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Review

Molecular markers in prostate cancer. Part II: potential roles in management

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Abstract

Predicting treatment responses in advanced prostate cancer (PCa) currently centres on prostate-specific antigen (PSA) kinetics and on being able to visualize measurable changes in imaging modalities. New molecular markers have emerged as potential diagnostic and prognostic indicators; these were summarized in Part I of this review in the *Asian Journal of Andrology*. A number of molecular markers are now being used to enhance PCa imaging and staging. However, management options for advanced and hormone-resistant PCa (HRPC) are limited and additional therapeutic options are needed. Molecular markers have been proposed as potential therapeutic targets using gene therapy and immunomodulation. Additionally, markers identified in early PCa and precursor lesions may offer novel targets for chemoprevention and vaccine development. This review summarizes the current advances regarding the roles of these markers in the management of PCa.

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1 Introduction

Managing advanced prostate cancer (PCa) is challenging, and the median survival in hormone-resistant PCa (HRPC) is still less than 2 years [1, 2]. Taxane-based agents are the main chemotherapeutic options for HRPC, and molecular markers have been identified in their mechanism of action and seems to involve induction of apoptosis and inactivation of Bcl-2 [2]. The advent of new PCa molecular markers and the identification of genetic loci allow for the development of new therapeutic strategies, with additional applications including chemoprevention and targeted imaging. Research in these fields is summarized in Table 1 [2–38].

2 Molecular imaging

The use of molecular markers for direct imaging may help to detect PCa, micro-metastases and PCa precursor lesions at an early stage. Non-invasive imaging techniques that identify areas of tissue hypoxia have been described using magnetic resonance imaging or radio-labelled 2-nitro-imidazoles with positron emission tomography (PET). The ability to label areas of hypoxia or molecular change may offer potential therapeutic applications. Labelled agents could be used in conjunction with an appropriate sensitizing drug and precision intensity-modulated radiotherapy to guide treatment [35]. Molecular imaging has also been used to improve PCa staging. Currently, HSV1-tk is the most common reporter gene used with PET; it has also been used in antiviral suicide gene therapy [22, 39]. Other markers include the sodium iodide symporter (NIS). Overall, molecular imaging may help to aid disease staging, guide treatment and offer the additional potential for monitoring therapeutic outcomes [22].

3 Chemoprevention

Targeting and modulating molecular markers identified in PCa precursor lesions, such as prostatic intraepithelial neoplasia (PIN) and proliferative inflammatory atrophy (PIA), offer the potential for chemoprevention, along with

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the ability to monitor the outcomes. Current research has focused on the modulation of serum hormones with 5-α-reductase inhibitors (PCPT and REDUCE trials) [40, 41]. PIN has been used as a biomarker for chemoprevention with Toremifene, a selective oestrogen receptor modulator. This trial examined 514 men with HGPIN, in whom use of toremifene resulted in a 48% reduction of PCa incidence at 12 months, compared with placebo treatment ($P = 0.05$) [42]. Synthetic retinoids such as *N*-(4-hydroxyphenyl)-retinamide (4-HPR) have also been used in animal models to demonstrate a reversal of the tumour's malignant characteristics and a decreased PCa incidence. However, a subsequent phase II study reported an increase in PCa incidence and was stopped [42].

4 Prostate cancer vaccines

Gene vaccines based on identified markers have been used in clinical trials. The PROSTVAC vaccine takes advantage of a genetically engineered, recombinant vaccinia virus that expresses the prostate-specific antigen (PSA) gene. Several early studies have shown PROSTVAC to be safe and well tolerated, leading to stable disease in patients for up to 19 months. Phase II and III (PARADIGM) studies are currently underway [10]. Prostate specific cell antigen (PSCA)-based vaccines have been under trial in a Pca-prone, transgenic-adenocarcinoma-mouse-prostate (TRAMP) mice model having PIN. PSCA-vaccinated TRAMP mice showed a 90% survival rate at 12 months compared with 0% in the control mice [43].

