Tumor markers in prostate cancer

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Novel tumor markers for prostate cancer are still needed to improve the ability to detect prostate cancer, predict prostate cancer related morbidity and mortality and monitor response to treatment. Current markers used in research and even in the clinic remain controversial (Table 1).[1] The most widely applied biomarker in prostate cancer is PSA. Because of its limitations, multiple new markers have been evaluated to compensate for these limitations. Unfortunately many of these markers have not made it into the clinic, which shows that identification of better markers remains a challenge.[2]

Table 1. Current tumor markers for prostate cancer

Marker		Biological function	Biochemical analyte	Marker ability
PSA	Prostate specific antigen	Serine protease	Protein	Screening/ Diagnosis/Prognosis
%fPSA	Percentage free PSA		Protein	Diagnosis/prognosis
PSAD	PSA Density		Protein	Diagnosis/prognosis
PSAV	PSA Velocity		Protein	Diagnosis/prognosis
[-7],[-5],[-4],[2] ProPSA	PSA isoforms		Protein	Diagnosis
hK2/KLK2	Human Kallikrein 2	Peptidase, cleaving proPSA to mature PSA	Protein	Diagnosis
PCA3	Prostate cancer antigen	Non-coding mRNA without a functional protein	RNA	Diagnosis
ETS	E twenty six gene family	Chromosomal rearrangement without a function	DNA	Prognosis
TMPRSS2:ERG	Trans membrane protein serine 2 (TMPRSS2) and ETS related gene (ERG)		DNA Protein (ERG)	Prognosis
AMACR	Alpha-methylacyl coenzyme A racemase	Metabolization of fatty acids and bile acid biosynthesis	RNA Protein	Diagnosis/prognosis
GSTP1	Glutathione S-transferase pi 1 (methylated)	Detoxification of carcinogens	DNA	Diagnosis/prognosis
PSMA (FOLH1)	Prostate specific membrane antigen	Peptidase, hydrolyzing peptides in prostatic fluids	RNA Protein	Prognosis
PSCA	Prostate stem cell antigen	Membrane based glycoprotein	RNA Protein	Diagnosis/prognosis
CgA	Chromogranin A	Proteolytic protein	Protein	Prognosis
B7-H3	Transmembrane protein family B7, member H3	Regulation of T-lymphocytes	Protein	Prognosis
CAV1	Caveolin-1	Molecular transport, cell adhesion and signal transduction	Protein	Diagnosis/prognosis
GOLPH2	Golgi phosphoprotein 2	Sorting and modification of proteins through the Golgi apparatus	RNA Protein	Diagnosis

Table 1. Current tumor markers for prostate cancer (continued)

Marker		Biological function	Biochemical analyte	Marker ability
CRISP3	Cysteine-rich secretory protein 3	Unknown	RNA Protein	Diagnosis/Prognosis
Sarcosine		Metabolite produced after enzymatic transfer of a methyl group from S-adenosylmethionine to glycine	Protein (metabolite)	Prognosis
Exosomes	Nano-sized vesicles, 100 nm in diameter containing RNAs and proteins	Intercellular communication, part of degradation pathway	RNAs and Proteins	Diagnosis/prognosis

PSA

Since its discovery in 1970, PSA has revolutionized the diagnosis and management of prostate cancer.[1] Subsequently, after its application in urological practice it has proven to be a valuable tool for (early) detection, staging and monitoring of men diagnosed with prostate cancer (Figure 1A).[3,4] Especially the use of PSA as a screening tool has increased the identification of prostate cancers and also improved curability with treatment.

PSA, also known as KLK3 or hK3, is a member of the human Kallikrein family. This gene family consists of 15 members and is described with a distinct nomenclature.[5] The first three members (hK1, hK2 and hK3) encode for serine proteases that have diverse physiological functions. Expression of PSA and some other Kallikrein members is androgen regulated. PSA protein has a half-life of 2-3 days and is secreted by prostatic epithelial cells into seminal fluid. Most likely through tissue leakage, PSA can be found in serum, but with a concentration of about 10^6 times less as compared to seminal fluid.

Initially, PSA is produced as a 261 amino acids preproenzym with a 17 amino acid signal peptide that is removed during synthesis (Figure 1B).[6] After this step, proPSA is formed which contains 244 amino acids, from which subsequently 7 amino acids are cleaved so it is processed to PSA that contains 237 amino acids. When shed in serum, PSA is unbound (free PSA or fPSA, 5-35%) or bound (complexed PSA or cPSA) to complexes with the anti-proteases α(alpha)1-antichemotrypsin (PSA-ACT), α(alpha)2-macroglobuline (PSA-A2M) or α(alpha)1-protease inhibitor (PSA-API) which inactivate its function.[7] In seminal fluids it functions as a protease that liquefies semen by interacting with semenogelin and fibronectin.[8,9] Although PSA is highly specific for prostate epithelial cells, in much smaller concentration it can be measured in malignant breast cells, salivary gland, bowel, other urological tissues and renal carcinoma cells.[10-12] Nevertheless for practical and clinical purposes PSA is organ specific because after removal of all prostate tissue PSA values become immeasurable in serum. Although PSA is organ specific, it cannot be ascribed

as prostate cancer specific because other urological conditions such as benign prostate hyperplasia (BPH), prostatitis or mechanical damage also contribute to aberrant PSA-values in serum.[13] It is noteworthy that the production of PSA by prostate cancer cells is not higher than benign prostate epithelial cells, but higher serum values is a result of an altered prostate-blood barrier.[14] In fact, production of PSA by prostate cancer cells is generally lower.[15]

