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REVIEW ARTICLE

Prostate cancer: Promising biomarkers related to aggressive disease


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Abstract

Background: In spite of a rapidly growing number of candidate biological markers of prognosis and/or response to specific treatments in prostate cancer, none have to date showed ability to completely prognosticate prostate cancer on evidence based urology.

Objective: To review the pertinent literature on the issue.

Acquisition of evidence: A comprehensive review of the current literature was done focusing on promising biomarkers related to aggressive prostate cancer.

Summary of evidence: Combined with the heterogeneous nature of the disease, mixed case series are the most common study design, impeding robust results and the development of an effective therapeutic strategy. Improvement in prostate cancer patient survival requires not only the identification of new therapeutic target based on detailed understanding of the biological mechanisms involved in metastatic dissemination and tumor growth but also strong clinical studies as well.

Conclusion: Better study design involving potential markers and including well-classified and staged patients with robust methodology and adequate outcomes (mainly survival) are necessary to the field evolution.

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PALABRAS CLAVE

Agresividad; Próstata; Pronóstico; Marcadores

Cáncer de próstata: biomarcadores prometedores relacionados con la enfermedad agresiva

Resumen

Antecedentes: A pesar del número rápidamente creciente de marcadores biológicos de pronóstico y/o respuesta a tratamientos específicos posibles en el cáncer de próstata, ninguno ha mostrado hasta la fecha la capacidad de pronosticar por completo el cáncer de próstata en la urología basada en la evidencia.
Prostate cancer overview

Prostate cancer (PCa) is the most common non-dermatological cancer in men, and it is the second leading cause of cancer-related death for this gender in Western industrialized countries. Its estimated incidence around the world is over 200,000 new cases each year, with 35,000–40,000 deaths. Retrospective studies suggest that by the age of 80, more than half of all American men have some cancerous cells in their prostate. Most of these men are asymptomatic, have an indolent and very well differentiated disease, and treatment is not needed.

In contrast, some patients harbor cancer cells exhibiting aggressive behavior characterized by possessing a more poorly differentiated phenotype, heightened proliferative index, and increased motility and invasiveness. These have the propensity to quickly spread locally and metastasize. Androgen ablation therapy remains the most effective treatment for disseminated disease. However, this treatment is not curative since it results in only a temporary tumor regression. Inevitably, some cancer cells become resistant to hormonal ablation, and relapsed tumor growth emerges usually within 12–18 months. These have been referred to as ablation-resistant, hormone refractory, androgen-independent, or, more recently, castrated-resistant.

Between these extremities, a substantial number of prostate cancer patients have localized but clinically significant tumor. These patients are usually treated with some local therapy, such as radical prostatectomy, radiotherapy, or brachytherapy. In some instances, based on clinical and pathological data, a combination of more than one form of therapy may be used, and sometimes added to hormone therapy. However, about one-third of these patients eventually have recurrence and, within an unpredictable period, will succumb to the disease.

The mechanisms underlying the development and progression of PCAs are complex and largely unclear. Clinical, laboratorial and pathological information is helpful, but far from precise to identify those patients who have a more aggressive disease and therefore should be treated more aggressively. A more accurate method (a biomarker, for example) is clearly necessary to better stratify these patients. The prostate specific antigen (PSA) is valuable in the diagnosis, staging, and follow-up, but it is not very helpful in tumor aggressiveness and it does not predict its evolution.

Combined with the heterogeneous nature of the disease, this has prevented the development of an effective therapeutic strategy. Improvement in prostate cancer patient survival requires the identification of a new therapeutic target based on detailed understanding of the biological mechanisms involved in metastatic dissemination and tumor growth.

Biomarkers and prostate cancer

Several studies have delineated clinical and pathological factors that may predict the outcome for men diagnosed with prostate cancer on biopsy and after a variety of treatments for the clinically localized disease.

However, there is a clear need for novel molecular markers specifically associated with biologically aggressive disease to improve staging and prognostic information, and perhaps provide information to facilitate treatment selection. Any new prognostic marker must be measured in the context of accepted predictors of prostate cancer recurrence and death and provide value additional to, and possibly independent of, that provided by those established factors.

The measurable aberrant activity of several regulatory pathways has been detected in prostate cancer, and it is currently being investigated as a potential prognostic factor of the disease.