5 Gene therapy

A number of gene-modulation strategies exist, including the use of DNA/RNA vectors, antisense oligonucleotides, suicide/pro-apoptotic/anti-angiogenic genes and corrective gene therapy [9, 10, 22].

DNA vectors can be transferred to a cell using an adenovirus (Ad), which transduces target cells efficiently even during cellular quiescence. However, the virus carries a low potential for inducing oncogenesis. RNA viral vectors are designed to target specific cells and to be replication deficient; examples include a retrovirus and a lentivirus. However, low transduction rates, dependence on cell division and rapid inactivation by human complement may limit their use in patients [9]. Antisense oligonucleotides are nucleic acid sequences considered to bind to oncogenic DNA in a manner that prevents mRNA transcription. This type of therapy has been used for c-MYC and Bcl-2 (Table 1).

Suicide gene therapy involves transduction of a suicide gene, followed by administration of a prodrug. The prodrug is converted to a toxic metabolite that induces tumour cell death by suicide gene-related products. Phase I studies have investigated the use of systemic ganciclovir with a replication-deficient Ad, Ad-HSV-tk (herpes simplex virus type 1 thymidine kinase adenovirus). Results included a low toxicity with > 50% reduction of PSA in some patients [9, 39] and a significantly prolonged median PSA doubling time, increasing from 2.9 to 6.2 months ($P = 0.041$) [44]. *Escherichia coli* nitroreductase (NTR)/HSP 70 is thought be another promising cytotoxic/immunomodulatory gene [22, 45]. Pro-apoptotic Ad vector gene strategies aim to introduce tumour necrosis factor genes into target cells, which could include Fas ligand and tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) [22]. Further examples include melanoma differentiation associated gene-7/interlukin-24 (mda-7/IL-24) and the RTVP-1 gene (related to testis-specific, vespid and pathogenesis protein) [22].

Oncolytic gene therapy is based on viral cytotoxity that is secondary to conditional viral replication within tumour cells, where this process induces cell lysis. The strategy employs tissue-specific promoters to direct viral expression and replication towards specific cells. One way that this strategy could be accomplished is through the control of Ad proteins E1A and E1B [22]. CG7870 Ad (previously CV787) is being evaluated in phase I/IIa trials in combination with docetaxel and has resulted in a mean PSA reduction of 44% in HRPC [9]. Murine osteocalcin (OC) promoters have also been used to restrict E1A (Ad OC-E1A virus) to the prostatic epithelium and osseus metastasis. This study was performed in mice and the results indicated undetectable PSA levels and a 40% cure, with no evidence of skeletal metastasis [22, 23]. Current oncolytic gene therapy phase I/II trials have been reviewed and are promising, but they require further evaluation [9, 22]. Corrective gene therapy aims to repair or replace defective genes. A replication-deficient Ad containing wild-type p53 injected intra-prostatically has been under trial. The tumour size was reduced by 25% in 3 of 17 cases [10].

6 Immunomodulation

PCa immunotherapy aims to induce antibody and/or cytotoxic T lymphocyte (CTL) immune responses with cytokines and interleukins. Two mechanisms exist for this type of therapy. Active or direct immune system stimulation uses autologous dendritic cells, whereas the passive creation of immune cells involves the administration of monoclonal antibodies to specific cancer receptors [9]. Dendritic cells are harvested by leukapheresis. Targets include growth factor receptors and components of various signalling pathways. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine that promotes dendritic cell uptake of tumour antigens by regulating macrophages, granulocytes and monocytes [46]. In phase II trials, GM-CSF-secreting vaccines using allogenic PCa cell lines

Table 1. Summary of molecular markers with potential roles in prostate cancer (Pca) management.