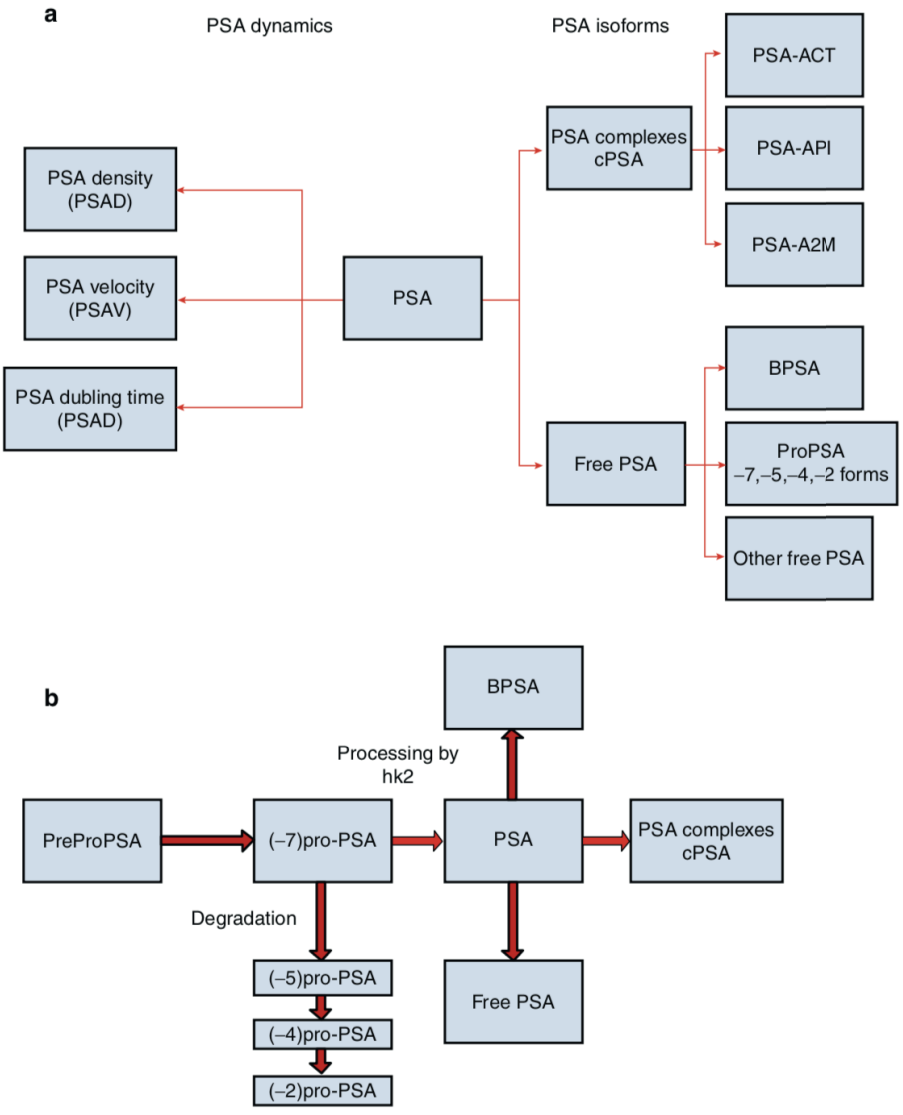

Figure 1. A. Different measurements contributing PSA including PSA dynamics. B. Processing of PSA to its subforms.

Large studies showed that 97% of all men older than 40 years have PSA serum levels lower than 4 ng/mL, which gave rise to the idea that this value should be the threshold when it is used in a diagnostic setting.[16] Furthermore, it was shown that PSA serum values could increase when prostate cancer is present.[17,18] Initially PSA was used as a reliable marker to prove residual disease or progression after radical prostatectomy for prostate cancer.[19] Patients with lower values preoperative had higher rates of organ-confined disease.[20,21]

In a screening setting it has been shown that PSA can increase the detection rate of prostate cancer in men without symptoms.[22] By using PSA, the percentage of men who were found with metastases at diagnosis was reduced from 16% to 4%, but also late-stage disease and prostate cancer related mortality was observed to be less.[23] During the last decades it is shown that with the use of PSA the detection of prostate cancer has increased dramatically, but that prostate cancer mortality was only reduced with 20%. Therefore it was concluded that using PSA for the detection of prostate cancer results in a substantial overdiagnosis and overtreatment.[24]

As a diagnostic tool PSA has a high sensitivity but low specificity for prostate cancer, where the positive predictive value (>4.0 ng/mL) is limited to 25%.[25,26] Serum PSA levels are influenced by tumor grade, volume and site of origin (primary tumor or metastases) and it is capable to predict pathological features.[13] On the other hand, in 15% of men with low PSA levels, prostate cancer is present.[27] So, in order to improve identification of prostate cancer and gain specificity, changes in variant forms of PSA have been investigated and introduced into the clinic.

FREE PSA

The proportion of free PSA (%fPSA) is lower when compared to total PSA in healthy men or men with BPH.[28-30] Therefore, %fPSA has been suggested as a marker for prostate cancer.[31] The exact cause for this occurrence is not fully understood, but it is thought that in patients with prostate cancer PSA 'escapes' proteolytic activity and stays bound to ACT, A2M or API. An extensive meta-analysis that compromised 66 studies showed that %fPSA and cPSA have better diagnostic potential compared total PSA (tPSA) in the intermediate range of 2-10 ng/mL.[32] In studies where %fPSA is combined with serum PSA levels between 2.5 and 4 ng/mL, more specificity can be obtained in diagnosing prostate cancer.[33] The use of %fPSA could contribute to a more reliable diagnosis and therefore maybe reduce biopsies by 20% and lessen the overdiagnosis.[34] Furthermore, a better stratification could be made of patients who are more eligible to undergo active surveillance and therefore decrease overtreatment.

