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TOPIC PAPER

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# Molecular and genetic prognostic factors of prostate cancer

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**Abstract** Prostate cancer is the most commonly diagnosed cancer in Western males, responsible for 3% of all deaths in men over 55 years of age and second only to lung cancer as a cause of cancer death. Biomarkers have become an important diagnostic tool in prostate cancer. The discovery of the serum marker prostate-specific antigen (PSA) significantly facilitated the detection and management of prostate cancer. As we enter into the post-genomics era, novel biomarkers of prostate cancer of therapeutic significance will invariably emerge. Here we review a series of existing and emerging molecular-based prognostic markers particularly with radiotherapy.

**Keywords** Prostate cancer · Radiotherapy · Prognosis · Molecular/genetic biomarkers · Cell cycle · Apoptosis Angiogenesis · Signal transduction · Invasion/adhesion · Tumor suppressor genes

Morphology-based approaches, especially Gleason scoring, have provided clinicians with important prognostic information, especially when combined with clinical parameters of prostate-specific antigen (PSA) and T stage [1, 2, 3, 4, 5, 6, 7]. However, the prognostic value of the Gleason score is limited by the fact that the vast majority of prostate cancer patients present with moderately differentiated tumors (e.g., Gleason score of 6) in the PSA era, limiting the prognostic utility of morphologic features.

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Recent successes in functional genomics and proteomics have served to cultivate the growing interest in discovering more molecular-based prognostic factors that could be utilized to assay the original needle biopsy specimen to tailor the primary treatment for individual prostate cancer patients [8, 9, 10, 12]. As targeted therapy in oncology becomes increasingly powerful, there is a significant interest in finding prognostic markers in prostate cancer that could be used as targets for novel biotherapies. Many molecular- and genetic-based biomarkers have been discovered over the last 2 decades and are summarized in Table 1and also in Abate-Shen and Shen [89]. This article discusses their prognostic significance in prostate cancer treatment.

## Cell cycle makers

Cell cycle regulators have been found to associate with adverse outcome in prostate cancer [11]. For instance, cyclin D1 appears to have an impact on both breast and prostate tumorigenesis by mediating hormone receptor signaling [11]. Cyclin D1 overexpression is significantly associated with the subsequent development of distant metastasis in prostate cancers [24]. The Cip/Kip and INK4 groups of cyclin-dependent kinase inhibitors that regulate the G1 to S phase transition of the cell cycle [11] have been found to play roles in prostate cancer [11, 25, 26, 27, 28, 29]. p21 immunoreactivity was found to be associated with improved survival [25, 26, 27]. Loss of p27 expression has been linked with adverse disease outcome in a number of studies [11, 28, 29]. In prostate carcinoma, the tumor suppressor gene CDKN2/MTS1 (p16 INK4) has a very low frequency of point mutations, but deletions of 9p21 and inactivation by promoter methylation are observed more frequently. In a recent study, Halvorsen et al. evaluated the expression of p16 and CDK4 proteins and their prognostic significance in patients with clinically localized prostate carcinoma treated surgically [30]. In their univariate survival analysis of the first 5 years, high levels of p16 protein

**Table 1** A list of prognostic markers categorized based on their biological functions and discussed in this review. Category I: markers that have been proven to be reliable in prognosis and generally used in patient management. Category II: markers that show promise as a result of extensively biological and/or clinical studies but with few clinical outcome studies. Category III: markers that have some scientific evidence to support their adoption as diagnostic or prognostic agents but are not currently recommended; or those factors with uncertain significance

| Type of marker | Prognostic factor | CAP category | Reference |
|---|---|---|---|
| Cell cycle | cyclin D1 | III | [11, 24] |
| | p21 | III | [25, 26, 27] |
| | p27 | III | [11, 28, 29] |
| | p34cdc2 | III | [31] |
| | Ki-67 (MIB-1) | III | [16, 17, 18, 19, 20, 21] |
| Angiogenesis | Tumor vascularity | III | [42] |
| | Microvessel density | III | [43, 44] |
| | VEGF | III | [49, 50] |
| | bFGF | III | [49, 50] |
| | Endothelin-1 | III | [119] |
| Apoptosis | Bcl-2 | III | [71] |
| | Bax | III | [71] |
| | Bcl-x | III | [71] |
| Oncogenes + tumor suppressor | *ras*. *myc* | III | [11, 87] |
| | p16(INK4) | III | [30] |
| | p53 | III | [90] |
| | PTEN/MMAC1 | III | [101, 102] |
| Tumor invasion + cell adhesion | Cathepsin D | III | [82, 83] |
| | Metalloproteases | III | [85] |
| | E-cadherin | III | [74, 75, 76, 77, 78, 79, 80, 81] |
| Signal transduction | Caveolin-1 | III | [107, 108, 109, 111] |
| | HER-2/*neu* | III | [11, 32, 92] |
| | EGF | III | [72] |
| | EGFR | III | [116] |
| | TGF-$\beta$ | III | [11, 72, 73] |
| | MAPK&MKK4 | III | [14, 105] |
| Epigenetics | DNA ploidy | II | [32, 33, 34, 35, 36, 37, 38, 39] |
| Other types | GST-$\tau$ | III | [91] |
| | Syndecan-1 | III | [112, 113, 114, 115, 117, 122] |
| | EZH2 | III | [110] |

expression, tumor greatest dimension, WHO histologic grade, capsular penetration, seminal vesicle invasion, positive surgical margins, lymph node involvement, and preoperative serum prostate-specific antigen $>20$ ng/ml all were significant predictors of biochemical failure. In their multivariate survival analysis, high p16 protein expression, age, WHO histologic grade, capsular penetration, and seminal vesicle involvement remained as independent predictors of biochemical failure. This study suggests that elevated expression of p16 protein, but not CDK4 protein, may play a significant role in the development of prostate carcinoma and represent an independent predictor of biochemical failure after radical prostatectomy. However, loss of p16 expression was found to be associated with adverse outcome (e.g., reduced overall and disease-specific survival) in patients with locally advanced prostate cancer treated by radiation therapy ± neoadjuvant hormones [116]. The observed discrepancy in the prognostic significance of p16 expression between radiation and surgery-treated patients suggests that the type of treatment delivered may be an important factor determining the significance of a given molecular marker.

Overexpression of $p34^{cdc2}$ cyclin-dependent kinase in the S to $G_2M$ transition of the cell cycle was shown to have connection with aggressive high-grade disease with increased incidence of biochemical failure after primary therapy [31]. As for the cell proliferation markers, immunohistochemical analysis using MIB-1/Ki-67 antibody is very useful for measuring cell cycle progression in human tissues [16, 17]. It is usually accepted that $>16$–20% MIB-1 staining is associated with a high proliferation rate and an adverse prognosis although clinically defined proliferation ranges remain under study for prostate cancer [16, 17, 18, 19, 20, 21]. Primary therapy failure has been attributed to MIB-1 overexpression, which has appeared useful in predicting prognosis even in patients with existing lymph node involvement [15, 22].