Gene/Protein	Action	Selected Literature	Potential Use
AR	Androgen receptor (AR), nuclear transcription factor mediates steroid hormones and stromal cell growth. AR activation in luminal cells surpresses growth [3].	Stimulates early PCa growth, found in 30% androgen independent (AI) tumours [4]. Vorinostat a histone deactylase inhibitor may reduce AR expression and act synergistically with bicaltamide (AR antagonist) to inhibit PCa [5].	Therapeutic & Prognostic
AKT & PTEN	Prostate specific phosphatase/tensin homologue, PTEN loss of function induces AKT & p53 senescence, inhibits apoptosis [4, 6].	PTEN null mice develop high grade PCa/metastasis [7]. PTEN alteration seen in 10% PCa, > 30% metastases. AKT may cause tubule regeneration with prostatic intraepithelial neoplasia (PIN) [6]. Possible gene vector [8].	Therapeutic & Prognostic
Bcl-2	B-cell CLL/lymphoma 2, Anti-apoptotic protein found in basal cells and stem cells [6]. Loss of expression associated with PIN, progression and androgen independence [2].	Antisene oligonucleotides (Oblimersen) against Bcl-2 delay progression, improve chemo-sensitivity. Docetaxel combination trial underway (NCT00085228) [9, 10].	Therapeutic & Prognostic
c-MYC	Oncogene transcription factor linked to cellular proliferation and apoptosis [11]. Chromosome 8q24. High levels linked to androgen independence.	c-MYC RNA increased in PCa, variable reports, linked to PIN [4]. Antisene oligonucleotides against c-MYC, decreased PCa growth *in vivo*/*vitro* [12, 13].	Therapeutic
Calcitriol receptor	Vitamin D cellular modulator of growth/differentiation, reduced calcitriol level may allow malignant transformation/metastases *in vitro* & *in vivo* [14].	Randomized double blind trial of high dose calcitriol with docetaxel improved overall survival ($P = 0.035$ univarate analysis) [15]. Phase III ASCENT trial with Docetaxel and calcitriol stopped due to increase death rate.	Therapeutic
Endothelin-1 (ET-1)	Secreted by PCa cells, autocrine/paracrine activity promoting tumour growth/osteoblastic metastasis [16]. ET-1 increase and loss of ETA endothelin receptor may inhibit apoptosis in PCa [10].	Altrasentan (ET-1 antagonist) variable results, may delay progression in metastatic hormone-resistant Pca (HRPC) [17]. Possible role in bone metastasis [15]. Phase III trial with Docetaxel, Zolendronic acid underway (SWOG 0421).	Therapeutic
EGFR (Erb B1 Her-2/Neu (Erb 2)	Epidermal growth factor receptor. associated with proliferation, malignant transformation, relapse, progression and AI [4].	Higher levels in PCa than BPH [18]. Monoclonal antibodies directed against specific binding domains anti-EGFR eg: cetuximab, anti-HER2 eg: trastuzumab [19]. Lack significant role in PCa [9].	Therapeutic & Prognostic
HDAC	Histone deacetylase by acetylation inhibitors can activate tumour suppressor genes [10]. Histones are nuclear proteins that organize DNA regulating gene expression by reversible acetylation.	Early inhibitor phenylbutyrate (PB) resulted in cell-cycle arrest, apoptosis and reduction in DNA synthesis with fragmentation. Multiple HDACs may have additive effect. PB, 13-cis-retinoic acid (CRA) and pacitaxel shown to inhibit PCa growth *in vivo*. Newer agents trials underway (Vorinostat) [10].	Therapeutic
KLK3/PSA	Encodes prostate-specific antigen (PSA) a kalikrein (serine protease subgroup) on chromosome 19 [9].	Combination rV-PSA and fowl pox primed rF-PSA vaccines trialled. Median time to PSA progression increased from 9.2 to 18.2 months (Study ECOG7897). Phase III PARADIGM trial underway [9].	Therapeutic & Prognostic
LMTK2	Encodes neuronal cyclin dependent kinase 5 (cdk5)/p53-regulated kinase [20].		Therapeutic
MSR1 (SR-A)	Macrophage scavenger-1 gene, Ch 8p22. Binds variety of antigens including gram-negative bacteria lipopolysaccharides [21].	May have role in prostate carcinogenesis [21]	Prevention

(To be continued)

Table 1. Summary of molecular markers with potential roles in prostate cancer (Pca) management (continued).