As a prognostic marker, high %fPSA correlated with smaller and lower grade prostate cancer.[34] Vice versa low %fPSA resulted in a more aggressive form of prostate cancer, even when measured up to 10 years before diagnosis.[35] Prostate cancers with Gleason scores of >7 and extra capsular extension also showed a correlation with low %fPSA.[36,37]

PSA DENSITY

In a majority of men with slightly elevated PSA levels, the main contributor is probably BPH and only in a small percentage of men, prostate cancer.[36] To differentiate better between these two condition a method was introduced that compensated for the increase of serum PSA levels by prostate enlargement.[38] This measurement, PSA density (PSAD) where serum PSA is divided by prostate volume (>0.15), has shown to have a direct relationship with the probability of having prostate cancer, especially with intermediate PSA levels and no abnormalities on DRE (digital rectal exam).[39,40] Although these primary reports embrace promising results, this measurement has shortcomings. When PSAD was compared to PSA it was not able to enhance the predictive value of PSA alone.[41] Furthermore, PSAD in not sensitive enough for prostate cancer detection, almost 50% of all cancers are missed.[42] The most plausible interpretation of these conflicting results is most likely the heterogeneity of prostate volumes in prostate cancer and BPH. Because PSAD is influenced by prostate volume, the number of epithelial cells has to be a correction for these factors. Correction for transition zone size has shown to be a very specific and sensitive technique to detect prostate cancer, but because of the variability of ultrasound measurements it has not gained wide acceptance in daily practice.[43] Also as prognostic marker, increased PSAD values were correlated with Gleason scores >7 and a greater risk of organ confined disease.[44]

PSA VELOCITY

Another approach for detecting prostate cancer in the intermediate range of serum PSA is by using PSA velocity (PSAV), where the rate of PSA change between two separate measurements is taken into account.[45] As a diagnostic tool, an increase of 0.75 ng/mL or more per year is correlated with the presence of prostate cancer, which has a high specificity with PSA values between 4-10 ng/mL (up to 90%).[45,46] To obtain a reliable PSAV result, the interval between the two separate measurement should be at least 18 months.[46] This interval seems not to be optimal for clinical daily practice because it can cause a delay in treatment. Furthermore, based on the characteristics of this marker, its use is limited. When initial PSA values are less than 4 ng/mL the sensitivity and specificity

is dramatically reduced.[47] As a prognostic marker, increased PSAV is significantly related to aggressiveness. One study showed that preoperative PSAV values of >2.0 ng/mL per year resulted in a nine times higher chance of prostate cancer related mortality after prostatectomy or external beam radiotherapy.[48,49] A recent study revealed that even a PSAV of >0.35 ng/mL per year correlated with a significant higher chance of biochemical progression.[50] On the other hand, when values of <0.4 ng/mL per year were used, it increased the likelihood of insignificant prostate cancer.[51] Besides these promising results, the exact role of PSAV in the stratification and characterization of specific subgroups of prostate cancer patients remains not fully elucidated. More research has to be performed to maximize its potential as a tumor marker and to establish the most ideal cutoff PSAV value for diagnosis and determining prognosis.

PSA DOUBLING TIME

Closely related to PSAV, PSA doubling time (PSADT) could also harbor some interesting capacities as a tumor marker. PSADT is defined as the time that serum PSA levels are doubled. As a diagnostic tool, so far no reports have been published. Nevertheless, the predictive abilities of this tumor marker has been the focus of multiple research efforts, but their results show no relationship between pretreatment PSADT and post treatment outcomes.[52] As a prognostic marker it has mainly been measured post prostatectomy and was correlated with survival results. The first study showed that fast PSADT values (<10 months) correlated with lower metastasis-free survival.[53] Others showed that if PSADT was <3 months within a period of 24 months after radical prostatectomy there was an associated with lower cancer specific survival.[54]

PSA ISOFORMS

ProPSA is an inactive precursor of PSA that is cleaved by hK2 or hK4, converting it into its active form.[55] The precursor form of PSA contains a 7 amino-acid proleader peptide and is therefore named [-7]proPSA. Incomplete cleavage of proPSA results in other subforms, such as [-2], [-4] or [-5]proPSA. Elevated levels of proPSA and its truncated forms were observed in prostate cancer tissue.[56,57] A possible explanation for this finding was the observation that proPSA is higher expressed in the peripheral zone of the prostate.[57]

Mainly in the intermediate range (2.5-10.0 ng/mL) of PSA, ProPSA could early detect more prostate cancers.[58-60] Even when these isoforms were used, it could avoid 59% of all biopsies taken, as compared to 33% when only %fPSA was used. Unfortunately, in a prognostic setting proPSA does not seem to be superior to %fPSA, but when combined

it is correlated with higher Gleason scores and non-organ defined prostate cancer.[61] All the single sub-isoforms of proPSA have been investigated and showed no better correlation in diagnosing or determining prognosis as compared to total proPSA or %fPSA.[60]

KLK2

Human Kallikrein 2 (hK2 or KLK2) is also a member of the Kallikrein family and shares 80% homology with PSA. It functions as a peptidase, cleaving proPSA to mature and active PSA.[62,63] Like PSA, it is highly and specifically expressed in the prostate and is androgen regulated. hK2 levels show a distinct expression pattern on immunohistochemical analysis, which was also observed in serum. These findings indicated that this marker could be indicative, independent of PSA.[64,65] The first studies on hK2 showed no correlation of this marker with prostate cancer.[66-68] Nevertheless, a review that also included all studies on hK2 performed in a later stage, revealed a significant higher expression of hK2 in serum from prostate cancer patients.[60] Especially for the intermediate elevated PSA values, it showed a better discrimination as compared to %fPSA. As a prognostic marker hK2 is capable of differentiating between low and high Gleason scores and also for extra-prostatic growth, even prior to radical prostatectomy.[69-71] Unfortunately, when this marker was analyzed in a multivariate model it had a very limited improvement on prognoses as compared to Gleason score alone.[72,73] One study revealed that hK2, together with other variables, was significantly predictor of biopsy outcome.[74]

URINARY PSA

In almost all reports, PSA as a tumor marker for prostate cancer was measured in serum. In contrast to serum PSA, also urinary levels of PSA were evaluated as a potential tumor marker for prostate cancer.[75] Although the first report was published in 1985, less is known about this PSA measurement. Just as serum PSA it was shown that elevated urinary PSA after radical prostatectomy was correlated with disease recurrence and therefore was suggested as a monitoring marker.[76] In a diagnostic setting, when a ratio was taken of urinary and serum PSA expression it was shown that it produced higher sensitivity and specificity as compared to serum PSA alone, especially in the intermediate range.[77,78] Unfortunately, reports on urinary PSA levels are few and more research is needed to fully elucidate if urinary PSA has any potential as a marker for prostate cancer.

