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REVIEW ARTICLE

Tumour markers in prostate cancer II: Diagnostic and prognostic cellular biomarkers

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Abstract

The main goal of prostate cancer tissue biomarkers is to improve diagnostic and prognostic accuracy. A particularly important question is whether the cancer needs immediate treatment or if treatment can be deferred. It is highly unlikely that a single biomarker that provides comprehensive prognostic information about a newly diagnosed prostate cancer will be forthcoming. Despite extensive research efforts, very few biomarkers of prostate cancer have been successfully implemented into clinical practice today. This can be partly explained by a lack of standardised methods for performance and interpretation of immunohistochemistry, but also by poor study design with insufficient biomaterial or inappropriate statistical analysis. Also appropriate cohorts to test prostate cancer biomarkers do not exist. It must be kept in mind that unsuccessful integration of new biomarkers in nomograms can also be explained by the good performance of the clinical and pathological base model with serum PSA as the only independent biomarker. A new biomarker must be powerful enough to improve this prediction model and not merely replace. *Material and methods*. In this report, we focus on diagnostic and prognostic cellular biomarkers in prostate cancer, recent advances and future aspects by reviewing currently available literature. *Results.* Similar to other malignancies, the proliferation marker Ki-67 seems to be a prognostic tissue biomarker and a strong candidate for integration in prediction models. Circulating tumour cells are promising markers of response to treatments in patients with metastatic disease. *Conclusion*. Important technical advances together with histological techniques of antibody or probes conjugated with different fluorophores will certainly improve standardisation and make immunohistochemical biomarker research more reliable and precise in the future. Cellular biomarker studies are also expected to change in the future towards a complexed individualised profiling of human tumours with integrative analysis using different technologies, genome-wide scanning and expression profiling.

A biomarker can be defined as a molecular test to provide additional information over current clinical data. There is a need for biomarkers in prostate cancer for several reasons:

1. to improve cancer detection and staging;
2. to identify subclasses of prostate cancer;
3. to predict outcome after treatment;
4. to select patients for different treatment options.

The introduction of the well established biomarker prostate-specific antigen (PSA) testing has impacted the detection rate of prostate cancer and is responsible for down-staging at diagnosis, with the vast majority (approximately 80%) of newly diagnosed tumours being localised to the prostate. Gleason score and clinical stage at the time of diagnosis are important factors to predict prognosis and outcome after therapy but additional accurate and reliable biomarkers are warranted. An increasing proportion of tumours fall into the category of clinical stage T1c, low Gleason score (6 or less) and PSA of less than 10 ng/ml and may be considered as indolent cancers. Despite extensive research efforts, very few biomarkers of prostate cancer have been successfully implemented and used in clinical practice today. In fact, the only prostate cancer biomarker routinely used in prediction models is PSA in blood.

The search for diagnostic or prognostic tissue biomarkers in prostate cancer was predominantly based on immunohistochemistry and a large number

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of tumour markers with prognostic information were proposed. However, a vast majority of these are not used in clinical practice, probably due to lack of standardised methods to perform and interpret immunohistochemistry, but also due to inadequate study design with insufficient biomaterial and inappropriate end points (e.g., biochemical recurrence instead of death) or misleading statistical analysis. The influence of tumour heterogeneity is yet another unsolved problem, especially when only a small part of the tumour volume is available for examination; i.e., in the case of prostate biopsies. Biomarker studies after radical treatment often lack sufficient end point data and only those from radical prostatectomy have plentiful tissue. Diagnostic tissue specimens are all that is available for those treated by radiotherapy, hormone manipulation or active surveillance. Subsequently, many reports on promising prognostic tissue biomarkers were never successfully reproduced and validation studies are still missing for most prostate cancer tissue biomarkers.

In 2005, the Statistics Subcommittee of the NCI-EORTC Working Group on Cancer Diagnostics encouraged transparent and complete reporting of prognostic tumour marker research by publishing the REMARK document *"Reporting recommendations for tumor MARKer prognostic studies"*, which led to a general reduction in the number of studies describing new immunohistochemical markers [1]. More recently, a systematic and comprehensive review recently focused on the use of classical and novel biomarkers as prognostic risk factors for localised prostate cancer and highlighted the poor quality and heterogeneity of studies, which render much of the results inconclusive [2].

