EXPERT REVIEWS

Prognostic and predictive biomarkers in prostate cancer

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Prostate cancer (PCa) is one of the leading causes of cancer death among males, especially in more developed countries. Diagnosis is often achieved at an early stage of the disease with prostate biopsy, following a screening test showing elevated serum levels of prostate-specific antigen or a positive digital rectal examination. Early detection of PCa has led to a substantial decline in the number of metastatic patients. However, the prostate-specific antigen screening test has proved to be a double-edged sword so far, as it also accounts for PCa overdiagnosis. Due to the variability of PCa features, accurate prognosis of PCa patients is very important for determining treatment options. Therefore, this review focuses on the most promising prognostic and predictive biomarkers in PCa, which are likely to play a pivotal role, alone or in panels, in the personalized medicine era that has recently emerged.

Keywords: Androgen receptor • kallikrein-related peptidase • KLK • miRNA • PCA3 • prognosis • prostate carcinoma • prostate-specific antigen • PSA • tumor marker

Prostate cancer (PCa) is the most frequently diagnosed cancer among men in developed countries and the second most common worldwide, after lung cancer. An estimated 1.1 million new PCa cases and 307 thousand PCa deaths occurred in 2012 worldwide.[1] PCa can be a serious disease, but most men diagnosed with this malignancy do not succumb to it. In fact, more than 2.9 million men in the United States who have been diagnosed with PCa at some point are still alive today. Relative survival rates of PCa patients are high, mostly due to early diagnosis based on the measurement of prostate-specific antigen (PSA) and to slow progression of the disease in many cases.[2]

Besides for diagnostic purposes, the PSA test is also used to monitor surgically treated PCa patients for disease recurrence. Thus, patients with a history of PCa and elevated PSA levels are considered to have a biochemical relapse, which typically appears months or years before other clinical signs and symptoms of PCa recurrence. Nevertheless, a single increased PSA value in a patient who has a history of PCa does not necessarily mean PCa relapse. Moreover, no other molecular biomarkers are used for PCa patient prognosis, monitoring or prediction of patient response to therapy.[3]

Staging is one of the most critical factors for defining prognosis and for decision-making with regard to PCa treatment options. PCa staging is based on the prostate biopsy results including the Gleason score, the PSA level and any other tests revealing how far PCa has spread. The most widely applied staging system for PCa is the TNM system of the American Joint Committee on Cancer. Recently, however, an alternative staging process, called stage grouping, has been established; stage grouping constitutes a combination of the determined T, N, and M categories with the Gleason score and PSA concentration.

Since there are many treatment options for men with PCa, it is important to have an accurate prognosis. Nevertheless, the currently used prognostic factors lead to overtreatment of PCa, especially in men of 66 years and older with a limited life expectancy. Thus, these men are likely to be aggressively treated, as age at PCa diagnosis and comorbidities are not integrated into patient prognosis and subsequent treatment decision-making.

Many research efforts have been focused on the discovery of novel protein and genetic biomarkers in order to refine prognostication in PCa and predict the benefit derived from the systemic treatment of PCa patients. This review

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summarizes current knowledge about promising prognostic and predictive biomarkers in PCa, which can play a major role in the personalized medicine era that has recently emerged.

Protein-coding genes as molecular biomarkers in PCa

Kallikrein-related peptidases

Tissue kallikrein (KLK1) and kallikrein-related peptidases (KLKs) are single-chain, secreted trypsin- or chymotrypsin-like serine peptidases. KLKs are mainly produced and secreted by epithelial cells composing the glandular epithelia of many organs, including prostate, breast, colon, ovary, pancreas, brain and skin. Secreted KLKs enter body fluids such as sweat, milk, saliva, seminal plasma, cerebrospinal fluid or remain in the pericellular space.[4] KLKs are implicated in a great variety of distinct physiological processes such as remodeling of the extracellular matrix, prohormone processing, neural plasticity, skin desquamation, regulation of blood pressure and electrolyte balance. Due to their ability to activate cell-surface receptors and other proteases by proteolytic cleavage, KLKs participating in proteolytic cascades, thus mediating signal transduction.[5] Although several *in vivo* targets of KLKs have already been described, no prostate-specific substrates have been identified, yet.