To establish the significance of MIB-1/Ki-67 staining as a prognostic marker of treatment outcome for prostate cancer patients treated by radiotherapy, Pollack et al. carried out a study with pretreatment archival prostate biopsy tumor tissue available from stage T1-T4 prostate cancer patients treated with external beam radiotherapy [23]. The percentages of Ki-67-positive tumor cells, the Ki-67 labeling index (Ki-67 LI), were determined using immunohistochemical staining for MIB1. Experimental analyses indicated that, for all patients, mean Ki-67 LI levels were higher with stage

T3/T4 disease, Gleason 7–10 disease, and in those that developed treatment failure. Comparable relationships were also observed when the Ki-67 LI was dichotomized into low and high groups. Their analysis using the approach of Cox proportional hazards regression demonstrated that dichotomized Ki-67 LI was an independent correlate of bNED (biochemical no evidence of disease) survival, along with pretreatment PSA, Gleason score, and clinical stage. This concludes that (1) Ki-67 LI could be used as a reliable independent predictor of failure after radiotherapy using biochemical criteria and (2) such a prognostic marker appears equally valuable for patients diagnosed by transurethral resection of the prostate (TURP) or needle biopsy

## Tumor suppressor genes

Many studies have demonstrated that p53 or genes that affect p53 function are mutated in most, if not all, human tumors. Several studies, however, have shown that p53 mutations are sporadic in prostate cancer and are associated with advanced disease [88, 92, 93, 99]. Recently, Grignon et al. [90] evaluated the prognostic value of p53 protein expression at unusual levels in the tumors of patients with locally advanced prostate cancer under either external beam radiation therapy alone or total androgen blockade before and during the radiation therapy. The study was undertaken through a population consisting of a subset of patients entered in Radiation Therapy Oncology Group protocol 8610 via immunohistochemical detection of abnormal p53 protein in pretreatment specimens. Relationships between p53 protein expression level and the time to local progression, the incidence of distant metastases, progression-free survival, and overall survival were assessed. Abnormal p53 protein expression, which is often an indicator of mutated p53, was observed in the tumors of 18% of patients. A statistically significant connection was revealed between abnormal p53 expression and elevated incidence of distant metastases, reduced progression-free survival, and reduced overall survival; however, no association was found between abnormal p53 protein expression and the time to local progression, which were independent of the Gleason score and clinical stage. For patients under both radiation therapy and hormone therapy, those with tumors displaying abnormal p53 protein expression underwent a reduced time to the development of distant metastases; for patients on radiation therapy alone, the time to distant metastases was independent of p53 protein expression status. They concluded that p53 protein expression level could yield significant, independent prognostic information concerning the development of distant metastases, progression-free survival, and overall survival for patients with locally advanced prostate cancer under ongoing radiation therapy. Therefore, the relationships of radiation and hormone therapies and aberrant p53 protein expression may well shed light on experimental evidence that radiation therapy and/or hormone therapy work, to some degrees, by the p53-dependent apoptosis.

The subsequent biological changes in recurrent prostate carcinomas following radiation treatment are far from being completely appreciated. In a different study, Cheng and his colleagues [91] demonstrated that p53 protein overexpression is associated with increased cell proliferation in patients with locally recurrent prostate carcinoma after radiation therapy. Their study determined the level of p53 protein overexpression and its association with cellular proliferation (Ki-67 LI), glutathione S-transferase-$\pi$ (GST-$\pi$) expression, and other clinical pathologic findings in patients with locally persistent prostate carcinoma after radiation therapy. The respective expression status of p53 nuclear accumulation, cellular proliferation activity, and GST-$\pi$ was investigated in patients with persistent or recurrent prostate carcinoma post radiation therapy. Experimental analysis revealed that p53 protein overexpression was associated with increased cell proliferation. A substantial proportion of recurrent cancer also showed GST-$\pi$ immunoreactivity. However, no apparent correlation was seen between p53 protein overexpression, cellular proliferation (Ki-67 LI), or GST-$\pi$ expression and Gleason score, pathologic stage, DNA ploidy, or patient outcome. Analysis also established an inverse correlation between GST-$\pi$ expression and Gleason score. The majority of prostate carcinomas (95%) were proliferative whereas concurrent prostatic intraepithelial neoplasia (PIN) had a lower Ki-67 labeling index. Sixty eight percent of the concurrent PIN demonstrated p53 immunoreactivity. A trend toward adverse clinical outcome was observed in patients with a higher Ki-67 LI in recurrent cancer. In their study, recurrent cancers were biologically aggressive following radiation therapy, which may represent selective persistence and regrowth of prognostically unfavorable tumor clonogens or stepwise clonogenic progression.

### PTEN/MMAC1

The PTEN/MMAC1 phosphatase is a tumor suppressor gene implicated in a wide range of human cancers [95, 96, 97]. The PTEN/MMAC1 tumor suppressor gene is deleted or mutated in a wide variety of cancers including prostate cancer cell lines, xenografts, and clinical samples [11, 98, 99]. Sawyers and his colleagues provided biochemical and functional evidence that PTEN/MMAC1 acted as a negative regulator of the phosphoinositide 3-kinase (PI3-kinase)/Akt pathway [100]. PTEN/MMAC1 was shown to exhibit the capacity of activating endogenous Akt in cells and inhibiting phosphorylation of 4E-BP1, a downstream target of the PI3-kinase/Akt pathway involved in protein translation, whereas a catalytically inactive, dominant negative PTEN/MMAC1 mutant enhances 4E-BP1 phosphorylation. Moreover, PTEN/MMAC1 suppresses gene expression in a way that was salvaged by Akt but not

PI3-kinase. More significantly, elevated levels of Akt activation were detected in human prostate cancer cell lines and xenografts lacking PTEN/MMAC1 expression when compared with PTEN/MMAC1-positive prostate tumors or normal prostate tissue. PTEN expression loss has been associated with downregulation of the cyclin-dependent kinase inhibitor p27 and adverse outcome [101] and increasing grade and stage [102] in prostate cancer.

### Caveolin-1

Caveolin-1 protein was initially discovered by differential gene expression display technique and has been associated with both signal transduction and cell motility in prostate cancer [41, 107, 108]. Recently, Yang et al. [111] established that Cav-1 expression is significantly increased in primary and metastatic human prostate tumors after androgen ablation therapy. And also, Cav-1 is secreted by androgen-insensitive prostate carcinoma cells, and this secretion is regulated by steroid hormones. In cases of clinically determined, localized prostate carcinoma, they found that Cav-1 expression is a novel prognostic marker with independent predictive value of biochemical recurrence. It was observed that the 5' promoter CpG islands of Cav-1 were more methylated in tumor than in adjacent normal prostate cells implying that Cav-1 may serve as a tumor suppressor gene in prostate cancer [109].