PCA3

The PCA3 transcript (prostate cancer antigen 3) was discovered in the late 90s as a new promising candidate marker for prostate cancer.[79] The PCA3 gene is located on chromosome 9q21-22 producing a (non-coding) mRNA that does not encode a protein.[80,81] After its discovery it was named DD3 (differential display clone 3) as a result of a differential display analysis that was used to compare mRNA expression between healthy prostate tissue and prostate cancer tissue.[82] 95% of prostate cancer specimens highly expressed PCA3, compared to no expression in normal prostate, BPH or other types of cancerous tissues. High grade PIN also revealed higher expression, up to 96% of the cases.[83,84] PCR on similar samples showed a 66-fold increase in PCA3 expression in prostate cancer samples with a sensitivity of 94% and specificity of 98%.[85,86] Furthermore, the expression of this marker is not influenced by age, prostate volume and infections.[82] The current PCA3 test is mRNA based and the outcome is a ratio between PCA3 mRNA and PSA mRNA multiplied by a 1,000.[86] This test is preferentially performed on urine samples that are collected after digital rectal examination or prostate massage.[87] When this test is performed on serum, it has less accuracy.[88]

Initially, the PCA3 test was launched to predict presence of PCa after negative biopsies. Subsequent reports on the urine test showed a sensitivity of 54-82% with a specificity of 66-83%, where PSA has a sensitivity of only 22-47% for the diagnosis of prostate cancer.[82,84,86,88-90] Multiple studies have shown that increased PCA3 is statistically significantly correlated with more tumor volume.[91-93] PCA3 also outperformed the diagnostic accuracy of %fPSA. This diagnostic accuracy can even further be increased when PCA3 is combined with other (clinical) variables such as PSA, physical characteristics during digital rectal examination, age and family history.[94] In a screening setting, PCA3 was capable of improving the performance characteristics and identification of serious disease compared with PSA.[95]

Although many reports describe the relation and prognostic features, such as histopathological outcome, generally no correlation could be observed between PCA3 and Gleason score and pT staging.[96] With these data it was suggested that PCA3 could be applied to predict histopathological outcome after biopsy, especially in patients with elevated PSA and a negative biopsy.[90,96,97] Furthermore, it was suggested that PCA3 could be used to determine multifocality of prostate cancer lesions and patients that are candidates for active surveillance.[82,98-100] The exact role of PCA3 in determining diagnosis and prognosis of prostate cancer remains to further investigated. Since the PCA3 detection assay is RT-PCR (reverse transcriptase PCR) based, the assay needs to be performed by expert labs and is much more expensive than protein-based ELISAs.

ETS

In prostate cancer, chromosomal rearrangements affecting the ETS (E twenty six) gene family members are common events; around 60-70% of all cases exhibit such an alteration.[101,102] In a majority of the rearrangements there is a fusion between the genes TMPRSS2 and ERG, the so called TMPRSS2:ERG fusion gene, which is unique for prostate cancer. Both TMPRSS2 and ERG genes are located in the same orientation on the long arm of chromosome 21. They are spaced by approx. 3 million base pairs and a deletion of this interstitial region can cause fusion of the two genes. Because the TMPRSS2 gene is androgen regulated, a fusion of this gene with ERG results in the androgen regulated and high expression of ERG. So far, this fusion is never observed in normal tissue and unique to prostate cancer.[103]

Multiple gene fusion partners that are related with either the TMPRSS2 part or the ERG part have been identified.[104] Other fusions of the TMPRSS2 gene occur in fewer cases with ETV1, ETV4 and ETV5. Although the TMPRSS2 gene is most often involved, other fusion partner such as the SLC45A3, ACSL3, HERV-K, FOXP1, EST14, KLK2, CANT1, DDX5 genes can rearrange with ETS family members.[105] All these gene fusions are unique to prostate cancer and seem to play an important role in the biogenesis and development of this disease. Therefore they could function as marker for diagnosis and prognosis. Recent studies showed that the fusion of TMPRSS2 to ERG is present in the precursor lesions PIN (prostatic intraepithelial neoplasia) and therefore must be an early event in cancer development.[106,107] Multiple studies that address the prognostic value of this marker have been performed, with several opposing conclusions.[102,105] Two studies examined 114 and 150 prostates after radical prostatectomy and revealed that expression of ERG or TMPRSS2:ERG correlated with a reduction of biochemical progression.[108,109] Gleason score are thought to be lower when TMPRSS:ERG is present.[110] No correlation was observed by other five studies that compromised similar sized study cohorts.[106,111-114] Also the presence of ETV1 rearrangements failed to correlate with progression of disease.[115] Most reports reveal an unfavorable correlation of gene rearrangements with outcome after treatment (radical prostatectomy). These studies showed an increased rate of biochemical recurrence, formation of metastases or even death.[114,116-124] Interestingly, one study showed that ERG rearrangement alone was associated with low grade prostate cancer, present with seminal vesicle invasion there seemed to be a poorer prognosis.[105,122] Expression of the TMPRSS2:ERG fusion gene was shown not to be able to predict response to endocrine treatment in hormone dependent and lymph node positive prostate cancer.[125,126]

Rearrangements of genes from the ETS family are potentially very useful diagnostic markers due to their prostate cancer specific occurrence if they can be measured in serum or urine. Like for PCA3, a test has been developed to measure fusion transcripts in

urine. For prognostic or predictive purposes, fusion gene-based tumor markers remain controversial.

Because measurements of the fusion transcripts and genes are performed with RT-PCR or FISH (fluorescent in situ hybridization) techniques, implementation in daily clinical practice is hampered. Recently, an antibody against the ERG protein was generated that can be used for immunohistochemistry.[127,128] Although the antibody has some cross-reactivity with FLI1, it gives the opportunity to easily and quickly assess thousands of retrospective and prospective patient samples. All three techniques (ERG antibody on protein level, RT-PCR on mRNA level and FISH on DNA level) provide their own unique information on the status of the fusion event and are likely complementary in their diagnostic and prognostic value.