Measurement of total PSA has been demonstrated to be useful as a prognostic indicator, with high preoperative tPSA values being associated with advanced stages of PCa, thus predicting a poor clinical outcome. In addition to PSA, several other members of the KLK family (*KLK2, KLK4, KLK5, KLK11, KLK14* and *KLK15*) have been intensively studied for their ability to assist PCa prognosis and/or therapy response monitoring.[6] Perhaps the most important finding with regard to prediction of risk of biochemical recurrence in men with serum PSA values of ≤10 µg/L is the fact that total KLK2 concentration in serum provides improved and independent prognostic information compared with PSA.[7] Artificial neural networks combining tPSA, fPSA/tPSA, KLK2, KLK2/fPSA and KLK2/(fPSA/tPSA) as input variables have also been shown to provide increased diagnostic and prognostic value for PCa.[8] Moreover, high *KLK4* mRNA levels are associated with advanced PCa and strongly correlated with elevated tPSA concentration values measured in preoperative sera. The investigation of the prognostic potential of *KLK5* and *KLK11* mRNA expression in needle biopsies from PCa patients led to the conclusion that these two genes represent two promising, independent prognostic biomarkers in PCa. Furthermore, *KLK15* mRNA expression has also been suggested as a novel molecular biomarker predicting treatment response in PCa cells. Specific *KLK15* transcripts were found to be aberrantly expressed in more aggressive prostate tumors, thus implying an unfavorable prognostic role for this gene in PCa.[6]

Androgen receptor

The androgen receptor (AR) is a member of the nuclear receptor superfamily that becomes activated when it binds either of the androgenic hormones, testosterone or its metabolite, dihydrotestosterone. After its activation, AR can translocate from the cytoplasm into the nucleus and act as a transcription factor, or exert its action directly in the cytoplasm, interacting with and activating the nonreceptor tyrosine kinase SRC. Besides cytoplasmic AR, there is also a membrane-associated AR isoform that, like cytoplasmic AR, potentiates proliferative and survival responses in PCa cells.[9]

AR regulates the expression of genes involved in the proliferation and differentiation of PCa cells, not only by binding with androgens in the epithelium but also in the prostate stroma.[10] The loss of AR in the stroma surrounding the malignant prostatic epithelium is supposed to exert a pivotal role in PCa progression and to hold prognostic significance, especially among castration-resistant PCa patients. It has also been suggested that the similarity of the loss of stromal AR expression in newly diagnosed and hormone-resistant PCa implies that the mechanisms accounting for the acquisition of hormone independence are established early in the malignant transformation.[11] Profound alterations in AR expression in the malignant epithelium and normal surrounding stroma are inversely associated with Gleason score, distant metastasis, tumor stage, response to castration therapy and biochemical relapse.[11,12] Furthermore, AR was shown to mediate PCa recurrence after androgen deprivation therapy.[13]

In addition to AR protein levels, post-translational modifications of AR hold their own prognostic significance in PCa. For instance, phosphorylation of AR at serine 213 is related to inferior overall survival of patients with hormone-refractory PCa.[14] Similarly, phosphorylation of AR at serine 515 is a predictor of shorter time to biochemical relapse.[15] On the contrary, phosphorylation of AR at serine 308 and serine 791 was a favorable prognosticator in terms of overall survival of patients with castrate-resistant PCa.[16]

Secreted frizzled-related proteins 1 (SFRP1) and 2 (SFRP2)

Secreted frizzled-related protein 1 (SFRP1), also known as SARP2, has been extensively studied in the past years for its biological significance as well as its potential value as a prognostic and/or predictive biomarker in PCa. SFRP1 is frequently downregulated in many types of cancer, including PCa. Alterations of the expression balance between SFRP1 and β-catenin plays a key role in both carcinogenesis and tumor progression.[17]