## Invasion and cell adhesion markers

### Tumor invasion associated proteases

Cathepsin D is a lysosomal protease and autocrine mitogen and has been demonstrated to associate with prognosis in breast cancer [82]. In prostate cancer increased tumor cathepsin D immunoreactivity has been connected with pathologic stage [83], tumor grade, and DNA content [84]. Elevated serum levels of soluble urokinase plasminogen activator receptor have been connected with progressive prostate cancer. Localization of matrix metalloproteases has been linked to prostate cancer development [85]. Upregulated expression of tumor collagenases has also been linked to adverse disease outcome [13].

### Cell adhesion molecules

E-cadherin is a cell adhesion molecule whose aberrant cellular expression has been linked to adverse disease outcome in prostate cancer [74, 75, 76, 77, 78, 79, 80, 81]. For instance, reduced expression of E-cadherin has been correlated to high tumor grade and aneuploidy [74]. A study by Sandberg a decade ago implied that a major chromosomal deletion on chromosome 16 may be a major event in the development of prostate cancer [79]. Further investigation showed that this deletion event may involve the E-cadherin gene; its expression product E-cadherin protein may serve as a tumor suppressor protein. As a result of the establishment of the relationship of $\beta$-catenin degradation and association with the APC (adenomatosis polyposis coli) gene, the E-cadherin interaction with $\beta$-catenin has been a subject of great interest [11, 81].

## Signal transduction markers

### Dominant oncogenes

The contributions of dominant oncogenes in prostate cancer development and progression appear to be diminutive in view of their significance in adenocarcinomas of the respiratory and gastrointestinal tract [40, 86]. For instance, the *ras* genes, frequently mutated in the malignancies of the gastrointestinal, hepatobiliary, and respiratory tracts, often remain unaltered in human prostate cancers or cell lines or experimental models [87]. Although *Myc* gene mutations may be associated with development of hormone refractory disease [11], study has also shown that the amplification of the *myc* gene was implicated in prostate cancer but without any direct linkage to disease progression [87].

### HER-2 oncoprotein overexpression

For breast cancer patients, HER-2 oncoprotein overexpression has been linked to adverse outcome in breast cancer [92]. This fact has led to the clinical testing of HER-2 as a standard of practice status for guiding treatment with various therapies. For prostate cancer, previous studies found that overexpression of HER-2 was associated with an adverse outcome [11, 32, 92]. HER-2 mRNA levels have recently been correlated with metastatic disease and androgen-independent hormone refractory progressive disease [93]. More significantly, in a recent study, the prognostic relevance of Her-2 protein expression in patients undergoing curative radiotherapy (RT) was compared to the traditional prognostic factors such as pretreatment PSA levels, biopsy Gleason score, and T category of the primary tumor [94]. The prognostic relevance of Her-2 expression was univariately associated with adverse outcome in terms of biochemical or clinical progression-free survival (B/C-PFS), clinical progression-free survival (C-PFS) and disease-specific survival (DSS). Her-2 expression, T category, and Gleason score were independently associated with C-PFS, whereas only Her-2 expression and Gleason score were associated with DSS. Her-2 expression and Gleason score together discriminated two groups of patients with 5-year DSS of 95% and 79%, respectively. Pretreatment PSA levels

were associated only with B/C-PFS but not with C-PFS or DSS. This investigation demonstrates that expression of Her-2 is of prognostic relevance in localized prostate cancer undergoing RT and implicates that analysis for Her-2 might improve prognostic algorithms for clinically relevant endpoints other than biochemical relapse.

### Growth factors

A recent RTOG protocol examined epidermal growth factor receptor (EGFR) expression patterns in locally advanced prostate cancer and found less than 5% expression (Chakravarti, personal communications). It has been shown that increased expression of basic fibroblast growth factor (bFGF) is linked to adverse outcome [13] and that overexpression of transforming growth factor (TGF)-$\beta$ is involved in the growth of prostate cancer cell lines [11] and a reduction in disease-free survival in clinical trials [72]. In a recent interesting study [73], the transcription factor early growth response (EGR)-1 expression was monitored to determine whether it, in the primary tumor, correlates with radiation response in terms of complete local tumor control with no evidence of disease or recurrence and no evidence of metastasis; whether it, in the post-irradiated biopsies, correlates with residual tumor; and whether it correlates with the expression of EGR-1 target genes such as TP53, pRB, and Bax. The authors analyzed pretreated surgically resected paraffin-embedded primary adenocarcinomas of the prostate for the presence of EGR-1 expression and mutation, and correlated this with clinical endpoints such as serum PSA levels and current clinical status. They also analyzed post-irradiated biopsies of prostate for the presence of EGR-1 expression, and correlated these findings to the residual tumor status. They finally analyzed prospective prostate tumor specimens for EGR-1 expression and its target genes. EGR-1 expression was measured by immunohistochemistry and mutations were screened in two regions of the EGR-1 gene: trinucleotide AGC repeats in transactivation domain (TD) and poly A tract in 3'UTR. EGR-1 overexpression correlated with treatment failure. No correlation with EGR-1 overexpression and its target genes was found, which may imply that overexpressed EGR-1 may lack transactivation function. This investigation indicated that EGR-1 overexpression in the mutant form might offer an indication of clinical failure, either local recurrence or metastasis.

### MAPK and MKK4

The mitogen-activated protein kinase (MAPK) cascade of protein phosphorylation and dephosphorylation has been linked to cell proliferation in prostate cancer [14]. Using an antibody specific for dually phosphorylated extracellular-regulated kinases 1 and 2, Weber's group has examined primary and metastatic prostate tumor specimens for the presence of activated MAPK [105]. Nonneoplastic prostate tissue produced little or no staining with activated MAPK antiserum. In prostate tumors, elevated levels of activated MAPK have been associated with increasing Gleason scores and tumor stage in prostate cancer [105]. In a separate analysis, tumor samples from two patients showed no activation of MAPK before androgen ablation therapy. Following androgen ablation treatment, high levels of activated MAPK have also been detected in patients with recurrent disease [105]. This study suggested an increase in the activation of the MAPK signal transduction pathway as prostate cancer progressed to a more advanced and androgen-independent disease. Mitogen-activated protein kinase kinase 4 (MKK4) is a potential prostate cancer metastasis suppressor [106]. Downregulated MKK4 expression has been associated with increasing Gleason grade implying that MKK4 protein may be downregulated during prostate cancer progression [106].