In the era of "omics" (genomics, proteomics, metabolomics, pharmacogenomics, etc.), tissue-based biomarker research has evolved dramatically. Recent advances in high-throughput technology, microarrays and bioinformatics have opened the door to gain much more information from an individual tumour and to develop multiplex tests to obtain a complete characterisation and to predict the behaviour of prostate cancer.

In this report, we focus on a selection of established and promising tissue biomarkers in prostate cancer, recent advances and future aspects. Areas of research based on novel and complexed technology like proteomics and genomics will not be covered in this report.

Diagnostic prostate cancer markers

The diagnosis of prostate cancer in histopathological specimens is mainly based on a combination of architectural and cellular atypia. In the vast majority of cases a confident diagnosis can be made based on morphology alone. However, in some cases the morphological findings are insufficient for a conclusive diagnosis, either because the atypia is too mild or the atypical focus too small. In a review of the literature, atypia suspicious for cancer was found to have been reported in an average of 5% of needle biopsy series [3]. The diagnostic accuracy can be improved in some of these cases by immunohistochemistry using one or several biomarkers, either on consecutive sections or using a staining cocktail.

High molecular weight cytokeratin

Invasive prostate cancer almost invariably lacks basal cell layer. The cytokeratin profile of basal cells of prostatic glandular epithelium differs from that of luminal cells. The basal cell layer can be labelled with antibodies against high molecular weight cytokeratin or cytokeratin 5. In rare cases (0.3%) there is an aberrant labelling of cancer cells by high molecular weight cytokeratin but the band-like basal cell distribution is not seen [4]. More problematic is that benign lesions such as partial atrophy may be negative for high molecular weight cytokeratin [5]. Thus, it is important that results with this biomarker is interpreted with great caution and carefully correlated with conventional morphology.

p63

An alternative to high molecular weight cytokeratin as basal cell marker is p63, a homologue to p53. This biomarker can be used either alone or in a cocktail with high molecular weight cytokeratin [6]. Similar to high molecular weight cytokeratin false negative stains of benign glands occur and correlation with morphology is of paramount importance. Aberrant diffuse expression of p63 in prostate carcinoma has been described [7]. The sensitivity of p63 and high molecular weight cytokeratin is comparable [8].

AMACR

Alfa-methylacyl-CoA-racemase (AMACR) is expressed in cancer and usually negative in benign glands [9]. Staining for AMACR in atypical cases with negative basal cell markers can convert the diagnosis from atypical to cancer in 50% of needle biopsy cases reviewed by an expert uropathologist. The sensitivity varies between 80–100% in small atypical foci on needle biopsy [10]. However, variants of prostate cancer that are more difficult to diagnose are unfortunately less often positive: 62–68% of foamy gland prostate cancers and 70–77% of pseudohyperplastic prostate cancers [11]. AMACR is also positive in PIN and occasionally in benign lesions such as atrophy and adenosis, hence, similar to basal cell markers, the results have to be interpreted with caution and carefully compared with hematoxylin and eosin stained sections.

Prognostic tissue biomarkers

Tissue biomarkers may be of value to predict the effect of certain treatments but also as a general prognostic tool. The natural history of prostate cancer has shown that the molecular determinants may change with time by clonal selection of tumour cell populations towards a more aggressive phenotype. However, we also know that some prostate cancers show an aggressive behaviour already at an early stage and there is an urgent need to identify tumours that need immediate treatment.