SFRP1 counteracts WNT/β-catenin signaling by binding to the WNT proteins through its cysteine-rich domain, which is homologous to the one of frizzled receptors. Loss of SFRP1 can result in activation of WNT/β-catenin signaling.[18] WNT proteins compose a major family of signaling molecules that orchestrate and influence a myriad of cell biological and developmental processes because of their participation in vital cellular functions such as cell proliferation, migration, differentiation, synaptic activity and embryogenesis. Moreover, SFRP1 has been shown to mediate stromal-to-epithelial paracrine signaling in PCa and is thus likely to account for the capacity of prostatic tumor stroma to provide a pro-proliferative paracrine signal to

adjacent epithelial cells.[19] In fact, forced overexpression of SFRP1 in prostatic epithelial cells did not modulate canonical WNT/beta-catenin signaling or activation of calcium/calmodulin-dependent protein kinase II gamma (CAMK2G), but resulted in sustained activation of JNK. Furthermore, blockage of JNK activity inhibited the SFRP1-induced proliferation of prostatic epithelial cells. Taken together, these data suggest that SFRP1 acts through the noncanonical WNT/JNK pathway in the prostate.[17] Besides compromising WNT/β-catenin signaling, treatment of a human prostatic epithelial cell line with SFRP1 led to enhanced cell proliferation and decreased apoptosis, *in vitro*.[19] On the other hand, loss of *SFRP1* expression has been reported in several distinct types of cancer, including PCa.[20] In general, promoter CpG hypermethylation as well as chromosomal deletions are the two main causes for dramatic downregulation or loss of *SFRP1* gene expression.[20,21] Nevertheless, hypermethylation of *SFRP1* is rare in PCa tissues,[22] suggesting that chromosomal deletion may account for the transcriptional silencing of *SFRP1* in PCa.

A very recent study focusing on the prognostic and predictive ability of SFRP1 has revealed that the expression levels of SFRP1 and β-catenin are associated with the Gleason score, survival rate and response of PCa patients to endocrine therapy. Furthermore, mRNA and protein expression of the *SFRP1* gene was significantly higher in the androgen-dependent PCa cell line -LNCaP than in the androgen-independent PCa cell lines DU 145 and PC-3. Therefore, SFRP1 expression, which inversely correlates with the expression of β-catenin, appears to be a favorable prognostic biomarker in PCa.[23] In accordance with these findings, restoration of normal SFRP1 levels in PCa cell lines treated with genistein – achieved through DNA methylation or histone modifications – reduced their proliferation, invasion and migration potential.[24] Similarly, a combination of DNA demethylation and EZH2 inhibition managed to trigger re-expression of the silenced tumor-suppressor *SFRP1* gene and hence showed an additive inhibitory effect on growth of PCa cells *in vitro*, rendering *SFRP1* a promising target for cancer cell-specific epigenetic therapy.[25]

Besides SFRP1, 14 other WNT/β-catenin signaling-related genes were shown to have a significantly altered expression profile in PCa. The majority of these genes were upregulated in malignant prostate tumors, in contrast to *SFRP1* and its paralog, *SFRP2*. The observed downregulation of *SFRP2* gene expression was mainly due to hypermethylation, a common oncogenic event in PCa. Perhaps more importantly, *SFRP2* methylation in combination with other epigenetic markers was suggested as a useful biomarker in PCa.[26]

Phosphatase and tensin homolog

The phosphatase and tensin homolog (PTEN) is a tumor suppressor protein, responsible for the dephosphorylation of lipid-signaling intermediates and deactivation of PI3K signaling, thus controlling cell growth and proliferation. PI3K signaling plays a pivotal role in PCa cell survival and progression to the androgen refractory state. Loss of the tumor suppressor PTEN and subsequent activation of AKT1 enhances prostate tumor growth and bone metastasis through stimulating the CXCL12/CXCR4 signaling axis.[27] Thus, ablation of PTEN expression is definitely a critical factor in progression toward metastatic disease and has been suggested to constitute an early prognostic biomarker for PCa metastasis.[28] The strong association of aberrant PI3K signaling with metastasis and poor survival of PCa patients highlights the enormous putative impact of inhibition of the PI3K signaling pathway on PCa patient survival.[29] For instance, AKT1 inhibitors could be employed as anticancer agents to counteract expansion of bone metastases in PCa.