AMACR

AMACR (alpha-methylacyl coenzyme A racemase) is an enzyme that is encoded by the P504S/AMACR gene. In cells, this protein is located in the mitochondria and peroxisomes and although the function has not been revealed completely it is related to the metabolization of fatty acids and bile acid biosynthesis.[129-131] The AMACR transcript and protein are known to be highly expressed in a variety of cancers with a very high (up to nine times higher) expression in 86% off all prostate cancers.[132-134] In 2002, AMACR was introduced as a new marker for prostate cancer.[135] A meta-analysis of multiple mRNA expression arrays revealed that AMACR is over expressed in prostate cancer with high sensitivity and specificity.[136,137]

In a diagnostic setting, the use of the AMACR protein on immunohistochemical analysis of prostate biopsy samples has been limited to a valuable complement to other known markers.[138] Unfortunately, samples that did not contain prostate cancer also had AMACR expression, but generally lower compared to the cancer samples.[139] In 18% of the prostate cancers, AMACR is false negative.[140] When unusual histopathological subgroups of prostate cancer had to be identified, the increased expression was only limited to 62-77%.[132,141]

In a prognostic setting it has been shown that untreated metastasis and hormone-refractory prostate cancers were strongly positive for AMACR. In this specific prostate cancer stages, AMACR has a sensitivity of 97% and a specificity of 92-100%.[135,142] Furthermore, decreased expression of AMACR has been shown to have prognostic value in predicting biochemical recurrence and prostate cancer related death.[143]

In order to assess this marker in non-invasive derived patient materials (not biopsies) such as serum or urine, expression of AMACR mRNA could also be identified in 69% of the cases. Unfortunately, AMACR is not specific to cancer of the prostate, because

serum levels can also be elevated in other urological disorders like BPH or auto-immune diseases.[144] When used in a diagnostic setting as an additive to PSA, sensitivity and specificity can be increased when measured in urine, especially when the PSA is in the midrange (4-10 ng/mL).[145-147] Unfortunately, when AMACR mRNA was normalized to PSA mRNA, AMACR did not accomplish to be a statistically significant predictor of prostate cancer.[148] New promising serum tests for prostate cancer which comprehend the AMACR gene are evaluated. With these tests a ratio is calculated between the expression of the AMACR gene and the PSA gene.[131] Until now, one report has been published were it was shown that the AMACR protein is detectable in serum with an ELISA, but elevation of this protein was not specific for prostate cancer.[149] Although more research has to be performed, it is also shown that circulating antibodies against the AMACR protein in combination with PSA could function as a useful tool for diagnosis.[146,150]

GSTP1

During aging, DNA damage occurs as a result of oxidative stress, exposure to chemical substances or ionizing radiation.[151] These damages can result in mutations or alterations of oncogenes and tumor suppressor genes. In healthy cells the cytoplasmic enzyme glutathione S-transferase pi I (GSTP1) plays an important role in detoxifying the cell from carcinogens. GSTP1 is a member of the glutathione S-transferase family, which contains four different classes. All these classes are expressed in prostate tissue.[152] Although GSTP1 expression is increased in various cancers, in prostate cancer GSTP1 is down regulated.[153] This is caused by hypermethylation of the GSTP1 promoter, a mechanism well known in cancer to decrease expression of tumor suppressor genes. Hypermethylation of GSTP1 was observed in all stage of prostate cancer, from high grade PIN to metastases.[154,155] Such methylation was not observed in benign prostate epithelial cells.[151] Based on these findings and the presence of methylation in 90% of prostate cancers and 67% in high grade PIN, it was concluded that GSTP1 methylation might function as a tumor marker for prostate cancer.[156,157] Subsequently, methylation of this gene could be observed in serum, urine and ejaculate of prostate cancer patients when analyzed by methylation specific PCR, which gave rise to the idea that it could even be applied in a clinical setting.[158-161]

As a diagnostic marker it was shown that GSTP1 DNA methylation in urine has a sensitivity of 75% (after DRE) and a specificity of 98% for prostate cancer and is comparable to its expression in biopsy specimen.[162] Similar values for sensitivity and specificity were observed in other studies. It is notable that sensitivity in urine is increased by collection directly after digital rectal exam or prostate massage and functions independent of

PSA.[163-165] To increase sensitivity even more, a relative ratio of GSTP1 methylation over methylated MYOD6 can be determined.[153]

For prognostic purposes, 100% of the locally advanced or metastatic tumors showed hypermethylation. Biochemical recurrence after prostatectomy seems to appear more and faster when the epigenetic alteration is present.[166] In a small study cohort it was shown that methylation of GSTP1 is a statistically significant predictor for time to recurrence.[167] Androgen deprivation therapy does not seem to influence GSTP1 methylation in 87% of the cases.[168] Unlike other genetic alterations, methylation of this gene is reversible after therapeutic intervention. Because no reports have been published which describe this effect, more research is needed.

Methylation of GSTP1 seems to function very well as a diagnostic and prognostic tool, but because the number of reports describing this marker is lacking, we should be careful in jumping to conclusions. As more results are being published, more allusions are made regarding the use of a set of hypermethylated genes for optimal diagnosis and determining prognosis in prostate cancer patients.

PSMA

PSMA (Prostate specific membrane antigen), or also known as FOLH1, is a androgen regulated gene that encodes a type II transmembrane glycoprotein. PSMA belongs to the M28 peptidase family and has a intracellular and extracellular domain.[169] Its function is limited to hydrolyzing peptides in prostatic fluid and generating glutamate and also acts as a folate hydrolase.[170,171] This protein is expressed in a number of tissues such as prostate, nervous system and kidney.[172,173] Furthermore, it has been shown to have a higher expression in prostate cancer. This finding could possibly be related to its enzymatic activity and thus invasiveness growth of prostate cancer.[174,175]

In the field of prostate cancer, PSMA has been the focus of many research groups. It has mainly been suggested as a prognostic tool.[176] Immunohistochemical analysis in a group of 232 patients showed higher expression in prostate cancer (79.3%) and metastases (76.4%) as compared to benign prostate tissue (46.2%).[177] Other studies showed an increased expression in progressive prostate cancer and hormone independent prostate cancer.[178-183] In serum from prostate cancer patients, the PSMA protein is increased, with a higher expression in advanced stages of cancer.[184-186] Nevertheless, contradicting studies show that PSMA is not prostate cancer specific and does not discriminate between localized prostate cancer and advanced disease.[187] A possible explanation for these different findings could be the fact that in those studies different types of antibodies have been used in various assays. Also studies that investigated the expression of PSMA mRNA have shown varying and inconclusive results, probably because of different assays used.

