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Blood and tissue biomarkers in prostate cancer: state of the art

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The prevalence of prostate cancer (PCa) is high and increases with age. It is the most common non-cutaneous cancer in American men with and estimate of 192,280 new cases diagnosed in 2009 but with only 27,360 deaths, and a leading cause of male cancer-related death, second only to lung cancer, representing 10% of all cancer deaths in men in the United States (http://www.cancer.org/docroot/STT/STT_0.asp).

Multiple factors contribute to the high incidence and prevalence of PCa. Risk factors include age, family history, environmental exposures and race. Prostate Specific Antigen (PSA) screening has impacted the detection of PCa and is directly responsible for a dramatic decrease in stage at diagnosis, with over 80% of PCa being localized to the prostate. Gleason's score and stage at the time of diagnosis, remain the mainstay to predict prognosis, in the absence of more accurate and reliable tissue or blood biomarkers.[1] Because of PSA screening, however, these parameters are beginning to lose discriminatory power for patients with organ confined disease and intermediate tumor differentiation. As a result, we are over treating most men diagnosed today in this post-PSA screening era and inadequately treating those men with the most aggressive form of the disease-metastatic PCA.

Despite extensive research efforts, very few biomarkers of PCa have been introduced to date in clinical practice. Recently, even screening with PSA has been questioned.[2,3]

In the '80s and '90s the search for diagnostic or prognostic markers in human solid tumors focused predominantly on immunohistochemical markers. The vast majority of these, however, never found a clinical application probably as a result of underpowered databases, lack of validation sets, inappropriate endpoints (e.g. PSA failure instead of death) or to the introduction confounders in statistical analyses such as inappropriate case selection or too many biomarkers analyzed at once. The application from 2005 by the major biomedical journals of the REMARK recommendations for reporting tumor marker studies has radically changed this approach leading to a general reduction in the number of studies describing immunohistochemical markers.[4] At the same time, a steady increase in the number of studies dealing with prognostic or diagnostic circulating blood/serum tumor biomarkers was observed reflecting the need for new and non-invasive tests to predict the behavior of PCa. A thorough analysis of all tissue and serum biomarkers in the field of prostate cancer cannot

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be easily synthesized and goes beyond the scope of the present review. Therefore we decided here to focus on the most recently reported tissue and circulating biomarkers for PCa whose application in the clinical practice is either current or expected in the next future.

TISSUE BIOMARKERS

The field of immunohistochemical biomarker in solid tumors has rapidly progressed alongside the development of the tissue microarray (TMA) and imaging technologies. The use of TMA allows the simultaneous analysis of hundreds of cases resulting in a more uniform staining procedure while reducing costs. [5] The development of automated image analysis systems with high throughput and more precise quantitation is now progressively replacing the subjective, semi-quantitative manual scoring previously performed by pathologists. Computerized image analysis allows the translatation of the number of tumor cells expressing the biomarker and its intensity of immuno-staining into numerical data, more amenable to large scale bioinformatic analyses.[6] Furthermore, the application to in situ histological techniques of antibody and/or probes conjugated with nanoparticles (quantum dots) permits the simultaneous use of multiple biomarkers in tissue or TMA sections.[7] These important technical advances have recently made immunohistochemical biomarkers more reliable and quantifiable in most solid tumors including PCa. The TMA technology also allows to ascertain the power of single biomarkers in much larger series of formalin-fixed, paraffin-embedded (FFPE) archival tumor samples. The application of tissue biomarkers to the largest cohorts of PCa patients traditionally studied by epidemiologists, has led to the establishment of the new discipline of patho-epidemiology. Patho-epidemiology combines the information from tumor registries and cancer statistics to the knowledge of morphology, biomarker expression and molecular genetics. The access to these well annotated cohorts of patients is particularly valuable in the field of PCa where patients must be followed for decades rather than years in order to obtain meaningful prognostic information.

Technologies of genome wide scanning like gene-expression profiling, comparative genomic hybridization (CGH) and single nucleotide polymorphism (SNP) arrays are now also applicable to nucleic acids extracted from archival tissues.[8] Genome-wide analyses are now able to quickly unravel expression signatures, specific genomic losses or gains and the activation or silencing of entire cell pathways on large series of PCa patients.