Homozygous deletion of the *PTEN* gene and loss of heterozygosity followed by a second inactivation event are frequent in malignant prostate tumors. Moreover, *PTEN* haploinsufficiency is likely to contribute in the transition from preneoplastic prostatic intraepithelial neoplasia to PCa.[30] Interphase FISH analysis of *PTEN* in histologic sections of malignant prostate neoplasms revealed that genomic loss of *PTEN* is indicative of advanced-stage disease at surgery and a poor prognosticator, predicting shorter time intervals to biochemical recurrence of the disease.[31] Furthermore, its combination with copy number alterations of other genes such as allelic gain of *MYC* is increasingly prognostic for relapse.[32] A previous effort to genetically distinguish between PCa patients with local risk and those with systemic risk, based on the application of array-based comparative genomic hybridization on prostate tissues and lymph node metastases after radical prostatectomy, clearly demonstrated that genomic losses on chromosome 10q (*PTEN*) and gains on 8q (*MYC*) were associated with locally aggressive and metastatic disease.[33] Stratification of PCa patients based on the copy number alterations of specific genes within pretreatment biopsies may improve the use of systemic therapies to target subclinical metastases or locally recurrent disease and improve clinical outcomes.

Several studies have shown that PTEN genomic deletion is associated with decreased PTEN protein expression, as assessed by IHC, both in localized and metastatic cancer. In fact, IHC-determined PTEN protein loss, assessed as a dichotomous variable, was highly reproducible and strongly associated with adverse clinicopathological features such as high Gleason score and advanced pathologic stage. Interestingly, PTEN protein loss was found occasionally in the absence of apparent genomic loss, as well.[34] In particular, loss of PTEN protein expression at the time of biopsy predicts time to development of metastasis, PCa-specific mortality, castration-resistant PCa and response to androgen deprivation therapy after radical prostatectomy.[35] Moreover, in patients with conservatively managed, localized PCa, absence of PTEN protein adds prognostic value to the Gleason score, PSA, Ki-67 and tumor extent.[36] Especially in patients with clinically localized PCa treated by prostatectomy, negative PTEN expression status is a predictor of increased risk of recurrence, independent of known clinicopathological parameters.[37] These findings support the validity of IHC to interrogate PTEN expression status in clinical prostate specimens and suggest its application in large multicenter studies,

clinical trials, and even perhaps in clinical routine as an early marker of aggressive PCa.

Transforming growth factor beta isoforms

Transforming growth factor beta (TGF-β) is an important regulator of the prostate physiology. In the normal prostate, this secreted pleiotropic protein controls cell growth, proliferation, differentiation, and apoptosis. TGF-β is also a potent immunosuppressor, secreted by cells of the immune system. [38] When activated, this ligand binds to the type II receptor (TGF-βRII), which in turn recruits type I receptor (TGF-βRI). Subsequently, the activated TGF-βRI phosphorylates its downstream targets, the members of the SMAD family of signal transducers, thus mediating SMAD signaling. Besides that, TGF-β can induce other non-SMAD signaling pathways, including the MAPK pathway. On the other hand, perturbation of TGF-β signaling is related to autoimmunity, inflammation and cancer.[39]

Three distinct isoforms of TGF-β are currently known: TGF-β1, TGF-β2 and TGF-β3. TGF-β1 constitutes an important regulator of PCa pathobiology. Being highly expressed in PCa cells, TGF-β1 facilitates tumor growth and metastasis, both by promoting angiogenesis and by counteracting immune response directed against tumor cells. Moreover, PCa-derived TGF-β1 is likely to induce the expression of DNMTs in PCa, resulting in methylation of the *TGFBR1* and *TGFBR2* genes.[40] Thus, by gradually losing their TGF-β receptors, PCa cells become resistant to the antiproliferative and proapoptotic effects of TGF-β1. The loss of TGF-βRI and TGF-βRII may also account for the ability of some malignant prostate neoplasms to avoid castration-induced apoptosis. Accordingly, high TGF-β1 protein levels accompanied by loss of TGFβRII expression have been associated with angiogenesis, metastasis and poor clinical outcome of PCa patients.[41] For patients subjected to radical prostatectomy, elevated preoperative plasma TGF-β1 concentration is associated with positive regional lymph nodes, presumed occult metastases at the time of primary treatment and disease progression,[42] while TGF-β1 overexpression by prostate tumor cells is associated with pathological features of the tumor and biochemical progression.[41] Additionally, TGF-β1 is overexpressed in metastatic primary PCa, compared to non-metastatic PCa.[43]