The sensitivity of diagnosing prostate cancer with PSMA mRNA is more or less similar to that of PSA mRNA.[174] As a prognostic marker no correlation was observed between PSMA mRNA and Gleason score, pT staging and serum PSA. In a study on patients with clinically localized prostate cancer, a combined PSMA/PSA mRNA analysis in peripheral blood samples showed that this could be an independent predictor to biochemical progression after radical prostatectomy.[188]

Although PSMA seems to be not prostate and prostate cancer specific, there is an upregulation of PSMA in prostate cancer and probably more in its aggressive forms. Therefore its function as a marker for prostate cancer is limited. A more promising feature of PSMA is its application in tissue targeted therapy such as prostate specific cancer vaccine therapy or radioimmunotherapy.[189,190]

PSCA

Prostate stem cell antigen (PSCA) is a gene that encodes for a membrane based glycoprotein. PSCA has been found to be relatively highly present in prostate, but also in other cell types such as bladder, placenta and gastrointestinal tissues.[191] The expression is also elevated in malignant tissues such as prostate cancer, bladder cancer and gastrointestinal cancers.[192,193] In prostate the expression of the PSCA mRNA is influenced by puberty, androgen deprivation and androgen restorement.[194] Although the exact involvement of PSCA in prostate cancer is fairly unknown it was shown that PSCA protein and mRNA are higher expressed from high grade PIN through all stage of prostate cancer.[195,196] Nevertheless, knockout of the PSCA gene in mice resulted in a normal urogenital development without an increased risk of prostate cancer.[197]

As a diagnostic or predictive marker it was shown that expression of PSCA in negative biopsies before TURP (transurethral resection of the prostate) is associated with higher risk of having prostate cancer in the TURP specimen. Especially when serum PSA levels >4.0 ng/mL or with a suspicious DRE.[198]

In a prognostic setting, immunohistochemical analysis showed that expression of the PSCA protein was present in 94% of all tumors and was significantly associated with adverse prognostic features, such as high Gleason score and extra-capsular extension.[199,200] Furthermore, PSCA was identified in bone metastases and lymph node metastases.[201,202] These findings suggest that there is a positive correlation of the PSCA protein with advancement of disease status in prostate cancer. When PSCA mRNA was measured in peripheral blood it corresponded with a reduced disease free survival time.[203] Compared to PSA and PSMA it was noticed that specificity and independent prognostic value were very high.[203] Unfortunately this transcript could only be identified in 13.8% of the patients, which limited its ability to differentiate between benign and malignant prostate

tissue. When this marker was investigated for its post-treatment monitoring value, it was shown that after EBRT PSCA mRNA is decreased.[204] Therefore it was proposed as in interesting marker for follow-up after treatment.

Besides the properties of being a possible diagnostic or prognostic marker for prostate cancer, it has also been found that PSCA is a possible target for prostate specific virus therapy.[205,206] When PSCA is used, it was possible to inhibit tumor growth and formation of metastases.

CHROMOGRANIN A

Chromogranin A (CgA), is a gene that encodes for a proteolytic protein that is a member of the chromogranin/secretogranin family of neuroendocrine secretory proteins. CgA is one of the most frequently produced proteins in neuroendocrine cells in the prostate and can be easily measured by a radioimmunoassays.[207] Serum levels of Chromogranin A could reflect neuroendocrine activity of prostate malignancies, therefore it holds an interesting potential to function as a marker for prostate cancer and especially for neuroendocrine differentiation.[208,209] Unfortunately, Chromogranin A is not prostate specific, it is also elevated in various neuroendocrine tumors and neuroblastomas.[210-213] The exact function of Chromogranin A in prostate cancer is unknown, but it has been shown that it influences the growth of prostate cancer cells.[214]

Despite conflicting results as a diagnostic tool, when measured in serum, high Chromogranin A levels seem to correspond with the presence of (organ confined) prostate cancer. [216] In combination with PSA a better diagnostic accuracy could be established.[215] An interesting report showed that Chromogranin A is able to predict conversion of hormone naïve prostate cancer to hormone refractory disease and the presence of hormone independent prostate cancer itself.[216,217] A small prospective study on 50 prostate cancer patients showed that high Chromogranin A serum levels prior to radical prostatectomy were able to predict higher Gleason scores, extra capsular extension and eventually treatment failure.[218-220] Especially in patients with hormone independent prostate cancer this marker correlates with adverse outcomes and decreased overall survival.[221] Furthermore, this marker could function as a predictor for chemotherapy response in hormone independent prostate cancer.[222] In a prognostic setting, high levels of CgA correspond with factors such as a higher Gleason score, advanced pT stage and metastases.[223,224] Immunohistochemical analysis showed similar results.[225,226] No decrease in Chromogranin A serum levels were observed after radiotherapy or hormone therapy, Therefore the use of this marker in as a monitoring tool seems not to be sefull.[227,228] Specific antibodies against Chromogranin A can suppress its function through apoptotic pathways, leading to programmed cell death. Therefore Chromogranin A antibody mediated apoptosis was

suggested as an alternative treatment for prostate cancer.[214] A derivate of this marker, Chromogranin A velocity was introduced as a marker for predicting time to androgen independence after hormonal treatment.[228]

B7-H3

The transmembrane protein family B7 has gained publicity with its role in regulation of T lymphocytes.[229] Subsequent reports showed that a total of four subtypes (B7-H1, B7-H2, B7-H3 and B7-H4) could be identified in cancers and might play a role in the mechanism by which human malignancies evade host immune responses.[230-232] Higher expression of some of these subtypes are correlated to more aggressive behavior and poor clinical outcome.[233,234] The B7-H3 has also been identified in healthy placenta and malignant tissues.[235] Although there was expression in benign tissue, the expression in cancerous lesions was significantly higher.[230]

B7-H3 could be identified as an independent prognostic factor in 338 patient samples after radical prostatectomy that were followed with a median of 3.9 years. The patients which showed elevated B7-H3 expression had a shorter time to cancer progression.[236] This indicated that B7-H3 could function as a prognostic marker. Furthermore, B7-H3 expression is higher in metastases and hormone refractory prostate cancer. The expression is not hampered by hormone treatment.[237] Also, this marker could have prognostic value for biochemical recurrence after salvage radiotherapy, especially with low primary TNM staging, low Gleason score and low pre-radiotherapy PSA.[238] Because this marker is membrane-bound in cells it also harbors a function in targeted therapy. Chemotherapy or radionucleotide therapy that is directed against B7-H3 makes it possible to specifically engage prostate cancer cells.