## Apoptosis markers

### Bcl-2, bax, and bcl-x

The bcl-2 family, consisting of two members, bcl-2 and bax, plays a critical role in the control of apoptosis via dimerizations of bcl-2 and bax, governing the cellular apoptosis in response to genotoxic stress rather than cell cycle arrest and repair [50, 51, 52, 54, 59, 64]. Recent studies in bcl-2 family proteins have related the overexpression of the bcl-2 anti-apoptosis protein to decreased expression of the proapoptotic protein, bax and adverse outcome in prostate cancer associated with resistance to cytotoxic chemotherapy in patients with hormone-refractory disease [53, 103, 104]. It had been established a long time ago that radiation induces apoptosis [65], and overexpression of bcl-2 has been associated with resistance to radiation [66]. It has been observed recently that locally recurrent tumors can upregulate bcl-2 expression upon radiation [68, 69, 70]. Given the experimental link of bcl-2 overexpression to clinical treatment failure, it is argued that bcl-2 can be viewed as a marker of aggressive tumor attributes and as an etiologic factor of resistance to androgen ablation and radiation [48].

More relevant to radiation therapy treatment, bcl-2 overexpression is related to patient outcome after radiotherapy in the form of radiation resistance, although the results are contradictory. It was reported that patients with bcl-2-positive diagnostic biopsy results had a significantly longer DSS [55], whereas in other studies that bcl-2 overexpression was correlated with treatment failure after radiotherapy [56, 57, 58]. These existing data support further studies of pretreatment bcl-2 levels as a marker for patient stratification. Bax is a proapoptotic protein, and it was expected that its overexpression in tumors treated by

radiation would give rise to increased cell death and improved outcome compared with tumors that had normal bax expression. For bax expression, prior studies on the usefulness of bax as a pretreatment predictor of outcome after radiotherapy in patients with prostate carcinoma are limited and the results were inconsistent [56, 58]. In the first study, the expression levels of bax and bcl-2 were related inversely to freedom from recurrence and that a high bcl-2:bax ratio was correlated with poor therapeutic responsiveness [56]. Pollack and his colleagues have demonstrated that bax expression appears to be a significant correlate of bNED, independent of the conventional pretreatment factors of clinical stage, Gleason score, and PSA level [48]. Their results also indicate that altered bax expression is a strong predictor of bNED after radiotherapy and bax was a much stronger predictor of bNED and was independent of bcl-2. Both bcl-x and bax belong to the bcl-2 family of apoptosis-regulatory proteins. While bax is proapoptotic, bcl-x has both proapoptotic (bcl-xS) and antiapoptotic (bcl-xL) effects [60, 61, 62, 63, 67]. There is still no evidence that the quantification of bcl-x has predictive value, which may be related to the lack of specificity of the antibody used in the Pollack et al. study where both proapoptotic (bcl-xS) and antiapoptotic (bcl-xL) components were measured [48]. This is a significant study in the evaluation of biomarkers for prognosis of patients who are treated with radiation therapy although further investigation is needed to determine the optimal methods for staining classification and to establish the reproducibility in a larger group of patients who are treated with radiotherapy for prostate carcinoma.

## Angiogenesis markers

### Tumor vascularity and microvessel density

As with other solid tumors, angiogenesis is a critical component of prostate cancer development and progression. Weidner et al. [42] showed a close correlation between increased microvascular density and Gleason score in advanced prostate cancer. Tumor angiogenesis has been associated with adverse outcome in prostate cancer as assessed by microvessel counting with the observation that significantly higher microvessel counts have been found in areas of adenocarcinoma than in the benign tissues of radical prostatectomy specimens [43, 44]. The infrequency of necrosis in prostate cancer may be explained by the fact that prostate cancers encompass the greatest concentration of microvessels in the tumoral areas. While augmented, microvascularity has been correlated with the pathologic stage of the disease [43, 45, 46, 47]; microvessel density has appeared to have association with the presence of metastasis [43]. The application of microvessel counts to needle biopsies of prostate cancer—where it could be used prospectively to plan therapy—is worth further investigation.

### Angiogenic growth factors

Latest analyses have focused on the use of specific angiogenic factors—such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and endothelin—as prognostic serum markers for prostate cancer. While VEGF is a peptide growth factor specific for the tyrosine kinase receptors, VEGF receptor-1 and -2 have been associated with growth of endothelial cells in prostate cancer [49, 50]. The levels of VEGF and bFGF in bodily fluids could serve as the indicators of the degree of tumor-related angiogenesis that occurs in a cancer patient. A reliable immunoassay for bFGF was not available until 1991 [118], and commercial assays are now available with good sensitivity for detecting bFGF in urine and blood.

Endothelin-1 (ET1) found in high concentrations in seminal fluid appeared to be another angiogenic factor important in prostate cancer development and progression [119]. It has been demonstrated that it stimulates prostate cancer cell proliferation in vitro, and boosts the mitogenic effects of IGF1 and other growth factors, and shows increased expression in prostate cancer primary tumor specimens and metastases [120]. Endothelins seem to play a major role in the growth and angiogenesis of prostate cancer, and in the pathophysiology of its metastasis to bone [121].

## Genetic/epigenetic makers

### DNA ploidy determination

Aneuploid DNA content in prostate cancer may independently predict poor prognosis for the disease [32, 33, 34, 35, 36, 37]. DNA ploidy measurements have been performed on needle biopsy specimens, and the ploidy status determined on needle biopsies has successfully correlated with the ploidy status of corresponding radical prostatectomy specimens and independently predicted disease outcome [38]. Despite such a solid establishment of these data, only a few studies have used pretreatment diagnostic material and have involved RT-treated patients. A significant analysis of RTOG 8610 in prostate cancer DNA ploidy and response to salvage hormone therapy after radiotherapy was carried out to supplement the knowledge [39].

In their retrospective study, the predictive value of DNA ploidy was assessed in patients treated under RT alone comparing with those under RT plus short-course neoadjuvant and concurrent total androgen blockade. Their results indicated that DNA ploidy was not associated with any of the other prognostic factors. It was shown that no relationship was observed between DNA ploidy and distant metastasis, and aneuploidy was independently associated with reduced overall survival. The patients treated with RT plus total androgen blockade (TAB) with nondiploid tumors had reduced survival after salvage androgen ablation.

This RTOG study clearly showed the close association of aneuploidy with shorter survival that is related to reduced response to salvage hormone therapy for those previously exposed to short-term TAB.

### Repressor of gene transcription EZH2

It has been associated through gene expression profiling that the polycomb group protein enhancer of zeste homolog 2 (EZH2) was overexpressed in hormone-refractory, metastatic prostate cancer, and clinically localized prostate cancers that express higher concentrations of EZH2 showed a poorer prognosis [110]. This suggests that dysregulated expression of EZH2 may play a role in the progression of prostate cancer, which may be considered as a marker that distinguishes indolent prostate cancer from those aggressive forms [110].