With regard to TGF-β2, this TGF-β isoform seems to constitutively activate NF-κB in PC-3 cells, thus blocking apoptosis and leading to sustained tumor cell survival. Since *TGFB2* silencing via specific siRNAs was shown to trigger PC-3 cell death, TGF-β2 could be exploited as a potential therapeutic target to inhibit growth of tumor cells or to sensitize them to cytotoxic drugs.[44]

Interleukin-6

Interleukin-6 (IL-6) is a cytokine playing a pivotal role in regulation of the immune system, hematopoiesis, acute phase responses, cell proliferation and differentiation. IL-6 exerts its pleiotropic action through binding its receptor (IL6R) and subsequently activating JAK/STAT and MAPK signaling pathways. Besides regulating immune and inflammatory responses, IL-6 regulates VEGFA expression and stimulates the growth of cancer cells, including PCa cells. Because of its overexpression in PCa tissues at early disease stage and its implication in autocrine and paracrine loops that trigger neuroendocrine differentiation in prostate, IL-6 has been in the center of PCa research.[45]

Not surprisingly, preoperative serum levels of IL-6 and its soluble receptor, sIL6R [official symbol: IL6ST (interleukin 6 signal transducer)], were found to be significantly associated with the extent of the disease, tumor volume, prostatectomy Gleason score, positive lymph nodes, and clinical stage.[42,46] Serum IL-6 level is also related to clinical stage and constitutes a significant prognostic factor in PCa.[47] In patients with clinically localized PCa and thereby undergoing radical prostatectomy, the preoperative levels of IL-6 and sIL6R in plasma independently predict biochemical progression following surgery, most probably due to occult metastases at the time of surgery. In support of this conclusion, plasma IL-6 and IL-6sR levels were found to be dramatically higher in PCa patients with bone metastases.[42] More interestingly, plasma IL-6 concentration retained its high prognostic significance in the group of patients with hormone-refractory, metastatic PCa.[48] These findings support the notion that IL-6 concentration in serum or plasma could be incorporated into multiparametric prognostic models. IL-6 has also been suggested as a biomarker for docetaxel response monitoring, as dramatic reduction in IL-6 levels early after treatment onset are indicative of clinical response of PCa patients to this drug.[49] Nevertheless, further studies are needed to elucidate the potential clinical significance of IL-6 inhibition in human PCa.

Chromogranin A

Chromogranin A (CHGA), also known as parathyroid secretory protein 1, belongs to the chromogranin/secretogranin family of neuroendocrine secretory proteins. CHGA, a compound found in the secretory vesicles of neurons and endocrine cells, is a precursor to several functional peptides, including vasostatins 1 and 2, pancreastatin, parastatin, catestatin, and chromofungin. The first four of these biologically active peptides act as autocrine or paracrine negative modulators of the neuroendocrine system, while the other two peptides have antimicrobial properties.

Elevated expression of CHGA is indicative of neuroendocrine malignancies, including PCa with neuroendocrine differentiation. Several studies have shown that CHGA may be a promising prognostic biomarker in PCa. IHC-assessed CHGA expression in prostate tissue is associated with Gleason score. [50] Furthermore, CHGA expression in PCa needle biopsies at the time of diagnosis constitutes an independent prognostic factor of survival.[51] It also correlates with circulating levels of CHGA, particularly in PCa patients with metastatic disease. [52] High CHGA concentration in plasma is very common in patients with hormone-refractory PCa and, more importantly, constitutes a predictor of poor prognosis, especially in patients

with metastatic castration-resistant PCa treated with abiraterone or enzalutamide.[53,54] In PCa patients with nonmetastatic carcinoma, elevated circulating levels of CHGA predict biochemical progression and, when combined with PSA, effectively predict poor survival after endocrine therapy.[55] In accordance with these findings, Krauss *et al.* have recently shown that neuroendocrine differentiation, as indirectly assessed by CHGA immunostaining, is a predictor of distant metastases and disease-specific survival in newly diagnosed PCa patients treated with definitive radiotherapy.[56] Therefore, it appears that CHGA is a very promising blood-based biomarker in PCa deserving more clinical evaluation.