CAV1 (CAVEOLIN-1)

Caveolin-1, is a major structural component of caveolae. These caveolae are specialized membrane invaginations that are abundant in adipocytes, endothelium and smooth muscle cells. Caveolae are involved in molecular transport, but also in cell adhesion and signal transduction.[239,240] Caveolin-1 has been linked to prostate cancer since the late 90s, where it was identified as a marker.[241] The exact relation of caveolin-1 and prostate cancer remains unclear, but it is known that caveolin-1 in prostate acts as a tumor suppressor by keeping Akt dephosphorylated in the Akt-pathway.[242] Subsequently it was shown in *in vitro* experiments that downregulation of the expression of this gene resulted in cells turning from androgen-independent to androgen-dependent.[243] This

implicated that there is a role for Caveolin-1 in the development of castration resistance. It is also known that this protein plays a role in the malignant characteristics of prostate cancer cells by changing the microenvironment and promoting angiogenesis.[244] Studies showed that Caveolin-1 is also expressed in normal prostate stromal cells, but minimally expressed in normal epithelial cells.[245] The protein expression of Caveolin-1 is higher in prostate cancer cells compared to normal prostate epithelial cells.[241] The expression of this marker in epithelial cells upregulates when prostate cancer grading increases.[245] Furthermore, the protein Caveolin-1 also has higher serum values in patients with prostate cancer, which makes it possible to measure it with a very sensitive and reproducible ELISA.[246] Median serum Caveolin-1 levels are significantly higher in localized prostate cancer compared to men with BPH.

Caveolin-1 levels could harbor a predictive potential in men undergoing radical prostatectomy.[247] Higher expression of Caveolin-1 was correlated with an increased risk of developing aggressive recurrent tumors after surgical treatment. Pre-operative high Caveolin-1 serum levels resulted in a 2.7 fold higher risk of developing biochemical recurrence.[248]

When Caveolin-1 was investigated as a prognostic tool, in samples retrieved after radical prostatectomy it was shown that a positive immunohistochemical staining correlates with a significant worse prognosis.[249] In patients with lymph node negative prostate cancer, Caveolin-1 expression is an independent prognostic factor for a Gleason score >7, extra prostatic extension, positive surgical margins. When combined in a multivariate model with other variables such as Gleason score it is possible to more accurately predict the chance of biochemical recurrence. Unfortunately, another study showed in 1458 cases no correlation between high post-operative Caveolin-1 values in serum and aggressiveness of prostate cancer or adverse prostate cancer events.[250]

GOLPH2

GOLPH2 (Golgi phoshoprotein 2), also known as GOLM1 or GP73, is a type II Golgi membrane protein and involved in the sorting and modification of proteins that are exported from the endoplasmatic reticulum through the Golgi apparatus. Recent findings suggest that changes in structure and function of the Golgi apparatus may play an important role in the development or behavior of malignant cells. This protein has already been shown to be elevated in liver diseases as a result of viral infections, but also as a potential marker for hepatocellular carcinoma.[251,252] Immunohistochemical experiments on prostate cancer samples revealed that the GOLPH2 protein also is upregulated in prostate cancer.[253,254] An interesting finding was that this specific marker is present, even when AMACR is negative. Therefore it was mainly introduced as an additive protein

marker for prostate cancer, next to other known markers. Preceding mRNA profiling studies, research already showed that GOLPH2 mRNA is upregulated in prostate cancer tissues.[255,256] When this gene transcript is used in a marker profile to detect prostate cancer in urine, it seems to be capable to outperform PSA measured in serum.[148]

MYO6 (MYOSIN IV)

Myosin IV is a Golgi apparatus-associated protein that is involved in intracellular vesicle and organelle transport and is required for the structural integrity of the Golgi apparatus. Furthermore the protein has been suggested as an important factor for cell migration and even cancer invasion.[257-259] Based on a microarray experiment it was discovered that the MYO6 mRNA is upregulated in prostate cancer, next to GOLPH2.[260] Interestingly, expression of the transcript goes down in androgen-independent and more aggressive prostate cancers.[260] With Immunohistochemical analysis it was shown that a strong protein expression is present in a PIN, the majority of prostate cancer cells, and weak or absent expression in neighboring benign prostate cells.[254]. In a prognostic setting, no differences were observed between the different Gleason scores or other pathological indicators for aggressiveness.[260] Based on these results, the transcript could be used as a diagnostic marker, but further research has to be performed to reveal the true potential of this marker and to assess its possible role in prognosis.

CRISP3

Cysteine-rich secretory protein 3 (CRISP3), also known as specific granule protein 28 (SGP28), has recently been implicated as potential marker in prostate cancer. Relatively little is known about its function and role in prostate cancer. The CRISP3 mRNA has shown to be present in high concentrations in salivary glands, pancreas and prostate.[260-262] Furthermore, its expression has been shown in secretory epithelium in the male urogenital tract, including the epididymis and the ampullae of the ductus deferens.[263] Regarding prostate cancer, multiple studies have shown that the expression of the CRISP3 mRNA is high[264]er (20-300 times) in prostate cancer as compared to healthy prostate tissue.[262,265,266] Also on the protein level, CRISP3 was shown to be higher expressed.[267] The protein also has been identified by ELISA in multiple bodily fluids, such as serum, saliva and seminal plasma.[268] Unfortunately, serum concentrations were not different between prostate cancer samples and healthy controls.

