [62]. A number of studies support the theory of PIA as a PCa precursor. The odds ratio of PCa in prostatitis is 1.7, with a relative risk of 2.5 for bacterial prostatitis [63]. PIA is thought to be a regenerative lesion associated with a low apoptotic rate and elevated Bcl-2 expression [31]. Another study has detected bacterial DNA sequences in 19.6% of patients with PCa [64]. Inflammation is thought to produce free radicals and oxidative stress, inducing protein, tissue and vascular damage leading to PIA. Anti-inflammatory agents are associated with a > 50% reduction in PCa risk, and COX-2 inhibitors have been shown to slow progression and recurrence of PCa [12]. Presently, COX-2 inhibitors have been withdrawn from the market owing to cardiovascular side effects. Other anti-inflammatory agents, such as epigallocatechin-3-gallate found in green tea, may also play a chemo-preventive role [65]. These observations suggest potential genetic targets for prevention and treatment. Potential markers involved in the susceptibility of the prostate to infection include macrophage scavenger receptor 1, TOLL-like receptor-4 and 2'-5'-oligoadenylate-dependent RNasel (RNasel) and the loss of glutathione S-transferase (a detoxifying enzyme linked to genomic instability and damage) [43].

5 Stem cells

The cancer stem cell theory, which proposes that PCa

originates from prostate stem cells (PSCs), was first suggested in 1875, supported by work in the 1960s [19, 66]. This theory is supported by recent observations. In acute myelogenous leukaemia, only minor subpopulations of cells have been shown to be capable of self-renewal, disease initiation and propagation when transplanted into immune-deficient mice [67]. Clonality has also shown the ability of these cells to differentiate into the various original leukaemic cells. In the prostate, cyclical atrophy and regeneration of rodent prostate glands with intermittent androgen deprivation have been described, showing the capacity for self-renewal and suggesting the presence of PSCs [2]. In addition to these PSCs being androgen independent, cancer stem cells have been shown to be relatively chemo/radiotherapy insensitive, hence acting as a possible reservoir for recurrent and androgen-independent (hormone-resistant PCa [HRPC]) disease [20, 68, 69]. Four cellular types exist within the prostate, which are identifiable by specific markers (basal cells, transient amplifying cells, luminal and neuroendocrine cells) [4]. PSCs are hypothesized to reside in the basal cell layer and may produce intermediate transient amplifying cells, which exhibit both luminal and basal cell markers. The neuroendocrine cell lineage is still unknown, but may arise from PSCs [2, 70]. Previous studies cited by the authors support this concept owing to the absence of a prostate in mice null for basal layer marker p63. Stem cell markers have also been identified in human and murine prostatic epithelial cells (Sca-1, CD133, α2β1, integrin, CD44, Oct4, Nanog, breast cancer resistance protein and SOX2) [4, 19, 20]). PSC research is in its infancy, with stem cell culture reported to be demanding, relatively unproductive and subject to variation in different environments/culture media [71]. PSCs are also rare in circulating blood, and adequate sampling requires biopsy or bone marrow aspiration [20]. However, therapies directed at selectively targeting PSC surface markers and their signalling pathways may offer a novel and highly specific treatment approach to PCa. The challenge will be to ensure tissue and organ specificity. Finally, Hedgehog pathway inhibitors have been shown to halt growth of PCa in murine models [2].

6 Conclusion

The genetic and molecular basis of PCa is complex; however, technological advances have increased our knowledge in this field. The identification of multiple genetic and biological markers has reinforced the concept of a 'multi-hit theory' for PCa, with some patients being genetically predisposed. Other genetic loci may only be altered under specific dietary or environmental conditions. Bacterial and viral infectious triggers may also be involved. PSA is the current mainstay for diagnosis and prognosis, but has a sensitivity of 20% and a specificity of 80% (PSA range, 4–10 ng mL^{-1}) [72]. Biomarkers for risk stratification, prediction of invasion and metastases are emerging, with multiple marker assays potentially offering improved diagnostic benefits. New markers such as PCA3, AMACR, EPCA, GSTP1, RNASEL and hK2 offer the potential to improve sensitivity and specificity, with some now having achieved commercial and clinical acceptance.

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