Gene/Proteins	Action	Selected Literature	Potential use
Osteocalcin (OC)	Androgen independent bone specific protein associated with metastasis. Offer potential target of bone metastasis.	OC-E1A virus gene therapy used in mice. PSA dropped to undetectable levels, 40% cured with no evidence of skeletal metastasis at study end [22, 23]. Ad-OC-TK (recombinant adenoviral vector carrying an osteocalcin promoter-driven HSV-tk gene) with valacyclovir trialled [24].	Therapeutic
PSMA	Prostate specific membrane antigen. AI prostatic epithelium transmembrane protein found in PCa/lymph node metastasis [25].	DCVax-prostate vaccine target. Phase I/II trial underway [9].	Therapeutic & Prognostic
PDGFR	Platelet derived growth factor is overexpressed in the presence of bone metastasis. Suggested role in osteotropism [26].	PDGFR inhibited by tyrosine kinase inhibitor, imatinib mesylate. Long-term combination therapy with docetaxel, PSA < 50% in 14/21 patients [27].	Therapeutic
PAP	Prostate acid phosphatase, glycoprotein more specific to prostatic tissue than PSA [9].	APC8015 autologous vaccine. Phase I/II trials in HRPC. 31 patients, 38% developed immune response to PAP, 3 had PSA reduction > 50%. Phase III trial improved overall survival by 4.5 months (P = 0.01). Phase III IMPACT trial ongoing [9].	Diagnostic & Therapeutic
p27Kip1	Cell cycle inhibitor found in basal compartment. Chromosome 12p12-13.1 [2].	Functional loss linked to Pca progression/androgen independence [4, 28]. proliferative inflammatory atrophy (PIA) association [29]. Gene therapy use with recombinant adenovirus [2].	Therapeutic & Prognostic
p53	Tumour supressor gene allows DNA repair/cell apoptosis in cellular stress conditions [3].	Less significant in PCa, uncommon mutation in early/localized PCa [30]. Frequent in late stage PCa, independent prognositic marker [31]. Concomitant homozygous PTEN and p53 inactivation lead to PCa lethality in mice [32].	Prognostic & Therapeutic
Sex hormones & binding-globulin	Testosterone is essential for prostatic development and maintanence. Ostrogens are associated with low risk of PCa [33].	High testosterone levels = lower PCa risk (non Gleeson > 7, Stage 4, N+,M+) (P = 0.003). Serum testosterone < 300 ng/100mL predicts PSA failure after radical prostatectomy. High levels SHBG predicts extracapsular extension (P = 0.006) [33].	Prognostic & Prevention
TMPRSS2:ETS	Transmembrane protease, serine 2 Fusion gene (Ch 21), upregulates ETS target genes modulates cell proliferation, differentiation, apoptosis and transformation [4, 6].	May be an early marker, as seen in 20% of PIN lesions [34].	Prognostic & Prevention
VEGF/HIF-1μ	Tissue hypoxia inducible factor, HIF-1μ, normally degraded by von Hippel Lindau E3 ubiquitin ligase. Stabilised by hypoxia and promotes hypoxia responsive genes, angiogenesis (vascular endothelial growth factor [VEGF]), metastasis and reduces chemotherapy sensitivity [35]. Implicated as novel mechanism for tumor escape from radiation damage [36].	Therapeutic targeting VEGF/HIF-1μ, along with anti-androgens may overcome hypoxia. VEGF/HIF-1μ staining density linked to Gleeson score following radical prostatectomy [35]. Monoclonal antibodies against VEGF (Bevacizumab), Phase II trial, PSA reduced to < 50% in 65% HRPC patients. Phase III CALGB 90401 enrolling [37]. Possible radiosensitiser, and prevention role through DNA repair. PX-478 an oral agent against HIF-1, phase I clinical trial ongoing [38]. Combined VEGF/PDGF receptor inhibition shown to reduce required radiation treatment doses to around 20% [36].	Therapeutic & Prognostic