### Syndecan-1 identified via tissue microarray

Syndecan-1 (CD-138), a proteoglycan that has been found to be of prognostic importance in various human tumors [112, 113, 114, 115, 117] has not been analyzed in prostate cancer until very recently. Zellweger et al. tested several molecular markers on a prostate tissue microarray (TMA) [122]. The respective expression status of Ki-67, bcl-2, p53, CD-10 (neutral endopeptidase), and syndecan-1 (CD-138) was investigated by immunohistochemistry. This study showed that Gleason grade and Ki-67 labeling index (Ki-67 LI) were independent predictors of early recurrence and poor survival while Bcl-2 predicts early recurrence and p53 was associated with poor survival. Syndecan-1 overexpression also predicted early recurrence and was much associated with tumor-specific survival, high Gleason grade, Ki-67 LI, and bcl-2 overexpression. The results of this TMA study confirmed a central prognostic importance of Gleason grading and Ki-67 LI in prostate cancer, as compared to a less pronounced role of Bcl-2, and p53. More remarkable, they identified syndecan-1 as a new prognostic factor and provided evidence for an androgen-dependent regulation of CD-10.

## Conclusions

Many prognostic markers have been discovered over the last 2 decades and are described in this review as summarized in Table 1. While the morphology-based factors of Gleason grade and clinical features of PSA and T stage are widely utilized as the standard of practice, the Ki-67 cell proliferation index, p53, and bcl-2 immunostaining and DNA ploidy analysis are now exploited in many laboratories as adjuncts for predicting outcome in the disease. At the same time, still many of the remaining prognostic factors listed in this review are now under evaluation to further investigate their abilities to determine prognosis and guide the selection of therapy for prostate malignancies. Additional prognostic and predictive tests that can predict efficacy of a specific therapy will invariably become part of the standard of care. Genomic and proteomic high-throughput array strategies will help accelerate the discovery of more molecular-based diagnostic tests that potentially drive the selection of the type, intensity, and duration of treatment. This trend will help the realization of personalized medicine to the care of newly diagnosed prostate cancer patients.

## References


1. Polascik TJ, Pearson JD, Partin AW (1998) Multivariate models as predictors of pathological stage using Gleason score, clinical stage, and serum prostate-specific antigen. Semin Urol Oncol 16:160–171
2. Partin AW, Kattan MW, Subong EN, Walsh PC, Wojno KJ, Oesterling JE, Scardino PT, Pearson JD (1997) Combination of prostate-specific antigen, clinical stage, and Gleason score to predict pathological stage of localized prostate cancer. A multi-institutional update. JAMA 277:1445–1451
3. Partin AW, Yoo J, Carter HB, Pearson JD, Chan DW, Epstein JI, Walsh PC (1993) The use of prostate specific antigen, clinical stage and Gleason score to predict pathological stage in men with localized prostate cancer. J Urol 150:110–114
4. Partin AW, Steinberg GD, Pitcock RV, Wu L, Piantadosi S, Coffey DS, Epstein JI (1992) Use of nuclear morphometry, Gleason histologic scoring, clinical stage, and age to predict disease-free survival among patients with prostate cancer. Cancer 70:161–168
5. Hammond ME, Fitzgibbons PL, Compton CC, Grignon, DJ Page DL, Fielding LP, Bostwick D, Pajak TF (2000) College of American Pathologists Conference XXXV: solid tumor prognostic factors-which, how and so what? Summary document and recommendations for implementation. Cancer Committee and Conference Participants. Arch Pathol Lab Med 124:958–965
6. Ross S, Sheehan CE, Fisher HA, Kauffman RA, Dolen EM, Kallakury BV (2002) Prognostic markers in prostate cancer. Expert Rev Mol Diagn 2:129–142
7. Ross JS, Sheehan CE, Dolen EM, Kallakury BV (2002) Morphologic and molecular prognostic markers in prostate cancer. Adv Anat Pathol 9:115–128
8. Bubendorf L (2001) High-throughput microarray technologies: from genomics to clinics. Eur Urol 40:231–238
9. Bok RA, Small EJ (2002) Bloodborne biomolecular markers in prostate cancer development and progression. Nat Rev Cancer 2:918–926
10. Sidransky D (2002) Emerging molecular markers of cancer. Nat Rev Cancer 2:210–219
11. Amanatullah DF, Reutens AT, Zafonte BT, Fu M, Mani S, Pestell RG (2000) Cell-cycle dysregulation and the molecular mechanisms of prostate cancer. Front Biosci 5:D372–390
12. Alers JC, Rochat J, Krijtenburg PJ, Hop WC, Kranse R, Rosenberg C, Tanke HJ, Schroder FH, van Dekken H (2000) Identification of genetic markers for prostatic cancer progression. Lab Invest 80:931–942
13. Isaacs JT (1997) Molecular markers for prostate cancer metastasis. Am J Pathol 150:1511–1521
14. Gopalkrishnan RV, Kang DC, Fisher PB (2001) Molecular markers and determinants of prostate cancer metastasis. J Cell Physiol 189:245–256
15. Newmark JR, Hardy DO, Tonb DC, Carter BS, Epstein JI, Isaacs WB, Brown TR, Barrack ER (1992) Androgen receptor gene mutations inhuman prostate cancer. Proc Natl Acad Sci U S A 89:6319–6323