Cell cycle control

Aberrations in cell cycle control, mainly in the progression of G1 to S phase, are present in virtually all human cancers. p16INK4A, an endogenous cycline kinase inhibitor (CDK inhibitor), has a controversial prognostic role. In fact, two studies in which protein expression was quantified by immunofluorescence flow cytometry and immunohistochemistry, respectively, using paraffin-embedded material of patients submitted to radical prostatectomy, showed that increased p16INK4A expression, compared with that observed in benign prostate hyperplasia, was independently associated with an adverse prognosis in terms of increased rate of biochemical failure and local progression.
Concerning p21WAF1, also an endogenous CDK inhibitor, Rigaud et al. reported that protein overexpression was independently associated with increased biochemical failure in patients treated with neoadjuvant androgen deprivation before salvage radiotherapy. Several studies have examined the relationship between p27Kip1 expression and clinical outcome. Results have consistently shown that low p27Kip1 protein expression was an independent predictor of treatment failure after prostatectomy defined by prostate-specific antigen recurrence.13-16

Prostate cancer cell proliferation depends on the regulatory action of steroid hormones and growth factors. Experimental evidence supports the existence of complex cross talk between the HER-2/neu and AR pathways. A comprehensive review demonstrated that there is a consistent association of HER-2/neu over expression and Gleason lower than 7 with a higher RR of death and recurrence in patients with prostate cancer.18

Furthermore, in the radical prostatectomy specimen, a well recognized marker for cell proliferation was selected as an independent time-influencing factor for biochemical progression; 649 days for Ki-67 negative (≤10%) versus 345 days for Ki-67 positive (>10%) specimens (p = 0.003).19

Apoptosis

Apoptosis-related markers and their relation to prostate cancer outcome have been assessed in many cohort studies. In response to stress stimuli, p53 promotes apoptosis by upregulating the transcription of pro-apoptotic genes, including Bax, PUMA, and Noxa.

The overexpression of p53 as detected immunohistochemically in preoperative biopsies of patients with localized prostate cancer who underwent radical prostatectomy was found to be associated, although not independently, to an increased rate of biochemical failure. In addition, Grignon et al. reported that a high p53 expression significantly predicts a poor outcome, in terms of an increased rate of distant metastases and decreased progression-free survival and overall survival, in patients who received radiotherapy with or without hormonal treatment in the context of the RTOG-8610 trial.

Several studies have examined the prognostic significance of Bcl-2 (an antipapoptotic protein) expression in prostate cancer prognosis. In two studies, an association between Bcl-2 overexpression and increased rate of biochemical failure in patients submitted to radical prostatectomy was consistently observed. More recently, an association, which was only appreciable in the univariate analysis, between Bcl-2 overexpression and increased rate of distant metastases and decreased overall survival in patients enrolling the RTOG-9202 trial, was observed. Finally, in the same trial, altered Bax (a pro-apoptotic member of the BCL-2 protein family) expression was found to be associated with poor clinical outcome, in terms of increased rates of local progression and distant metastases.

Cytokeratin 19 (CK-19) is an epithelial marker involved in several cellular functions, including apoptosis. Also, CK-19 expression by RT-PCR in the peripheral blood mononuclear cell fraction of prostate cancer patients correlates with time to prostate-specific antigen progression.

Signal transduction pathways

In prostate cancer, altered signal transduction pathways have been found, a common feature of most neoplasms. In addition to aberrant expression or activity of androgen receptor, altered expression of tyrosine kinase receptors belonging to the epidermal growth factor receptor (EGFR) family, such as EGFR and HER-2, is also present. These molecules drive a cell signaling pathway that through the PI3K/AKT mTOR pathway on the one hand and the RAS/ERK pathway on the other hand, stimulate cell cycle progression and proliferation. A higher tumor-to-benign ratio of the expression of androgen receptor mRNA, as detected by quantitative reverse transcriptase polymerase chain reaction in specimens obtained from patients who underwent radical prostatectomy, was independently associated with disease progression in terms of increased biochemical failure.

It has been recently reported that high HER-2 protein levels, as detected in preoperative plasma by enzyme-linked immunosorbent assay, were significantly associated with an increased rate of biochemical failure in patients who underwent radical prostatectomy.

Adhesion/cohesion mechanisms

Expression of molecules involved with cell adhesion/cohesion has also been demonstrated to be deregulated in prostate cancer. Initial reports found that E-cadherin overexpression in tumor specimens, assessed by immunohistochemical staining, was significantly associated with a poor patient prognosis, in terms of increased rate of biochemical failure after radical prostatectomy.