In order to provide a panoramic view of the most promising tissue biomarkers we will describe the immunohistochemical prognostic markers and targets of therapy belonging to the following broad categories: biomarkers of pathway activation, chromosome 8 aberrations, TMPRSS2-ERG fusion, tumor metabolism, stem cell markers and SNP analysis/gene expression profiling/genomic comparative hybridization.

Pathway activation markers

Signal trafficking regulation through major cell hubs is central in the processes of tumor initiation and progression and represents a major target for tumor specific therapies. In prostate adenocarcinoma, the phosphatidylinosytol 3-kinase (PI3K) is the most frequently activated signal transduction pathway.[9] In contrast, aberrant activation of the MAPK pathway seems less frequently involved possibly due to absence of Ras and Raf mutation.[10] In PCa the most common mechanism of PI3K pathway activation is the deletion of the gene encoding the phosphatase and tensin homolog (PTEN) protein, whose inactivation in turns leads to phosphorylations of AKT and of the S6 ribosomal subunit, which are therefore powerful biomarkers of PI3K activation.[11] Up-regulation of the PI3K/AKT/mTOR pathway is estimated to occur in about 50% of PCa often, but not only through loss of PTEN. In addition, PTEN deletions appear to be more frequent in metastatic than in organ-confined disease.[12,13] Immunohistochemistry can easily detect the activation of the major

components of the PI3K pathway using antibodies against specific phosphorylation sites of the AKT, mTOR and S6 proteins. Decreased expression of PTEN can be identified by immunohistochemistry as well, while its genetic inactivation may be determined by FISH or by genome-wide analyses such as aCGH. In humans, down-regulation of PTEN and consequent activation of the PI3K pathway members in PCa tissue samples has been correlated to higher Gleason grade, advanced stage and development of androgen resistance.[11,14,15] Immunohistochemical detection of phospho-AKT in PCa tissue also predicts early biochemical recurrence and poor outcome.[16,17] Interestingly, in mice, inhibition of mTOR with rapamycin analogues results in complete reversal of the neoplastic prostate phenotype driven by activated AKT1.[18] Several phase I and II clinical trials with specific agents targeting key activated proteins in the PI3K pathway are ongoing in PCa patients, including selective inhibitors of p110, AKT1 and mTOR.[19] The TMPRSS2-ERG translocation (see below) is found in upwards of 60% of prostate cancers and is androgen-driven.[20] Since this translocation results in similar downstream effects to MAPK activation, PCa displaying the fusion may be considered MAPK active. The combination of androgen blockade with tyrosine kinase inhibitors targeting the PI3K and/or the MAPK pathway may represent alternative therapeutic choices in PCa. Selection of patients for these therapeutic regimens becomes therefore very important. This can only occur through the use of selective biomarkers.

Chromosome 8 aberrations and MYC

Losses of 8p and gains of 8q represent two of the most common chromosome aberrations in PCa.[21] In particular, the 8p locus appears to be the most commonly deleted region in PCa, occurring in 30% of organ confined tumors and in about half of advanced cases. Single genomic losses at 8p represent early events in prostate carcinogenesis as demonstrated by the occurrence of frequent 8p deletions in experimental prostate intraepithelial neoplasia (PIN).[22] In humans, most PIN lesions are also associated with loss of heterozygosity at chromosome 8p21, where the *NKX3.1* gene, encoding a validated tumor suppressor gene, resides. *NKX3.1* is an androgen-regulated homeodomain transcription factor that regulates the proliferation rate of prostate luminal epithelial cells.[23,24] The development of PIN lesions in NKX3.1 deficient mice has been recently associated with alterations of the PTEN/AKT axis.[25] Haploinsufficiency of NKX3.1 might extend the proliferative stage of regenerating luminal cells leading to epithelial hyperplasia and dysplasia. NKX3.1 has been utilized as a biomarker with conflicting results to date.[26]

On the other hand, 8q is the most commonly gained region in advanced PCa.[27] Genetic polymorphisms at 8q are consistently associated with prostate cancer risk across multiple ethnic groups with the highest susceptibility at the 8q24 region.[28] Specific genetic variants at 8q24 have also been correlated with higher Gleason grade and more aggressive prostate cancer behavior.[29] The coding region closest to 8q24 is the well known oncogene MYC. Although many attempts have been made to correlate polymorphism at 8q24 with MYC up-regulation none to date clearly demonstrated a relationship with MYC increased transcription.[30] MYC is a well known regulator of proliferation and biological activity in prostate cancer cells and its amplification is associated with the presence of PIN and poor clinical outcome of PCa.[31] In addition, transgenic expression of MYC results in PIN as well as invasive cancers in the prostate.[32] Nuclear overexpression of the MYC protein has been shown in PIN suggesting a role for MYC in early prostate carcinogenesis.[33]