As a prognostic marker, immunohistochemical analysis of prostate cancer specimen showed an increase in expression when Gleason scores increased. Expression in normal

prostate epithelial cells was weak of absent. A similar analysis on radical prostatectomy samples revealed that expression of CRISP3 eventually positively correlated with biochemical recurrence.[269] In a multivariate analysis this protein was still associated with recurrence. Nevertheless, when this marker was added in a model with other known markers, such as PSA, no improvement was observed. With the results acquired so far, CRISP3 does not seem to be a good prognostic marker for prostate cancer.[264,270]

An interesting observation was the decrease of CRISP3 after ochiectomy in some patient samples. This could reflect that CRISP3 could be partially androgen regulated and might function as a monitoring marker.

SARCOSINE

The discovery of Sarcosine as a marker for prostate cancer has only recently been made. Since a large number of research groups are exploring changes on the level of genomics, transcriptomics and proteomics, changes in the metabolomic field are novel and few. Sarcosine is a metabolite that is produced by the enzymatic transfer of a methyl group from S-adenosylmethionine to glycine. This reaction is catalyzed by the enzyme glycine-N-methyltransferase (GNMT), which is highly expressed in prostate, liver and pancreas. The first report on Sarcosine in prostate cancer showed that Sarcosine stimulates malignant growth of prostate cancer cells and has prognostic value.[271] With mass spectrometry they analyzed blood, urine and tissue samples from different well characterized prostate cancer patients and explored them for metabolites. In a relatively small patient cohort a total of 1126 metabolites were identified. Sarcosine was highly increased during prostate cancer progression to metastasis and could easily be identified in urine.[271] Subsequently they showed a decrease in disease progression when glycin-N-methyltransferase was knocked down.

Although these results look very promising, subsequent reports showed that Sarcosine as prognostic marker is debatable. On tissue samples the expression in cancerous samples was 7% higher compared to benign prostate samples. Unfortunately no statistical differences were seen regarding prostate cancer progression.[272] A drawback of this study was the fact that metastatic samples were not included. Also Sarcosine as a urine marker, normalized to creatinine, could not reproduce the original finding that Sarcosine functions as a prognostic marker. [273] When compared to PSA, urine derived Sarcosine was not able to outperform serum PSA on itself. When added to an algorithm with PCA3 or %fPSA diagnostic performances could be improved.[274]

Although Sarcosine was promoted as a promising new marker for prostate cancer, its exact clinical value and applicability is unclear. The conflicting reports are mostly based on a limited number of samples with limited follow-up and different technologies to

measure this metabolite. In order to resolve these contradictions we need to control some of the variables, such as the study cohort and tumor marker assays.[275]

EXOSOMES

Exosomes are small vesicles (50-150 nm) that are shed by almost all cell types in the human body into almost all body fluids. Initially, exosomes were discovered during studies on the loss of the transferrin receptor loss in sheep reticulocyte maturation.[276] Exosomes are formed by inward budding of the cellular membrane which results in the formation of a large endosome. After formation of the endosome it is subjected to a second step of inward budding. During this second step, cytoplasmic content is taken up in small vesicles. When the endosome (now referred to as multivesicular body) is filled with small vesicles it fuses with the cellular membrane and the small vesicles, or so-called exosomes, are shed in the extracellular space.[277,278] Because of this biogenesis pathway, exosomes contain proteins and RNA that are specific for the cell from which they are derived and thus represent the state of the cell.[279] By isolating prostate (cancer) derived exosomes one is able to search for new and specific tumor markers for prostate cancer. The reports on exosomes in prostate cancer are limited. One of the first clinically related studies showed their potential. The quantity of exosomes isolated from urine is higher in prostate cancer patients as compared to healthy controls.[280] Unfortunately, in this study nothing was reported about differences in exosomal content. RNA expression analysis revealed that known markers of prostate cancer such as the TMPRSS2:ERG fusion mRNA and PSA mRNA could be identified in exosomes.[279] This finding emphasizes their function as tumor marker containing structures.[277,281]

Although the reports are limited, the study populations are very small and the variation in number of exosomes, exosome research in prostate cancer could accelerate tumor marker discovery. Because they are present in body fluids, noninvasive technique can be applied to isolate exosomes and use them for diagnose or monitor the course of prostate cancer.[282] Unfortunately, when isolating exosomes from serum or urine no distinction can be made between the different tissues from which the exosomes are derived. Therefore more research has to be done to specifically isolate and profile prostate (cancer) derived exosomes.

SUMMARY

Currently, PSA is the best and most widely accepted prostate tumor diagnostic and monitoring marker we have available for daily medical practice. Nevertheless, its limita-

tions cause a need for new and more accurate markers. From the many discovery endeavors, there seems to be an inexhaustible source of new potential tumor markers that are being explored. Unfortunately, most of these candidate tumor markers still need to be evaluated more thoroughly to validate their diagnostic or prognostic value and demonstrate their added value over current practice.

Because of the heterogeneity of prostate cancer there is a fairly good chance that the use of single tumor marker will not cover all aspects of the disease and a combination of two or more markers is needed. In addition, multiple markers will be needed to address the different types of relevant clinical decision points, ranging from risk assessment, diagnosis and personalized therapy.[283] Importantly, different technologies including mass spectrometry and microarrays are being introduced into the clinical to measure novel markers and extend the types of markers from the typical proteins to metabolites, DNA and RNA.

Despite the large efforts invested in prostate cancer marker research in the past decade, the number of clinically valuable markers is very limited. We have learned that open and unselective searches in a discovery phase, generally result in many new candidate markers, but also that most of these are not validated in independent and larger cohorts. It has become painfully clear that the complexity of body fluids and tissues, a selection bias and inadequate number of samples for discovery and the variation between individuals are some of the major hurdles in the ongoing quest for novel markers. Despite these challenges, more accurate and reproducible technologies, more focused explorations and the growing number of samples in (consortium) tissue banks, improve the essential steps of excluding false positive candidates in an early stage and robustly validate novel markers.

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