Conversely, in a more recent study, cadherin switching (high N-cadherin and low E-cadherin), which indicates epithelial to mesenchymal transition, showed a strong association with disease progression, in terms of increased rate of biochemical failure and decreased progression-free survival after radical prostatectomy.

Genetic aberrations

The genetic alterations detected in prostate cancer are innumerable. Nevertheless, prognostic significance has been found only in some. An example is the fusion of the androgen-regulated gene TMPRSS2 with ETS genes for transcription factors. This fusion was associated with an increased risk of biochemical recurrence in patients who underwent radical prostatectomy for clinically localized prostate cancer. The patients with a tumor characterized by duplication of the fusion of TMPRSS2 to ERG sequences together with interstitial deletion of sequences 5’ to ERG exhibited extremely poor cancer-specific survival. Conversely, another study failed to detect an association between the presence of TMPRSS2 and/or ERG gene rearrangements and risk of biochemical failure in prostate cancer patients treated by radical prostatectomy. Aberrant DNA methylation, a common event in carcinogenesis, has been suggested to pose prognostic information in prostate cancer. Several studies carried out in tumor specimens as well as in preoperative serum DNA from patients who
underwent radical prostatectomy have demonstrated that the presence of CpG island hypermethylation in several genes (including GSTP1, APC, RAR-beta, and reprimo) was significantly associated with a poor prognosis.\textsuperscript{55-58}

Gene polymorphisms

Recent technological advances using a combination of genome wide association scans, linkage analysis, and fine mapping techniques have allowed researchers to identify inherited genetic variants known as single nucleotide polymorphisms (SNPs). These genetic variations can predispose or protect individuals against clinical events. Some have been clearly associated with an increased risk of PCa.

The variants contained in chromosomal regions along 8q24 and 17q are reproducibly associated with PCa risk.\textsuperscript{39-44} More recently, several studies have identified genetics variant specific for aggressive disease. Adverse pathological features, including higher Gleason grade and pathological stage, were more frequent in 17q12 carriers using a best fit additive genetic model.\textsuperscript{45}

A diverse study in the same issue as the former publication demonstrated that predicting biochemical recurrence after radical prostatectomy based on clinicopathological data can be significantly improved by including patient genetic information. They found the single nucleotide polymorphisms at the KLK2, SULT1A1 and TLR4 genes to be the most significant.\textsuperscript{46}

The identification of single nucleotide polymorphisms (SNP) in the CCL2 gene (in MSMB and 8q24) is associated with risk for metastatic prostate cancer, the most lethal form of this disease, but not with prostate cancer recurrence.\textsuperscript{47} Genetic variation at CCL2 is also associated with markers of disease aggressiveness. Three SNPs, each in strong linkage disequilibrium, are associated with a higher (>7) biopsy Gleason score and advanced pathologic stages.\textsuperscript{48} The specific combination of single nucleotide polymorphisms variations in miRNAs and miRNA target sites was significantly associated with disease progression, prostate cancer-specific mortality, and all-cause mortality in prostate cancer patients receiving androgen-deprivation therapy.\textsuperscript{49}

Circulating tumor cell detection

Circulating tumor cells (CTCs) are found in the peripheral blood and potentially be disseminated from either primary tumors or metastatic sites. In the prostate research arena, the detection of CTC has been incorporated into different fields of oncology as a prognostic marker, a tool to monitor response to therapy and a method to understand basic tumor characteristics in the prostate.\textsuperscript{50}

The Food and Drug Administration of the United States has recently accepted the CTC as a prognostic tool in advanced prostate cancer. The detection of CTC, however, did not predict PSA failure using strict criteria (e.g. two consecutive values of elevated PSA $\geq 0.2$ ng/mL). Instead, in castration-resistant prostate cancer patients, those with circulating tumor cells and/or tumor-related methylated DNA have a significantly poorer outcome.\textsuperscript{51}

In an elegantly designed study, the patients with metastatic prostate cancer that initiated a new line of therapy, those with more than 5 CTC/7.5 ml blood, had a significantly lower probability of survival.\textsuperscript{52} On the other hand, a threshold of 4 CTC/7.5 ml (compared with the FDA approved value of 5) as an optimal cut-off value with respect to correlation with survival outcomes as well as predictive of metastatic disease was recently suggested.\textsuperscript{53}