TMPRSS2-ERG fusion

One of the most important recent finding in PCa is the discovery of the TMPRSS2-ERG fusion. The fusion of the 5′ untranslated region of the androgen-regulated TMPRSS2 gene with the transcription factor ERG leads to the aberrant expression of ERG in an androgen

dependent manner.[34] TMPRSS2-ERG gene fusion is rare in normal prostate tissue while is consistently detectable in PIN, in organ confined as well as in metastatic hormone refractory PCa.[35] The presence of the TMPRSS2-ERG fusion has been associated, in some studies, with high tumor stage, presence of lymph-node metastases and poor outcome.[36,37] These reports, together with the finding that the fusion occurs in both PIN and adjacent invasive cancer cells apparently as an “all or none” phenomenon in individual invasive clones within the same prostate, led to the hypothesis that the TMPRSS2-ERG rearrangement might define, from the early steps of carcinogenesis, a subset of more aggressive PCa. Unfortunately, attempts to correlate the fusion status with high Gleason score or with outcome, have given conflicting results in subsequent studies.[38,39,40] This issue is further complicated by the presence of a growing number of different translocations involving the ETS transcription factor family.[41] Further work is required to understand the role that this prevalent translocation plays in prostatic carcinogenesis and its relationship to the biologic behavior of prostate adenocarcinoma.

Biomarkers of altered lipid metabolism in prostate cancer

More than 80 years ago the Nobel prize winner Otto Warburg proposed that tumorigenicity of cancer cells derived from their ability to switch from oxidative phosphorylation to glycolysis in order to satisfy their high energetic needs.[42] The products of glycolytic pathway and of the subverted metabolic pathways may provide the substrate for the structural need in the tumor cell enhanced proliferative state. Lipogenesis is therefore a distinctive feature of tumor cells.[43,44,45] Cancer cells synthesize *de novo* large amounts of fatty acids and cholesterol irrespective of the circulating lipid levels, and benefit from this increased lipid synthesis in terms of growth advantage, self-survival and drug resistance. Numerous studies have shown that inactivation of most lipogenic enzymes, such as ATP citrate lyase (ACL), fatty acid synthase (FASN) and acetyl-CoA carboxylase, results in either cytostatic or cytotoxic effects in tumor cells.[46,47,48] Cholesterol is critical for the composition and stabilization of cell membranes while fatty acids may also be required for post-transcriptional regulation of key signal transduction proteins through post-translational modifications such as palmitoylation and miristoylation. Fatty acid synthase (FASN) is the only enzyme that is able to synthesize fatty acids de novo in normal and cancer cells and its main enzymatic product palmitic acid is responsible for the acylation (palmytoilation) of key regulatory switches in most signal transduction pathways. FASN was proposed as the first *bona fide* candidate metabolic oncogene in the prostate.[49] The mere forced expression of FASN is able to transform immortalized prostate epithelial cells, to form invasive tumors in immunodeficient mice while transgenic mice expressing FASN in the prostate develop PIN.[50] The proposed mechanisms of FASN oncogenicity include structural needs of synthesized lipids, protection from ER stress, inhibition of the intrinsic (mitochondrial) pathway of apoptosis. and palmitoylation of Wnt-1 with subsequent stabilization of β-catenin and activation of the pathway.[50,51,52] Natural and irreversible inhibitors of FASN are able to reduce cell proliferation and induce cell-death in PCa cell-lines and reduce the volume of PCa xenograft tumors in mice.[53,54] FASN can be therefore considered an excellent and promising biomarker and therapeutic target in prostate cancer.