(GVAX vaccines) have improved the median survival of HRPC from 18 to 26.2 months, compared with patients treated with docetaxel. Phase III trials were initiated (Cell Genesys). VITAL I has been completed with interim analysis, and the study is continuing. However, VITAL II has been stopped because of an imbalance in deaths between the two groups. Further examples of autologous-dendritic cells pulsed with specific peptides include DCVax-prostate vaccine (prostate specific membrane antigen [PSMA] peptides) and Sipuleucel-T (PA2024, a PAP /GM-CSF recombinant protein). A phase III study for Sipuleucel-T in 127 men with HRPC did not alter the time to progression but significantly increased the median survival compared with a placebo. At 3 years, 34% and 11% of men were alive in the Sipuleucel-T and placebo groups, respectively. However, a possible increase in cerebrovascular events was reported [10, 47].

Down-regulating T-cell responses to self-antigens may offer the potential to boost immunotherapy agents. A key inhibitor of cytotoxic T-cells is the cytotoxic T-lymphocyte antigen-4 (CTLA4) molecule (CD152). Anti-CTLA4 therapy has been used in combination with immunotherapy, and this course of action has been shown to enhance rejection of tumour cells. However, unwanted autoimmune side effects may be a potential obstacle. Phase II trials involving anti-CTLA4 therapy are underway [10]. Overall, immunomodulation offers promise but requires further evaluation.

7 Combination therapy

Given the multiple genetic markers identified in PCa development, a combination approach to therapy may improve the outcomes even more than treatment with one agent alone. Current strategies under investigation include the combination of multiple molecular techniques or molecular agents with conventional chemotherapy or radiotherapy. Examples include cytoreductive strategies (suicide genes/oncolytic gene therapy), methods to improve radiosensitivity (VEGF/PDGF inhibitors) [10, 36] and taxane combination trials (CALGB 90401, VITAL I and ASCENT 2 [Table 1]). Multiple gene therapies include the use of numerous histone deacetylase by acetylation inhibitors (HDACs) (Table 1) or a double suicide gene (*Ad5-CD/Tkrep* [replication-competent adenovirus to CD/HSV-tk]) with prodrugs fluorouracil and ganciclovir (5-FC/GCV) [48]. In phase I trials using a trimodal therapy (double suicide gene, 5-FC/GCV plus radiotherapy), PSA levels were reduced to < 0.5 ng mL^{-1} in 5 out of 10 patients over 9 months [49]. In androgen-independent disease, *in vivo* studies using PSA/PSMA-positive PCa cells have also shown therapeutic benefits by combining GCV with AdIU1 (a prostate-restricted replicative adenovirus [PRRA] combined with HSV-TK) [50]. Suicide gene therapy has also been monitored with a combination approach including non-invasive imaging using the NIS [51]. A replication-competent Ad combined with two suicide genes and the NIS gene (Ad5-yCD/utTK(SR39)rep-hNIS/) was injected intra-prostatically into men with localized PCa. NIS gene expression (GE) was imaged using single-photon emission-computed tomography (SPECT). GE volume seemed to change over time, peaking at 1–2 days after injection. There was no evidence of extra-prostatic Ad activity seen on SPECT.

8 Conclusion

The advent of new molecular markers and advances in imaging techniques offers the potential to more accurately measure responses, predicts prognosis and directs therapy. Significant progress has been made in PCa gene therapy and immunomodulation. However, the long-term effects and benefits on overall survival remain to be determined. There is a growing interest in the use of molecular markers in chemoprevention with PCa vaccines targeting early PCa, and this strategy is potentially feasible [43]. A number of studies are currently entering Phase III trials and offer potential therapeutic options for patients with HRPC, especially for those who have failed taxane treatment. Promising markers for therapeutic targeting include OC, growth factors, PAP, PSMA and PSA. The results of larger multi-centre controlled trials are critical to help direct the future of these therapeutic options.

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