Kallikreins

The human tissue kallikrein *KLK* gene locus consists of 15 genes on chromosome 19q13-4 [12]. The best known of these genes is kallikrein 3 (KLK3), also known as prostate-specific antigen (PSA), that is a widely used clinical tumour marker in serum for detection and monitoring of prostate cancer progression. PSA is produced in luminal cells of the benign epithelium and in most tumour cells. Thus it has a prostate-specific but not a tumour specific expression, which explains the low specificity of serum PSA measurement to detect small tumours in patients with benign prostatic hyperplasia (BPH). Human kallikrein type 2 (hK2) is similar to PSA in structure and is overexpressed in tumour cells [13]. In an immunohistochemical study hK2 was expressed in every cancer, and the expression incrementally increased from benign epithelium to primary cancer and lymph node metastases [14]. Lintula et al. demonstrated that the ratio of hK2/PSA mRNA increased with grade of tumour [15]. Using a large tissue microarray (TMA) containing samples of 3 261 prostatectomy specimens Erbersdobler and co-workers showed that the loss of PSA expression in tissue samples of prostate cancer is associated with adverse pathologic features and clinical outcome but is not an independent prognostic factor for PSA recurrence after prostatectomy [16]. Immunohistochemical studies on other kallikrein family members have also been suggested to be of prognostic value in prostate cancer patients but validation studies are still lacking.

Prostate-specific membrane antigen (PSMA)

The prostate specific membrane antigen (PSMA) is a cell surface membrane protein. It is an attractive antigen for antibody-based diagnostic and therapeutic intervention in prostate cancer, since it is highly restricted to the prostate and overexpressed in all tumour stages [17]. In a TMA study, high PSMA expression was independently associated with PSA recurrence in a high-risk cohort and thus might provide insight into the additional use of adjuvant therapy in this group of patients [18]. Again, validation on other cohorts is required and further evaluation of this marker is needed to determine whether or not it has clinical utility for prostate cancer detection or treatment monitoring.

Ki-67

The Ki-67 protein is well known and widely used to assess the tumour proliferation rate and numerous studies have shown Ki-67 to be a prognostic marker in prostate cancer patients treated by radical prostatectomy, radiotherapy or androgen deprivation therapy (ADT). In a cohort of 808 patients diagnosed by TURP treated conservatively, Ki-67 expression was assessed immunohistochemically in two laboratories, by two different scoring methods and the results compared with cancer-specific and overall survival [19]. Using both methods, Ki-67 provided additional prognostic information beyond that available from Gleason score, PSA and tumour extent. Although it was confirmed to be the most promising biomarker, confirmatory studies need to occur in TRUS biopsy material in order for prospective studies to implement use into routine practice and prediction models.

Androgen receptor

Expression of kallikreins and several other gene products in the benign and malignant prostate gland are dependent on the androgen receptor (AR) function. Studies in animals and humans have demonstrated that AR plays a key role in prostate cancer progression with an overexpression in castration-resistant prostate cancer (CRPC) [20]. However, expression of AR may be of prognostic value also in hormone-naïve prostate cancer. Using a total of 640 cases in a TMA setting, Li et al. correlated levels of AR expression with some well-established clinical and pathologic parameters in patients treated with radical prostatectomy [21]. High levels of AR status correlated with proliferation (high Ki-67 index) and a high level of AR expression was an independent predictor of decreased biochemical recurrence-free survival. The prognostic role of AR in prostate cancer was recently confirmed in a study from an independent group who described that increased nuclear AR expression in either the diagnostic biopsy and/or radical prostatectomy specimen, from patients with advanced disease, was associated with a reduced time to prostate cancer-specific mortality [22]. Simple immunohistochemical analysis of AR may not be sufficient to evaluate AR function, because tumour cells may harbour differences in the AR genotype with variation in the length of CAG repeat, mutations and increased copy number.