Caveolae are plasma membrane microdomains rich in cholesterol, similar to lipid rafts but characterized by the presence of a protein family named caveolins. Both lipid rafts and caveolae are cholesterol- and sphingolipid-enriched microdomains in cell membranes that regulate phosphorylation cascades originating from membrane-bound proteins. Altered Cholesterol synthesis results in changes in membrane cholesterol and, in turn, in Akt signaling in both normal and malignant cells.[55] Caveolins possess a key regulatory activity in cell molecular transport and cell trafficking. The most studied member of this family, Caveolin-1, has been involved in prostate cancer initiation and progression and identified as

a marker of aggressive PCa. Caveolin-1 knockout in the PCa TRAMP mouse model significantly reduces prostate tumor size and the development of metastases.[56] This effect might be at least in part mediated by FASN which is downstream of Caveolin-1.[57] Caveolin 1 may therefore be an important biomarker of aggressive behaviour in prostate cancer.

Stem cells markers

Identification and isolation of Cancer Stem Cells (CSCs) in solid tumors, including PCa, has been an area of intense research efforts in the last ten years. Growing literature supports the CSCs hypothesis as a model to explain intratumoral heterogeneity and the development of resistance to therapy.[58]

CSCs (also known as tumor-initiating cells, TICs) are characterized by unlimited renewal potential when injected in immunodeficient mice in which neoplasms recapitulating the heterogeneity of the original tumor form at high efficiency.[59,60] CSC phenotype is usually defined by a panel of multiple rather than single surface markers. In PCa these cells have been identified as $CD44^+/\alpha2\beta1^{high}/CD133^+$ and androgen receptor negative.[61] This phenotype was widely employed to select and isolate putative CSCs from prostate cell-lines, xenografts and biopsies of human tumors in order to test their invasive property and to identify a specific CSC genetic signature.[62] $CD44^+/CD24^-$ cells isolated from LNCaP cells possess increased clonogenic properties, form tumors in NOD/SCID mice and show a gene signature of invasiveness originally identified in breast tumors.[63,64] There is also experimental evidence that a subpopulation of $CD44^+$ CSC-like cells is able to invade Matrigel suggesting that basement membrane invasion is a characteristic trait of these putative prostate CSCs.[65] Intriguingly, in human PCa tissues all the cells with neuroendocrine differentiation are $CD44^+$ suggesting a role for such cells in the resistance to androgen therapy and tumor recurrence.[66] Additional studies are required to better identify putative prostate CSCs in PCa tissue samples. To our knowledge there are no conclusive studies that have characterized the molecular phenotype and the clinical significance of CSCs in PCa patients. Based on the current knowledge it seems most plausible that prostate CSCs originate from cells in different stages of prostate epithelial cell differentiation, providing an alternative explanation for the well-known morphological and biological heterogeneity of human PCa.[67] Reliable recognition of prostate CSCs and development of novel markers for their identification might help the development of therapies specifically targeted at the CSCs compartment.

SNP analysis, gene expression profiling, comparative genomic hybridization

Single nucleotide polymorphism (SNP) array technology allows genome-wide and high throughput analysis of DNA polymorphisms with the intent of comparing different cancers, exploring tumor-normal differences and assessing polymorphic alleles with predictive behaviour. It is therefore the method of choice to assess genetic variants and allelic imbalances. Although in the prostate no susceptibility genes comparable to BRCA1 in breast or APC in colon have been discovered so far, multiple germ-line polymorphisms have been correlated to increased individual risk of developing PCa.[68] In addition to susceptibility studies, SNP arrays have been utilized to distinguish genetic subsets of PCa with different clinical behavior.[69] Progression of PCa towards metastasis and high Gleason grade in the primary tumor were recently correlated to gains of 8q, 1q, 3q and 7q, while androgen ablation therapy was associated with gains at 2p and 10q using SNPs array analysis.[70] In another large control study based on SNP arrays germ-line deletion at 2p24.3 was strongly associated with aggressive PCa.[71] Germline polymorphisms within genes encoding for androgen metabolizing proteins have been also correlated to response rate in PCa patients treated with hormone therapy.[72]

Array comparative genomic hybridization (aCGH) is the best technology to detect DNA copy number alterations in cancer compared to normal tissues.[73] Deletions in 8p, 13q, 6q and gains in 8q and 7q and specific losses at 10q24 (PTEN) and gains at Xq12 (AR) are the most common copy number alterations in PCa. Loss of 8p and gain of 11q13 have been associated with advanced stage and biochemical recurrence, respectively.[74] Interestingly, intermediate risk PCa was characterized by copy number alterations previously associated with high-risk disease.[75] The progressive optimization of the aCGH on DNA extracted from FFPE tissues will extend the applications of this technique with further expansion in the clinical practice.[76]