16. Goel A, Abou-Ellela A, DeRose PB (1996) The prognostic significance of proliferation in prostate cancer. J Urol Pathol 4:213–223
17. Scalzo DA, Kallakury BV, Gaddipati RV, Sheehan CE, Keys HM, Savage D, Ross JS (1998) Cell proliferation rate by MIB-1immunohistochemistry predicts post-radiation recurrence of prostatic adenocarcinomas. Am J Clin Pathol 109:163–168
18. Visakorpi T (1992) Proliferative activity determined by DNA flow cytometry and proliferating cell nuclear antigen (pcna) immunohistochemistry as a prognostic factor in prostatic carcinoma. J Pathol 168:7–13
19. Sadasivan R, Morgan R, Jennings S, Austenfeld M, Van Veldhuizen P, Stephens R, Noble M (1993) Overexpression of Her-2/neu may be an indicator of poor prognosis in prostate cancer. J Urol 150:126–131
20. Henke RP, Kruger E, Ayhan N, Hubner D, Hammerer P (1993) Numerical chromosomal aberrations in prostate cancer: correlation with morphology and cell kinetics. Virchows Arch Pathol Anat Histopathol 422:61–66
21. Kaibuchi T, Furuya Y, Akakura K, Masai M, Ito H (2000) Changes in cell proliferation and apoptosis during local progression of prostate cancer. Anticancer Res 20:1135–1139
22. Cheng L, Pisansky TM, Sebo TJ, Leibovich BC, Ramnani DM, Weaver AL, Scherer BG, Blute ML, Zincke H, Bostwick DG (1999) Cell proliferation in prostate cancer patients with lymph node metastasis: a marker for progression. Clin Cancer Res 5:2820–2823
23. Cowen D, Troncoso P, Khoo VS, Zagars GK, von Eschenbach AC, Meistrich ML, Pollack A (2002) Ki-67 staining is an independent correlate of biochemical failure in prostate cancer treated with radiotherapy. Clin Cancer Res 8:1148–1154
24. Drobnjak M, Osman I, Scher HI, Fazzari M, Cordon-Cardo C (2000) Overexpression of cyclin D1 is associated with metastatic prostate cancer to bone. Clin Cancer Res 6:1891–1895
25. Cheng L, Lloyd RV, Weaver AL, Pisansky TM, Cheville JC, Ramnani DM, Leibovich BC, Blute ML, Zincke H, Bostwick DG (2000) The cell cycle inhibitors p21WAF1 and p27KIP1 are associated with survival in patients treated by salvage prostatectomy after radiation therapy. Clin Cancer Res 6:1896–1899
26. Kuczyk MA, Bokemeyer C, Hartmann J, Schubach J, Walter C, Machtens S, Knuchel R, Kollmannsberger C, Jonas U, Serth J (2001) Predictive value of altered p27Kip1 and p21WAF/Cip1 protein expression for the clinical prognosis of patients with localized prostate cancer. Oncol Rep 8:1401–1407
27. Omar EA, Behlouli H, Chevalier S, Aprikian AG (2001) Relationship of p21(WAF-I) protein expression with prognosis in advanced prostate cancer treated by androgen ablation. Prostate 49:191–199
28. Yang RM, Naitoh J, Murphy M, Wang HJ, Phillipson J, deKernion JB, Loda M, Reiter RE (1998) Low p27 expression predicts poor disease-free survival in patients with prostate cancer. J Urol 159:941–945
29. Kuczyk M, Machtens S, Hradil K, Schubach J, Christian W, Knuchel R, Hartmann J, Bokemeyer C, Jonas U, Serth J (1999) Predictive value of decreased p27Kip1 protein expression for the recurrence-free and long-term survival of prostate cancer patients. Br J Cancer 81:1052–1058
30. Halvorsen OJ, Hostmark J, Haukaas S, Hoisaeter PA, Akslen LA (2000) Prognostic significance of p16 and CDK4 proteins in localized prostate carcinoma. Cancer 88:416–424
31. Kallakury BV, Sheehan CE, Ambros RA, Fisher HA, Kaufman RP Jr, Ross JS (1997) Prognostic significance of p34cdc2 and cyclin D1 protein expression in prostatic adenocarcinomas. Cancer 80:753–763
32. Ross JS, Nazeer T, Church K, Amato C, Figge H, Rifkin MD, Fisher HA (1993) Contribution of HER-2/neu oncogene expression to tumor grade and DNA content analysis in the prediction of prostatic carcinoma metastasis. Cancer 72: 3020–3028
33. Winkler HZ, Rainwater LM, Myers RP, Farrow GM, Therneau TM, Zincke H, Lieber MM (1988) Stage D1 prostatic carcinoma: significance of DNA ploidy patterns studied by flow cytometry. Mayo Clin Proc 63:103–112
34. Babiarz J, Peters JM, Miles B, Crissman JD (1993) The prognostic significance of the nuclear DNA content in localized prostatic adenocarcinoma. Anal Quant Cytol Histol 15:158–164
35. Peters-Gee JM, Miles BJ, Cerny JC, Gaba AR, Jacobsen G, Crissman JD (1992) Prognostic significance of DNA quantification in stage D1 prostatic carcinoma with the use of image analysis. Cancer 70:1159–1165
36. Foster CS, McLoughlin J, Bashir I, Abel PD (1992) Markers of the metastatic phenotype in prostate cancer. Hum Pathol 23:381–394
37. Montgomery BT, Nativ O, Blute ML, Farrow GM, Myers RP, Zincke H, Therneau TM, Lieber MM (1990) Stage B prostate adenocarcinoma: flow cytometric nuclear DNA ploidy analysis. Arch Surg 125:327–331
38. Ross JS, Figge H, Bui HX, del Rosario AD, Jennings TA, Rifkin MD, Fisher HA (1994) Prediction of pathologic stage and post prostatectomy disease recurrence by DNA ploidy analysis of initial needle biopsy specimens of prostate cancer. Cancer 74:2811–2818
39. Pollack A, Grignon DJ, Heydon KH, Hammond EH, Lawton CA, Mesic JB, Fu KK, Porter AT, Abrams RA, Shipley WU (2003) Prostate cancer DNA ploidy and response to salvage hormone therapy after radiotherapy with or without short-term total androgen blockade: an analysis of RTOG 8610. J Clin Oncol 21:1238–1248
40. Ross JS, Sheehan CE, Ambros RA, Nazeer T, Jennings TA, Kaufman RP Jr, Fisher HA, Rifkin MD, Kallakury BV (1999) Needle biopsy DNA ploidy status predicts grade shifting in prostate cancer. Am J Surg Pathol 23:296–301
41. Brinker DA, Ross JS, Tran TA, Jones DM, Epstein JI (1999) Can ploidy of prostate carcinoma diagnosed on needle biopsy predict radical prostatectomy and grade? J Urol 162:2036–2039
42. Weidner N, Carroll PR, Flax J, Blumenfeld W, Folkman, J (1993) Tumor angiogenesis correlates with metastasis in invasive prostate carcinoma. Am J Pathol 143:401–409
43. Weidner N, Carroll PR, Flax J, Blumenfeld W, Folkman J (1993) Tumor angiogenesis correlates with metastasis in invasive prostate carcinoma. Am J Pathol 143:401–409
44. Bigler SA, Deering RE, Brawer MK (1993) Comparison of microscopic vascularity in benign and malignant prostate tissue. Hum Pathol 24:220–226
45. Siegal JA, Enyou YU, Brawer MK (1995) Topography of neovascularity in human prostate carcinoma. Cancer 75:2545–2551
46. Silberman MA, Partin AW, Veltri RW, Epstein JI (1997) Tumor angiogenesis correlates with progression after radical prostatectomy but not with pathologic stage in Gleason sum 5 to 7 adenocarcinoma of the prostate. Cancer 79:772–779
47. Brawer MK, Deering RE, Brown M, Preston SD, Bigler SA (1994) Predictors of pathologic stage in prostatic carcinoma. Cancer 73:678–687
48. Gettman MT, Bergstrahl EJ, Blute M (1998) Prediction of patient outcome in pathologic stage T2 adenocarcinoma of the prostate: lack of significance for microvessel density. Urology 51:79–85
49. Jackson MW, Roberts JS, Heckford SE, Ricciardelli C, Stahl J, Choong C, Horsfall DJ, Tilley WD (2002) A potential autocrine role for vascular endothelial growth factor in prostate cancer. Cancer Res 62:854–859
50. Soker S, Kaefer M, Johnson M, KlagsbrunM, Atala A, Freeman MR (2001) Vascular endothelial growth factor-mediated autocrine stimulation of prostate tumor cells coincides with progression to a malignant phenotype. Am J Pathol 159:651–659
51. Korsmeyer SJ (1992) Bcl-2 initiates a new category of oncogenes: regulators of cell death. Blood 80:879–886