Gene fusions

The bioinformatic tool Cancer Outlier Profile Analysis (COPA) was developed to analyse DNA microarray data for outlier genes and used when they presented the first evidence of current rearrangements in common epithelial tumours as demonstrated by fluorescent *in situ* hybridisation (FISH) [23]. Gene fusions involving members of the erythroblast transformation-specific (ETS) family of transcription factors were reported to occur in prostate cancer and with the gene fusion of the 5′-untranslated region of the androgen-regulated gene TMPRSS2 and the ETS family gene ERG as the predominant finding (approximately 90%). Several studies have confirmed the presence of TMPRSS2–ERG fusions in 36–78% of prostate cancers from PSA screened surgical cohorts. Conflicting results have been reported regarding the prognostic significance of prostate cancers harbouring TMPRSS2-ETS gene fusions. Duplication of the fusion TMPRSS2 to ERG sequences was shown to identify fatal human prostate cancer in a cohort on 445 men conservatively treated for prostate cancer [24] but in a recent TMA study of 521 cases of clinically localised prostate cancer, Fine and co-workers found that TMPRSS2–ERG fusion was associated with low Gleason scores and not with high-grade morphological features [25]. Taken together, is seems like the TMPRSS2–ERG fusion gene is an early event related to development of prostate cancer rather than a marker for progressive disease. Duplication of the gene may be merely an indicator of overall genetic instability, aneuploidy and hence, poor prognosis. Several strategies for therapeutically targeting ETS fusions have been identified and are currently being pursued.

PTEN

The phosphatidylinositol 3-kinase (PI3K) is a frequently activated signal transduction pathway in prostate cancer cells [26] and the most common mechanism of PI3K activation is deletion of the gene encoding the phosphatase and tensin homologue (PTEN) protein. PTEN deletion has been reported to be more frequent in metastatic than in organ-confined prostate cancer [27]. Decreased expression of PTEN and consequent activation of the PI3K pathway members in prostate cancer tissue samples has been correlated to higher Gleason grade, advanced stage, and development of androgen resistance [28,29]. These discoveries have already been shown to have clinical impact and several phase 1 and 2 clinical trials with specific agents targeting key activated proteins in the PI3K pathway are ongoing in prostate cancer patients [30]. A recent finding that may have impact on future studies is that PTEN deletion, cooperates with aberrant ERG expression to promote prostate cancer progression [31]. Use of molecular biomarkers related to the PI3K pathway, may facilitate future selection of patients for these novel therapeutic regimens.

p53

The tumour suppressor gene p53 is mutated in half of human malignancies and has been intensively studied in numerous cancer models. Many of these studies have suggested that p53 may be of prognostic value in prostate cancer after different treatments. In a more recent study on patients treated conservatively for prostate cancer, p53 remained prognostically significant in a multivariate model though the risk ratios were less strong compared to Ki-67 [32].

SPINK1/TATI

Tumour-associated trypsin inhibitor (TATI), which is alternatively called pancreatic secretory trypsin inhibitor (PSTI) or serine protease inhibitor Kazaltype 1 (SPINK1), is expressed in various normal and malignant tissues, and is known as a prognostic tumour marker as reviewed by Paju and Stenman [33]. TATI was first shown to be overexpressed in high-grade prostate cancer [34] and later, outlier expression of *SPINK1,* the gene coding for the TATI protein was identified exclusively in a subset of ETS rearrangement-negative cancers (approximately 10% of total cases) [35]. A series of *in vitro* and *in vivo* experiments revealed *SPINK1*/TATI to be associated with prostate cancer aggressiveness and with invasive growth in a prostate cancer cell line (22RV1) with outlier expression. It was thus shown that *SPINK1* outlier expression defines an aggressive molecular subtype of prostate cancer (approximately 10% of cases) not attributable to known gene fusion events. Further support of the potential role of *SPINK1* expression as a prognostic biomarker was recently demonstrated by Leinonen et al. who studied a cohort of men with endocrine-treated prostate cancer [36]. Additional work is needed to understand the tumour biology behind the role of *SPINK1*/TATI as a tissue biomarker in prostate cancer.