Erythroblast transformation-specific family members

The members of the erythroblast transformation-specific (ETS) family of transcription factors are key regulators of embryonic development, cell proliferation, differentiation, angiogenesis, inflammation and apoptosis. Modulations in the ETS transcription factor activity in PPC-1 cells exert an inhibitory effect on anchorage-independent growth, survival and invasiveness of these prostate tumor cells.[57] Interestingly, recurrent gene fusions involving the 5′-untranslated region (UTR) of the androgen-regulated transmembrane serine protease 2 (*TMPRSS2*) gene and the ETS family genes *ERG, ETV1, ETV4, ETV5* and *FLI1* have been identified in most PCa cases.[58] From a mechanistic point of view, ERG plays a major role in PCa progression, as it activates the *MYC* oncogene and represses prostate epithelial differentiation genes such as PSA and prostein (solute carrier family 45 member 3 [SLC45A3]), thus abolishing prostate epithelial differentiation.[59] The same transcription factor enhances also prostaglandin-mediated signaling, which favors tumor progression.[60]

In fact, the detection of transcripts produced from the *TMPRSS2:ERG* chimeric gene in surgically treated PCa patients was initially shown to strongly predict disease recurrence, independently of grade, stage, and serum PSA levels.[61] In contrast with this finding, Saramaki *et al.* concluded that the *TMPRSS2:ERG* rearrangement in primary prostate tumors is an independent predictor of longer progression-free survival and favorable outcome in PCa patients treated by prostatectomy. Moreover, this chimeric gene was not associated with Gleason score, pathological tumor extent (pT), diagnostic PSA levels or cell proliferation activity in prostatectomy samples nor with the AR gene amplification in hormone-refractory malignant neoplasms.[62] This prognostic controversy could be partly explained by the fact that ERG expression is AR-dependent and hence prostate tumors overexpressing ERG due to *TMPRSS2:ERG* fusion are likely to progress in an androgen-rich environment but respond better to androgen suppression.[63] On the other hand, there are also studies implying no prognostic significance for this chimeric gene.[64]

Besides the *TMPRSS2* gene, ETS family members have many other 5′-fusion partners that are occasionally present in prostate tumors, including *SLC45A3, OR51E2, ERVK-17, UBTF, NDRG1*, and the strongly expressed housekeeping gene *HNRPA2B1*.[58] Nevertheless, the prognostic potential of chimeric genes containing ETS family members and their aforementioned fusion partners has not been extensively studied, so far.

α-*Methylacyl-CoA racemase*

α-Methylacyl-CoA racemase (AMACR) is an enzyme regulating the entry of branched-chain lipids into the peroxisomal and mitochondrial β-oxidation pathways. AMACR is significantly upregulated in PCa, compared to BPH and normal prostate, as shown by a meta-analysis of DNA microarray data.[65] Besides its high diagnostic value in PCa due to its high sensitivity and specificity, AMACR expression seems to be an important favorable prognostic biomarker in this malignancy. In more detail, low AMACR expression in patients with localized PCa is associated with an increased rate of biochemical recurrence and PCa-specific death.[66] However, additional studies are needed to fully uncover the prognostic significance of AMACR in PCa.

Endoglin

Endoglin (ENG) is a major homodimeric transmembrane glycoprotein of the vascular endothelium, mainly expressed in proliferating endothelial cells. As a component of the TGF-β receptor complex, ENG binds to TGF-β1 and TGF-β3 with high affinity. The presence of ENG is a feature of newly formed tumor vessels, rendering this protein a biomarker of ongoing angiogenesis in prostate.[67] In patients with organ-confined PCa treated with radical prostatectomy and subsequent neoadjuvant hormonal therapy, high tumor microvessel density – as evaluated by elevated ENG expression – constitutes a significant and independent predictor of biochemical recurrence.[68] Positive ENG immunostaining was also associated with high Gleason score, nodal metastases and advanced disease stage, suggesting a putative prognostic role for overall survival.[67]

Noncoding genes as molecular biomarkers in PCa

Prostate cancer associated 3

Prostate cancer associated 3 (*PCA3*) is a long noncoding RNA, synthesized almost exclusively in the prostate and overexpressed in PCa tissues and metastases, compared with BPH tissue. PCA3 is implicated in the control of PCa cell survival, partly through modulating AR signaling, and exerts its main action in the nuclei and microsomal cell fractions.[69] Although castration-resistant prostate tumors lack *PCA3* expression, the question whether disease progression *per se* affects PCA3 expression remains unanswered. PCA3 is useful as a biomarker in PCa as it is able to stratify patients based on prostatectomy tumor volume and Gleason score and may thus have clinical applicability in distinguishing those having low volume/low grade PCa and selecting patients eligible to undergo active surveillance and nerve-sparing surgery.[70] Nonetheless, the association between *PCA3* score and clinicopathological factors is controversial, as several studies have failed so far to demonstrate such associations or any prognostic significance for this long non-coding RNA in PCa.[71]