Gene expression profiling of up to 26K genes is now available also for RNA extracted from FFPE tissues.[77] Expression based models have been correlated to patient outcome and to biochemical recurrence in PCa.[79,80] A case control study comparing men who just had PSA failure with those who developed metastatic PCa after radical prostatectomy showed that the two groups could be separated by an expression model containing 17 genes with a specific enrichment in the 8q24 locus.[81] Unfortunately, the high number of genes utilized in constructing these predictive models and the variable cut-offs employed to estimate the signal to noise ratio in each model, together with the complex bioinformatic algorithms required to deconvolute and interpret the data, have prevented the routine use of gene expression analyses in clinical practice although these may become useful adjuncts to the commonly utilized Partin tables or the Kattan nomograms.[8] Once the RNA extraction techniques from FFPE tissues will be optimized and the relevant gene signatures applied, this technology will likely become necessary for stratification, prognostication and assessment of predictive behavior in PCa patients.

BLOOD BIOMARKERS

The focus of molecular diagnostics is rapidly moving from tissues to bodily fluids and particularly to blood. Detection of biomarkers with diagnostic and prognostic significance in bodily fluids may provide in the near future valuable clinical information while avoiding unnecessary invasive procedures. The current technological advances allow reliable extraction and separation of circulating tumor cells (CTC) in the blood and of tumor-derived nucleic acids and proteins in plasma, urine, sputum and stools. Enhanced tumor invasion during the metastatic process, active secretion of proteins by highly vascularised tumors or passive release of cellular breakdown materials in areas of necrosis could all result in tumor-specific nucleic acid or protein material in the bloodstream or in other fluids. PCa is relatively poorly vascularised with little or no necrotic areas. Therefore, the presence of tumor cells or tumor-derived material in the blood of PCa patients probably reflects increased tumor invasiveness. Tumor specific DNA mutations, genomic instability and epigenetic changes, detection of aberrant proteins, exploration of tumor-specific transcriptome signatures and microRNA expression profiles may all represent valuable biomarkers that can be found in bodily fluids in cancer patients.

While we will not address the paramount role that prostate specific antigen (PSA) plays as a serum biomarker, we will focus on two intriguing and novel blood biomarkers in PCa: the detection and characterization of the free tumor plasma DNA (FPDNA) and the isolation of CTC.

Free Plasma DNA

It has been known from the 50’s that free plasma DNA (FPDNA) circulates in the blood of normal individuals while increases in FPDNA may occur in pathological conditions including autoimmune diseases, liver cirrhosis, major traumas and malignant tumors.[82,83] The detection of circulating free plasma DNA appears to be a promising and non-invasive

test for early tumor detection, assessment of disease recurrence, and monitoring of therapy.[84,85,86] Increased FPDNA levels have been observed in patients with several epithelial malignancies including PCa when compared to healthy subjects.[87,88] In addition, FPDNA has been recently correlated with both tumor stage and the presence of CTCs suggesting a role of FPDNA as candidate biomarker for the monitoring of PCa patients.[89,90,91]

An important concern for the application of FPDNA tests are false-positives, particularly in patients with autoimmune or inflammatory diseases and a recent history of trauma or surgical procedures.[83] In order to hone in on the tumor origin of circulating DNA, specific genetic aberrations (e.g. loss of heterozygosity and microsatellite analysis) or epigenetic alterations (e.g. gene promoter hypermethylation) should be identified in FPDNA. This has been successfully reported in blood and bone marrow samples.[92,93] However, discrepancies between genetic aberrations in the primary tumor DNA and in FPDNA have been reported and might be ascribed to tumor hetrogeneity with various populations of cells with diverse genetic alterations undergoing lysis.[92,94] Further clinical validation of these findings may unravel role for FPDNA as a valuable new biomarker for monitoring the metastatic progression in PCa patients.