MSMB and EZH2

MSMB (also known as prostate specific protein of 94 amino acids (PSP94)) is expressed in benign and malignant prostatic epithelium and is along with prostate specific antigen (PSA) and prostate acidic phosphatase (PAP) one of the three most abundant proteins in human seminal plasma [37]. Additionally, *MSMB*, located at chromosome 10q11.2, has recently been reported as an important candidate gene for prostate

cancer susceptibility [38,39]. Several groups have employed a variety of approaches in both tissue and serum samples to demonstrate decreasing levels of MSMB in prostate cancer compared to normal controls, prompting the suggestion that MSMB may be a promising biomarker for prostate cancer. In a large TMA study of almost 1 000 patients with localised prostate cancer, MSMB was found to be an independent predictor of recurrence after radical prostatectomy, however, addition of MSMB did not importantly improve the performance of existing predictive models [40]. Molecular mechanisms behind the decreased expression of MSMB in prostate cancer is not yet fully clarified. In advanced, castration-resistant prostate cancer decrease expression of MSMB was found to correlate with an increased expression of the Polycomb gene (PcG) protein EZH2 (enhancer of zeste homolog 2), which represses transcription via trimethylation of histone H3 on Lys27 (H3K27) [41]. EZH2 has also been attributed the role of a useful tissue biomarker in prostate cancer [42]. Furthermore, EZH2 has also been suggested to silence expression of E-cadherin by trimethylation of H3 lysine 27, providing a functional link between dysregulation of EZH2 and repression of E-cadherin during cancer progression.

Epigenetic silencing of genes by EZH2 and activation of members of the PcG group may be common oncogenic events in pathogenesis of metastatic solid tumours and provide justification for development of small molecule inhibitors of the PcG chromatin silencing pathway as a novel therapeutic modality for treatment of metastatic prostate cancer [43].

Heat shock proteins

Heat shock proteins (HSPs) are molecular chaperones, protecting cells against stress-related injury [44]. Although HSPs are important for the function of normal cells, cancer cells frequently overexpress HSPs in response to stress and may thereby gain a survival benefit. It has been demonstrated by proteomics that prostate cancer overexpress HSP60 and HSP70 [45]. In a validation study using immunohistochemistry, HSP60 was an independent predictor of biochemical recurrence after radical prostatectomy in multivariate analysis including extraprostatic extension, margin status, seminal vesicle invasion and Gleason score, while HSP27 correlated with outcome in univariate analysis [46]. Interestingly, HSPs are potential therapeutic targets in prostate cancer patients. Inhibition of the function of HSPs may decrease tumour cell survival by blocking the antiapoptotic activity of HSPs [47]. HSP90 is suggested to play a key role in prostate cancer through interaction with AR and recently designed mitochondrial Hsp90 chaperones are potentially attractive therapeutic targets in advanced prostate cancer [48].

DNA methylation

Epigenetic events that can affect gene expression without altering the actual sequence of DNA include phenomena such as DNA methylation, chromatin remodeling, histone modification and RNA interference [49]. Many gene promoters are associated with GC rich regions of the DNA known as CpG islands. Abnormal methylation of CpG islands located within gene promoters is associated with decreased transcriptional activity and it occurs in many types of cancers. Abnormal methylation of genes such as those involved with control of cellular growth or detoxification is believed to have a critical role in early stages of PCa progression. Already in 1994, glutathione S-transferase P (GSTP1) hypermethylation was introduced as a central part of prostate carcinogenesis [50]. GSTP1 is a member of a large family of glutathione transferases that function to protect cells from oxidative insult thus, the biological rationale for selecting this marker is its role in preventing damage to cells by neutralising free radicals. GSTP1 has been extensively studied in prostate cancer, and its reduced expression, due predominantly to promotor hypermethylation, is the most common epigenetic alteration associated with this disease. Several studies have shown a high sensitivity for this marker to detect the presence of both PIN and prostate cancer, an ability to distinguish these from BPH, and a prevalence of methylation in the range of 60–80% in prostate cancer. Strengths of GSTP1 as a clinical marker are the ability to quantitate the methylation status of the *GSTP1* gene in biopsy/prostatectomy tissues and in cells derived from serum, urine, and seminal plasma. Recent studies using quantitative real-time methylation sensitive PCR demonstrate that epigenetically modified genes are candidate markers for early detection and post-treatment monitoring of prostate cancer, however it is more likely that urine and serum will be used instead of tissue samples. This role of hypermethylated genes as promising biomarkers was recently reviewed by Ahmed who emphasised that in addition to clinical validation, assays for methylated genes must be robust, simple, sensitive, specific, and made available at affordable costs [51].