Therefore, in spite of the fact that the clinical usefulness of the PCA3 test for the detection of PCa and the possible decrease of unnecessary biopsies has been well established,[72] additional studies are required to investigate the potential of *PCA3* expression as a prognostic biomarker in PCa.

MicroRNAs

MicroRNAs (miRNAs) are small noncoding RNAs of about 19–24 nucleotides that regulate protein-coding gene expression by inhibiting translation and/or destabilizing targeted transcripts. miRNAs recognize target sites in the 3'-UTR of mRNAs and probably in the 5'-UTR through imperfect base pairing. miRNAs play a very important role in prostate physiology, acting as oncogenes or tumor suppressors, whereas modulations in their expression contribute to prostate carcinogenesis.[73] Since miRNAs serve as phenotypic signatures of different cancers, they appear as potential diagnostic, prognostic and therapeutic tools.

The first large-scale study investigating the diagnostic and prognostic implications of microRNA profiling in PCa was reported in 2010. Among the 15 miRNAs (miR-16, miR-31, miR-96, miR-125b, miR-145, miR-149, miR-181b, miR-182, miR-182*, miR-183, miR-184, miR-205, miR-221, miR-222 and miR-375) that were differentially expressed between cancerous and normal prostate tissue, miR-31, miR-96 and miR-205 were the only ones to be associated with Gleason score, while miR-125b, miR-205 and miR-222 were associated with tumor staging.[74] One of the most promising prognostic biomarkers for PCa is the prostate-specific miR-221, an oncomiR that regulates proliferation, apoptosis and invasion of PCa cells by inhibiting IRF2 and SOCS3.[75] Low miR-221 expression was shown to be associated with clinicopathological factors, including the Gleason score and the clinical recurrence.[76] In fact, PCa patients with localized tumors expressing miR-221 at low levels have a greater risk for recurrence after surgery. Progressive loss of miR-221 expression has been designated as a hallmark of metastasis, which explains the high prognostic significance of miR-221, highlighted by independent studies.[75,76]

Another miRNA that seems to be highly significant in terms of prognostic capability is miR-145. This tumor suppressor miRNA regulates androgen-dependent prostate cell growth by suppressing AR, *in vitro*. Low miR-145 expression in PCa was associated with higher Gleason score, presence of metastases, advanced clinical stage and androgen deprivation therapy response. In accordance with these findings, miR-145 was a reliable predictor of biochemical relapse and poor DFS in PCa patients.[77] Similarly, loss of miR-378 expression was associated with aggressive PCa and shown to predict short-term relapse of PCa patients despite any treatment.[78]

Besides the aforementioned miRNAs, there are several others that have been proposed as putative prognostic biomarkers in PCa; one of them is miR-224. This PCa-related miRNA was shown to regulate the expression of calcium/calmodulin-dependent protein kinase 2 (CAMKK2) and apelin (APLN), a peptide that functions as an endogenous ligand for the G protein-coupled receptor APJ and that hence activates distinct tissue-specific signaling pathways.[79,80] Defects of the miR-224/APLN axis are considered to promote prostate carcinogenesis and aggressive progression of the disease. Thus, low miR-224 levels were significantly associated with advanced clinical stage and metastasis in PCa.[80] Nonetheless, the favorable prognostic role of miR-224 expression in PCa was not superior to the prognostic significance of conventional biomarkers that are used for prognostic purposes.

Expert commentary

The currently used staging system and other prognostic factors have inherent limitations and usually lead to overtreatment of PCa, especially in men of 66 years and older with a limited life expectancy. It is, therefore, evident that more "objective" biomarkers are needed to ameliorate the accuracy of patient prognosis and subsequent treatment decision making. Undoubtedly, the discovery of novel molecular biomarkers and their establishment in clinical practice could bridge this gap between prediction of outcome and true patient outcome.