Circulating Tumor Cells and Disseminating Tumor Cells

The occurrence of a metastatic, castration resistant tumor represents the major cause of PCa -related mortality. Metastatic spread has been typically considered a late process in malignant progression but several studies have recently suggested that dissemination of primary cancer cells to distant sites might be an early event in tumorigenesis. In addition, tumor cells can bypass the lymph-node filter and disseminate directly through the bloodstream to distant organs.[95] These findings led to the development of different assays for the detection of Disseminated Tumor Cells (DTC) in Bone Marrow (BM) and CTC in the peripheral blood. The two main techniques employed are the immunological and the PCR-based molecular approach.[96] In the immunological approach, immunochemistry using monoclonal antibodies against surface epithelial antigen is the most widely applied technique. This method is easy to perform and enables the evaluation of cell size and morphology, but sensitivity and specificity are antibody-dependent. Real time RT-PCR-based assays are extremely sensitive and able to detect aberrations at a single cell level. Prostate specific transcripts such as PSA, PSMA and PSCA are usually utilized as single or multiplexed surrogate markers in blood CTCs using RT-PCR or quantitative real-time PCR (qPCR). The major problem of the molecular approach is the false positive rate due to illegitimate transcripts and heterogeneous expression of target markers. The introduction of a cancer cell-enrichment step during the CTC isolation process and the establishment of a reliable cut-off value for analysis may overcome these problems.[97] Most clinical reports on DTC focused on bone marrow, the most common metastatic site in PCa. Some authors reported significant correlations between the presence of DTC and clinical-pathologic parameters such as high Gleason grade or metastatic disease.[98,99] In addition, the presence of DTC in the BM at the time of diagnosis represents an independent negative prognostic parameter in patients with localized PCa.[100] Since BM aspiration is invasive, uncomfortable for the patients and not suitable for repeated analysis, recent efforts have focused on the detection of CTCs in the peripheral blood. CTC can now be easily detected by PCR in the blood at the time of diagnosis, before, during and after therapy, and their increased number has been positively associated with high Gleason score and stage.[101] In addition, the detection of PSA mRNA by qPCR has been significantly correlated to time to progression and overall survival.[102] Technical limitations of the PCR technique and the need for a more standardized method for the detection of CTC in the peripheral blood led to the development of new technologies. The CellSearch™ (Veridex) is a device recently approved by the Food

and Drug Administration for the monitoring of metastatic breast, colon and prostate cancer able to isolate by immunomagnetic enrichment followed by fluorimetric count, the single CTCs. (http://www.accessdata.fda.gov/cdrh_docs/reviews/K073338.pdf). Data generated using this automated system showed that CTC could be detected in 55–62% of patients with castration-resistant Prostate Cancer (CRCP).[103,104] A baseline CTC count ≥5 cells/7.5 mL of blood before therapy represent a powerful predictor of poor overall survival (OS).[105] In addition, the study of CTC dynamics following therapy showed that the CTC count predicts clinical outcome better than the algorithms based on PSA.[102] Patients whose CTC counts decreased from ≥5 cells at baseline to <5 cells after treatment had a better overall survival compared with those showing an increase during therapy.[106] By contrast, in patients with organ-confined PCa the number of detectable CTC appears low and does not correlate with known prognostic factors.[107] Further molecular characterization of CTC or DTC in cancer patients could provide additional information on cancer biology and could improve the management of the disease by selecting effective targeted therapy in the individual patient context. Recent studies show that both high and low-resolution techniques such as FISH or CGH could be performed on isolated cancer cells to obtain a genomic profile of CTCs/DTCs that could be related to prognosis and response to therapy.[108,109,110] The ultimate goal of the research on DTC/CTC is their propagation in vitro after isolation from cancer patients. Such approach could provide a tool to test personalized oncologic treatments directly on the cells responsible for tumor progression of each specific patient.

CONCLUSIONS

The clinical nomograms based on Gleason grade, tumor stage and serum PSA are still the best predictors of PCa outcome. The biotechnological advancements achieved in the last decade represent an incredible source for new prognostic and predictive tissue and serum molecular biomarkers. In order to introduce new biomarkers in the clinical practice of PCa the three following requirements must be necessarily satisfied: 1. the value of each biomarker must be tested in large annotated series of patients with reliable validation sets; 2. The statistical analyses must be robust, taking into account all the possible confounders and the patient selection; 3. the tissues utilized for biomarker validation and for the extraction of protein or nucleic acids must be reviewed by pathologists for the precise assessment of tumor areas, pathological parameters and tissue preservation. Patho-epidemiology is the first example of the useful interconnection of two apparently distant branches of medicine with preventive, diagnostic and clinical overlap that will ultimately benefit prostate cancer patients.

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