HER2

Overexpression, or gene amplification of the human epidermal growth factor receptor 2 (HER2) is evident in 20–25% of breast cancers and correlated with disease outcome. Validated methods for determination of HER2-status by immunohistochemistry and/or FISH are used to identify patients with breast cancer who are eligible for treatment with trastuzumab, an HER2-targeted monoclonal antibody that inhibits the proliferation of tumour cells and induces tumour

cell death through multiple mechanisms. HER2 has also been linked to the clinical progression of CRPC. In a recent meta-analysis, Serpa Neto and collaborators [52] investigated the prognostic impact of HER2 over expression in patients with prostate cancer and its correlation with other pathological and clinical variables. Several databases were searched for studies of HER2 protein expression in primary prostate cancer tissue. The overall relative risk of death in those with HER2 over expression in the primary tumour was 1.63 (95% CI 1.47–1.82, $p<0.0001$) and the authors found a consistent association of HER2 overexpression with death and recurrence. The clinical usefulness of HER2-status in prostate cancer patients still has to be proven.

NKX3.1 and MYC

Losses of 8p and gains of 8q are two of the most common chromosome aberrations in prostate cancer [53]. Gene products related to these loci are candidates as new important biomarkers in prostate cancer. NKX3.1 (8p21) is an androgen-regulated transcription factor that regulates the proliferation rate of prostate luminal epithelial cells and functions as a tumour suppressor gene [54]. Experimental work from different groups shave demonstrated the importance of NKX3.1 in development of prostate cancer, but studies on its role as a tissue biomarker have generated conflicting results.

The well-known oncogene MYC resides on the short arm of chromosome 8. MYC is a well-known regulator of proliferation and biologic activity in prostate cancer cells, and its amplification is associated with the presence of PIN and poor clinical outcome of prostate cancer [55]. Similar to NKX3.1, additional studies are needed to understand how MYC can be clinically used as a tissue biomarker in prostate cancer.

Validation and implementation of tissue biomarkers

Examples of statistical models to predict outcome in prostate cancer patients are the clinical nomograms (www.nomograms.org) [56]. These models incorporate known prognostic variables, such as PSA, Gleason grade, extracapsular extension, lymph node involvement, and surgical margins to estimate the probability of disease recurrence following prostatectomy and other treatments. Unsuccessful integration of new biomarkers in nomograms can be explained by the already high performance of the clinical and pathological base models with strong independent biomarkers. Several questions must be satisfactorily met before a new biomarker can be implemented:

1. Can the marker be measured accurately and reproducibly? The value of each biomarker must be tested in large annotated series of patients with reliable independent validation sets. The assay must be robust and reproducible.
2. *Does the marker provide additional information to that already available to the clinician?* An important step in evaluating the value of a new marker is to compare the predictive accuracy of a model including only standard clinical variables with that of a model including standard clinical variables plus the new marker [57]. The statistical analyses must be robust and demonstrate a significantly improvement in receiver operation characteristics (ROC) analyses or Concordance Index (CI) when the new biomarkers is added to the base model.
3. *Is the marker associated with outcome in the sort of patients to whom the marker would be applied to in clinical practice?* Before we use a marker in clinical practice, we need to know whether it will improve clinical outcome. In other words, we need evidence that measuring the marker would change the decision a doctor would have made in the absence of the marker, and that this changed decision will benefit the patient. If an assay failed to give reproducible results, it would clearly be of little clinical value and would not be worthy of subsequent research.

Conclusions and future aspects

It is unlikely that a single biomarker will provide all information we need to tell how aggressive a newly diagnosed prostate cancer is. No immunochemical, or genetic marker is currently used to differentiate between aggressive and non-aggressive prostate cancers.