The clinical interest of the aforementioned molecules as potential biomarkers for prognosis and monitoring of treatment efficacy in PCa has been extensively studied during the last decades. The elucidation of the biochemical pathways involved in prostate carcinogenesis, tumor progression and metastasis are expected to assist the discovery of novel biomarkers in PCa and the development of more personalized treatment with increased possibilities for curative success. Additionally, some of the molecular biomarkers presented in this review could hold prognostic significance, alone or in panels, in particular subtypes of PCa. The frequently observed modulations in protein and/or mRNA expression in malignant prostate tumors compared to benign hyperplasia and/or normal specimens originating from the prostate suggest the notion that some of these novel promising biomarkers deserve further validation in large cohorts of PCa patients.

Five-year view

The emerging potential of several molecules as molecular tumor biomarkers – along with the need for more reliable prognosis and prediction of therapy response – will lead to the development of multivariate panels of biomarkers. Moreover, the use of high-throughput quantitative methodologies including next-generation sequencing will soon uncover the prognostic ability of several mRNAs, long noncoding RNAs and miRNAs that have not been studied yet as standalone biomarkers. The first efforts have already proven to be fruitful; for instance, a few long intergenic noncoding RNAs have just emerged as candidate biomarkers for PCa diagnosis and prognosis.[81–83] However, there is still much work to be done.

Besides miRNAs, there are several other classes of small noncoding RNAs that are more intensively studied during the last years and that are expected to add clinical significance to the existing PCa biomarkers. Such molecules include stress-induced

tRNA fragments (tRFs, also known as tiRNAs), small nuclear RNA fragments and small nucleolar RNA fragments. These tiny noncoding RNAs that have very recently emerged as regulatory RNAs are present at high levels in the prostate tissue as well as in blood and urine, either as circulating or enclosed in exosomes. Interestingly, some of them have already been shown to be heavily implicated in metastasis and other cancer-related processes. Moreover, extracellular vesicles such as PCa cellular fragments could also serve as a fluid biopsy for PCa. In parallel, the further unraveling of the functions of novel RNAs will significantly contribute to the clarification of the genetic and molecular basis of PCa.

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Key issues

- Besides prostate-specific antigen, several other members of the kallikrein-related peptidase (KLK) family, including *KLK2, KLK4, KLK5, KLK11*, and *KLK15*, have been intensively studied for their ability to assist (prostate cancer) PCa prognosis and/or therapy response monitoring.
- Remarkable alterations in androgen receptor expression in the malignant epithelium and normal surrounding stroma are inversely associated with Gleason score, distant metastasis, tumor stage, response to castration therapy and biochemical relapse.
- Although the diagnostic significance of the PCA3 test for the detection of PCa and the possible decrease of unnecessary biopsies has been well established so far, the potential of *PCA3* expression as a prognostic biomarker in PCa remains doubtful.
- SFRP1 and β-catenin expression levels are associated with the Gleason score, survival rate, and response of PCa patients to endocrine therapy; *SFRP2* methylation in combination with other epigenetic markers is a promising biomarker in PCa.
- Loss of phosphatase and tensin homolog protein expression at the time of prostate biopsy predicts time to development of metastasis, cancer-specific survival, castration-resistant PCa and response to androgen deprivation therapy after radical prostatectomy.
- For PCa patients treated with radical prostatectomy, elevated preoperative plasma transforming growth factor-β1 levels are associated with positive regional lymph nodes, presumed occult metastases at the time of primary treatment and disease progression; transforming growth factor-β1 overexpression by prostate tumor cells is associated with pathological features of the tumor and biochemical progression.
- In PCa patients undergoing radical prostatectomy due to clinically localized PCa, the preoperative levels of IL-6 and sIL6R in plasma independently predict biochemical progression following surgery, most likely because of occult metastases at the time of surgery.
- Chromogranin A expression in PCa needle biopsies at the time of diagnosis is an independent prognostic factor of survival and also correlates with circulating chromogranin A levels, particularly in PCa patients with metastatic disease.
- The prognostic potential of chimeric genes containing erythroblast transformation-specific family members such as the *TMPRSS2:ERG* fusion is controversial and merits further investigation.
- Several miRNAs, including miR-145, miR-221, and miR-224, have been proposed as putative prognostic biomarkers in PCa.

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