52. Raffo AJ, Perlman H, Chen MW, Day ML, Streitman JS, Buttyan R (1995) Overexpression of bcl-2 protects prostate cancer cells from apoptosis in vitro and confers resistance to androgen depletion in vivo. Cancer Res 55:4438–4445
53. Apakama I, Robinson MC, Walter NM, Charlton RG, Royds JA, Fuller CE, Neal DE, Hamdy FC (1996) Bcl-2 overexpression combined with p53 protein accumulation correlates with hormone-refractory prostate cancer. Br J Cancer 74:1258–1262
54. Gleave ME, Miayake H, Goldie J, Nelson C, Tolcher A (1999) Targeting bcl-2 gene to delay androgen-independent progression and enhance chemosensitivity in prostate cancer using antisense bcl-2 oligodeoxynucleotides. Urology 54:36–46
55. Bylund A, Stattin P, Widmark A, Bergh A (1998) Predictive value of bcl-2 immunoreactivity in prostate cancer patients treated with radiotherapy. Radiother Oncol 49:143–148
56. Mackey TJ, Borkowski A, Amin P, Jacobs SC, Kyprianou N (1998) Bcl-2/bax ratio as a predictive marker for therapeutic response to radiotherapy in patients with prostate cancer. Urology 52:1085–1090
57. Scherr DS, Vaughan ED Jr, Wei J, Chung M, Felsen D, Allbright R, Knudsen BS (1999) Bcl-2 and p53 expression in clinically localized prostate cancer predicts response to external beam radiotherapy. J Urol 162:12–17
58. Szostak MJ, Kaur P, Amin P, Jacobs SC, Kyprianou N (2001) Apoptosis and bcl-2 expression in prostate cancer: significance in clinical outcome after brachytherapy. J Urol 165:2126–2130
59. Oltvai ZN, Milliman CL, Korsmeyer SJ (1993) Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. Cell 74:609–619
60. Boise LH, Gonzalez-Garcia M, Postema CE, Ding L, Lindsten T, Turka LA, Mao X, Nunez G, Thompson CB (1993) Bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. Cell 74:597–608
61. Chao DT, Linette GP, Boise LH, White LS, Thompson CB, Korsmeyer SJ (1995) Bcl-XL and Bcl-2 repress a common pathway of cell death. J Exp Med 182:821–828
62. Sedlak TW, Oltvai ZN, Yang E, Wang K, Boise LH, Thompson CB, Korsmeyer SJ (1995) Multiple Bcl-2 family members demonstrate selective dimerizations with Bax. Proc Natl Acad Sci U S A 92:7834–7838
63. Clarke MF, Apel IJ, Benedict MA, Eipers PG, Sumantran V, Gonzalez-Garcia M, Doedens M, Fukunaga N, Davidson B, Dick JE, Minn AJ, Boise LH, Thompson CB, Wicha M, Nunez G (1995) A recombinant bcl-Xs adenovirus selectively induces apoptosis in cancer cells but not in normal bone marrow cells. Proc Natl Acad Sci U S A 92:11024–11028
64. Krajewska M, Krajewski S, Epstein JI, Shabaik A, Sauvageot J, Song K, Kitada S, Reed JC (1996) Immunohistochemical analysis of bcl-2, bax, bcl-X, and mcl-1 expression in prostate cancers. Am J Pathol 148:1567–1576
65. Hendry JH, Potten CS (1982) Intestinal cell radiosensitivity: a comparison for cell death assayed by apoptosis or by a loss of clonogenicity. Int J Radiat Biol Relat Stud Phys Chem Med 42:621–628
66. Pollack A, Salem N, Ashoori F, Hachem P, Sangha M, von Eschenbach AC, Meistrich ML (2001) Lack of prostate cancer radiosensitization by androgen deprivation. Int J Radiat Oncol Biol Phys 51:1002–1007
67. Sentman CL, Shutter JR, Hockenbery D, Kanagawa O, Korsmeyer SJ (1991) bcl-2 inhibits multiple forms of apoptosis but not negative selection in thymocytes. Cell 67:879–888
68. Huang A, Gandour-Edwards R, Rosenthal SA, Siders DB, Deitch AD, White RW (1998) P53 and bcl-2 immunohistochemical alterations in prostate cancer treated with radiation therapy. Urology 51:346–351
69. Rakozy C, Grignon DJ, Sarkar FH, Sakr WA, Littrup P, Forman J (1998) Expression of bcl-2, p53, and p21 in benign and malignant prostatic tissue before and after radiation therapy. Mod Pathol 11:892–899
70. Grossfeld GD, Olumi AF, Connolly JA, Chew K, Gibney J, Bhargava V, Waldman FM, Carroll PR (1998) Locally recurrent prostate tumors following either radiation therapy or radical prostatectomy have changes in Ki-67 labeling index, p53 and bcl-2 immunoreactivity. J Urol 159:1437–1443
71. Pollack A, Cowen D, Troncoso P, Zagars GK, von Eschenbach AC, Meistrich ML, McDonnell T (2003) Molecular markers of outcome after radiotherapy in patients with prostate carcinoma: Ki-67, bcl-2, bax, and bcl-x. Cancer 97:1630–1638
72. Wikstrom P, Bergh A, Damber JE (2000) Transforming growth factor-$\beta$1 and prostate cancer. Scand J Urol Nephrol 34:85–94
73. Ahmed MM, Chendil D, Lele S, Venkatasubbarao K, Dey S, Ritter M, Rowland RG, Mohiuddin M (2001) Early growth response-1 gene: potential radiation response gene marker in prostate cancer. Am J Clin Oncol 24:500–505
74. Bussemakers MJ (1992) Decreased expression of E-cadherin in the progression of rat prostatic cancer. Cancer Res 52:2916–2922
75. Giroldi LA, Schalken JA (1993) Decreased expression of the intercellular adhesion molecule E-cadherin in prostate cancer: biological significance and clinical implications. Cancer Metastasis Rev 12:29–37
76. Umbas R, Schalken JA, Aalders TW, Carter BS, Karthaus HF, Schaafsma HE, Debruyne FM, Isaacs WB (1992) Expression of the cellular adhesion molecule e-cadherin is reduced or absent in high-grade prostate cancer. Cancer Res 52:5104–5109
77. Bussemakers MJ, Van Bokhoven A, Tomita K, Jansen CF, Schalken JA (2000) Complex cadherin expression in human prostate cancer cells. Int J Cancer 85:446–450
78. Ross JS, Figge HL, Bui HX, del Rosario AD, Fisher HA, Nazeer T, Jennings TA, Ingle R, Kim DN (1994) E-cadherin expression in prostatic carcinoma biopsies: correlation with tumor grade, DNA content, pathologic stage and clinical outcome. Mod Pathol 7:835–841
79. Sandberg AA (1992) Chromosomal abnormalities and related events in prostate cancer. Hum Pathol 23:368–380
80. Kallakury BV, Sheehan CE, Winn-Deen E, Oliver J, Fisher HA, Kaufman RP Jr, Ross JS (2001) Decreased expression of catenins ($\alpha$ and $\beta$), p120 CTN and E-cadherin cell adhesion protein and E-cadherin gene promoter methylation in prostatic adenocarcinomas. Cancer 92:2786–2795
81. Harington KJ, Syrigos KN (2000) The role of E-cadherin-catenin complex: more than an intercellular glue? Ann Surg Oncol 7:783–788
82. Pujol P, Maudelonde T, Daures JP, Rouanet P, Brouillet JP, Pujol H, Rochefort H (1993) A prospective study of the prognostic value of cathepsin D levels in breast cancer cytosol. Cancer 71:2006–2012
83. Makar R (1994) Immunohistochemical analysis of cathepsin D in prostate carcinoma. Mod Pathol 7:747–751
84. Ross JS (1995) Quantitative immunohistochemical determination of cathepsin D levels in prostatic carcinoma biopsies. Am J Clin Pathol 104:36–41
85. McCabe NP, Angwafo FF 3rd, Zaher A, Selman SH, Kouinche A, Jankun J (2000) Expression of soluble urokinase plasminogen activator receptor may be related to outcome in prostate cancer patients. Oncol Rep 7:879–882
86. Wang YZ, Wong YC (1997) Oncogenes and tumor suppressor genes in prostate cancer: a review. Urol Oncol 3:41–46
87. Neto GJ, Humphrey PA (1994) Molecular biologic aspects of human prostatic carcinoma. Am J Clin Pathol 102 [Suppl 1]:S57–S64
88. Kleihues P, Schäuble B, zur Hausen A, Esteve J, Ohgaki H (1997) Tumors associated with p53 germline mutations: a synopsis of 91 families. Am J Pathol 150:1–13
89. Abate-Shen C, Shen MM (2000) Molecular genetics of prostate cancer. Genes Dev 14:2410–2434