PCA3

PCA3 is a non-coding mRNA molecule that is overexpressed in prostate cancer. PCA3 molecular urine assay is useful for risk stratification in men with a prior negative prostate biopsy.\textsuperscript{54,55} Withman et al. first suggested PCA3 detected in the post-digital rectal examination of urine of patients with prostate cancer correlated with pathological findings: extracapsular extension and total tumor volume.\textsuperscript{56}

However, in a subsequent study, the PCA3 test did not seem to predict adverse pathologic features, although it may have an association with perineural invasion.\textsuperscript{57} Also, in men with low-risk prostate cancer who were carefully selected for surveillance, the PCA3 score was not significantly associated with short-term biopsy progression,\textsuperscript{58} suggesting its greater value for diagnosis rather than identifying aggressive prostate cancer.\textsuperscript{59} The relationship of PCA3 to PCa aggressiveness and its role in prognostication requires further study.

Chromogranin-A

Chromogranin-A (CgA) is considered the best marker of neuroendocrine differentiation, which may play a key role in androgen-independent tumor progression and is related to tumor aggressiveness.\textsuperscript{60}

Proper assessment of the CgA results requires detailed knowledge about various factors, drugs, and pathological conditions influencing its concentration in blood and tissue. In addition, the sort of investigated biological material (whether it is serum or plasma, if fresh or processed tissue) is of importance.

The serum levels of CgA are significantly increased in patients harboring PCa compared with healthy men. However, low correlations between serum CgA and known prognostic factors (such as total and free PSA, age, Gleason score, and bone metastases) or clinical deterioration limit its clinical application.\textsuperscript{60}

Tissue CgA quantification evaluated by immunohistochemistry in prostate specimens recently proved to be an independent variable for predicting biochemical failure after radical prostatectomy ($p = 0.023$) in patients with localized prostate cancer.\textsuperscript{51}

In spite of presenting controversial results in the literature, a myriad of circumstances should be overcome to prove CgA clinical effectiveness in positively impacting the diagnosis and treatment of patients with PCa; among them are fundamental aspects as the best tissue quantification method as well as blood cut-off values to classify patients are to be better defined.

Biomarkers may improve predictive accuracy of nomograms

Nomograms, which are based on clinical and pathological data, have been introduced to complement the standard
modeling techniques, with the ability to predict various risks for individual patients. Despite numerous reports of promising new biomarkers in the prostate cancer literature, only 3 studies have to date demonstrated a statistically significant improvement in predictive accuracy when biomarkers were added to established predictors in the nomogram setting.

In this context, a prognostic model that incorporates preoperative plasma levels of transforming growth factor-b1 (TGF-B1) was validated on patients with localized prostate cancer and improved the predictive accuracy of biochemical recurrence from 75% to 83% relative to clinical variables alone.\(^\text{62}\)

Similarly, the addition of biomarkers (circulating plasmaminogen activator activation inhibitor-1 and gene expression profiling by oligonucleotide microarrays in tumor tissues,\(^\text{63,64}\) respectively) increased the predictive accuracy of the preoperative and postoperative nomograms by a clinically significant margin.

It is noteworthy that the combined approach provided a marked improvement for patients whose nomogram-predicted likelihood of disease recurrence was in the indeterminate range.

**Future directions**

There is a rapidly growing number of candidate biological markers of prognosis and/or response to specific treatments in prostate cancer. Nevertheless, none of them has to date showed ability to completely prognosticate prostate cancer on evidence-based urology.\(^\text{65,66}\) In this context, the available results indicate that integration of selected biological markers (or gene signatures) with clinical variables produces predictive models that perform significantly better than ‘standard’ nomograms. Once externally validated, such improved nomograms may assist clinical decision making regarding treatment choice and follow-up as well as identification of patients at high risk of failure who could benefit from neoadjuvant or adjuvant treatment.

Improvement in prostate cancer patient survival requires not only the identification of a new therapeutic target based on detailed understanding of the biological mechanisms involved in metastatic dissemination and tumor growth but also strong clinical studies, instead of the commonly mixed case series provided in the current literature. A better study design involving potential markers and including well-classified and staged patients with firm outcomes (mainly survival) is necessary for the field evolution.

**Conflict of interest**

The authors declare that they have no conflict of interest.

**References**