Recent discoveries in genetics, proteomics and bioinformatics using new high-throughput arrays indicate that new multiplex tests will soon be launched and some even commercially available, however, the above mentioned criteria for evaluation of new biomarkers must always be strictly followed. Tissue biomarker research will change in the future as illustrated in a recent report by Taylor and co-workers who used different new technologies for integrative genomic profiling of human prostate cancer and identified new candidate biomarkers in prostate cancer [58]. Technologies of genome-wide scanning such as gene expression profiling, comparative genomic hybridisation (CGH), and single nucleotide polymorphism (SNP) arrays are now also applicable to nucleic acids extracted from archival tissues on large series of prostate cancer patients.

Development of TMA for large-scale analysis and automated image analysis systems for more precise quantitation and a direct transformation of scoring data to biostatistical analysis are now progressively replacing the subjective, semiquantitative manual scoring previously performed by pathologist. These important technical advances together with histological techniques of antibody or probes conjugated with nanoparticles (quantum dots) and other fluorescent conjugates will certainly improve the standardisation and make immunohistochemical biomarker research more reliable and precise in the future.

Another challenge is to identify a specific tissue biomarker for prostate cancer stem cells. Today it seems like a combination of different markers is needed to identify a cancer stem cell population. Uglokov et al. [59] performed immunohistochemical analysis of proposed stem cell markers (CD44, CD133, Oct4, SOX2 and EZH2) in benign and malignant prostatic tissues and suggested that suggest that combined expression of embryonic stem cell markers EZH2 and SOX2 might identify potential cancer stem cells as a minor (<10%) subgroup in CD44+ prostatic adenocarcinoma. A new and different approach is aldehyde-dehydrogenase (ALDH) – based sorting of human prostate cells by flow cytometry (ALDEFLUOR assay) which is used to simultaneously select putative prostate cancer stem cells and metastasis-initiating cells [60]. Analysis of ALDH activity of clinical prostate cancer samples may thus become useful for the stratification of prostate cancer patients at risk of developing metastatic disease.

An important question that remains to be answered is the cellular origin of human prostate cancer. Using a combination of immunohistochemical and biochemical studies *in vitro* and *in vivo,* an American group recently provided evidence that prostate tumours originate from the basal cell layer and not from the luminal cell compartment as earlier believed [61]. Their discoveries may impact future strategies in search for new prostate cancer biomarkers.

Another complex but highly interesting area of research with great expectations in the near future relates to microRNA and other forms of non-coding RNAs which hypothetically can act as important regulators of other biomarkers, but can also function as biomarkers themselves and as therapeutical targets [62].

Circulating tumour cells (CTC)

The shedding of tumour cells into the circulation is a necessary condition for metastatic spread. Recently, the enumeration of circulating tumour cells (CTC) using the CellSearch™ system which has been cleared by the US Food and Drug Administration as a prognostic clinical biomarker that can be used to monitor the effectiveness of therapy in patients with metastatic prostate cancer [63]. Although changes in CTC counts over time are predictive of survival, it is the molecular profiling of these cells that offers insight into the biological status of the tumour. Effective molecular profiling of CTC is complicated by the relatively low cell numbers involved and by dilution from non-tumour material because the current enrichment procedures cannot eliminate all contaminating leukocytes. Further, as patients with advanced disease may shed tumour cells from multiple sites, the CTC detected may have diverse characteristics, further diluting potential molecular biomarkers and complicating their predictive utility. In a study of 77 patients with progressive castration-resistant disease, Scher and his group have shown that CTC numbers can be monitored in a routine clinical laboratory setting and that cells confirmed by immunohistochemistry to have features of prostate cancer can be sampled for genetic profiling by FISH [64]. FISH could be done on CTC (success rate >87%) supporting its use in the routine management of progressive castration-resistant prostate cancer [65]. The CTC technology is currently evaluated and validated in clinical trials as a predictor and surrogate end point of treatment response.

In conclusion, the search for new cellular biomarkers in prostate cancer has resulted in a proliferation of tumour marker studies. In order for markers to be successfully incorporated into clinical practice, we need evidence that measuring the marker would change the decision a doctor would have made in the absence of the marker, and that this changed decision will benefit the patient.

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