90. Grignon DJ, Caplan R, Sarkar FH, Lawton CA, Hammond EH, Pilepich MV, Forman JD, Mesic J, Fu KK, Abrams RA, Pajak TF, Shipley WU, Cox JD (1997) p53 status and prognosis of locally advanced prostatic adenocarcinoma: a study based on RTOG 8610. J Natl Cancer Inst 89:158–165
91. Cheng L, Sebo TJ, Cheville JC, Pisansky TM, Slezak J, Bergstralh EJ, Pacelli A, Neumann RM, Zincke H, Bostwick DG (1999) p53 protein overexpression is associated with increased cell proliferation in patients with locally recurrent prostate carcinoma after radiation therapy. Cancer 85:1293–1299
92. Ross JS, Fletcher JA (1998) The HER-2/neu oncogene in breast cancer: prognostic factor, predictive factor and target of therapy. Oncologist 3:237–252
93. Signoretti S, Montironi R, Manola J, Altimari A, Tam C, Bubley G, Balk S, Thomas G, Kaplan I, Hlatky L, Hahnfeldt P, Kantoff P, Loda M (2000) Her-2-neu expression and progression toward androgen independence in human prostate cancer. J Natl Cancer Inst 92:1918–1925
94. Fossa A, Lilleby W, Fossa SD, Gaudernack G, Torlakovic G, Berner A (2002) Independent prognostic significance of HER-2 oncoprotein expression in pN0 prostate cancer undergoing curative radiotherapy. Int J Cancer 99:100–105
95. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang S I, Puc J, Miliaresis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R (1997) PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 275:1943–1947
96. Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, Langford LA, Baumgard ML, Hattier T, Davis T, Frye C, Hu R, Swedlund B, Teng DH, Tavtigian SV (1997) Identification of a candidate tumor suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 15:356–362
97. Teng DH, Hu R, Lin H, Davis T, Iliev D, Frye C, Swedlund B, Hansen KL, Vinson VL, Gumpper KL, Ellis L, El-Naggar A, Frazier M, Jasser S, Langford LA, Lee J, Mills GB, Pershouse MA, Pollack RE, Tornos C, Troncoso P, Yung WK, Fujii G, Berson A, Steck PA (1997) MMAC1/PTEN mutations in primary tumor specimens and tumor cell lines. Cancer Res 57:5221–5225
98. Graff JR, Konicek BW, McNulty AM, Wang Z, Houck K, Allen S, Paul JD, Hbaiu A, Goode RG, Sandusky GE, Vessella RL, Neubauer BL (2000) Increased AKT activity contributes to prostate cancer progression by dramatically accelerating prostate tumor growth and diminishing p27Kip1 expression. J Biol Chem 275:24500–24505
99. McMenamin ME, Soung P, Perera S, Kaplan I, Loda M, Sellers WR (1999) Loss of PTEN expression in paraffin embedded primary prostate cancer correlates with high Gleason score and advanced stage. Cancer Res 59:4291–4296
100. Wu X, Senechal K, Neshat MS, Whang YE, Sawyers CL (1998) The PTEN/MMAC1 tumor suppressor phosphatase functions as a negative regulator of the phosphoinositide 3-kinase/Akt pathway. Proc Natl Acad Sci U S A 95:15587–15591
101. Heidenreich B, Heidenreich A, Sesterhenn A, Srivastava S, Moul JW, Sesterhenn IA (2000) Aneuploidy of chromosome 9 and the tumor suppressor genes p16(INK4) and p15(INK4B) detected by in situ hybridization in locally advanced prostate cancer. Eur Urol 38:475–482
102. Nguyen TT, Nguyen CT, Gonzales FA, Nichols PW, Yu MC, Jones PA (2000) Analysis of cyclin-dependent kinase inhibitor expression and methylation patterns in human prostate cancers. Prostate 43:233–242
103. Furuya Y, Krajewski S, Epstein JI, Reed JC, Isaacs JT (1996) Expression of bcl-2 and the progression of human and rodent prostatic cancers. Clin Cancer Res 2:389–398
104. Kallakury BV, Figge J, Leibovich B, Hwang J, Rifkin M, Kaufman R, Figge HL, Nazeer T, Ross JS (1996) Increased bcl-2 protein levels in prostatic adenocarcinomas are not associated with rearrangements in the 2.8 kb major breakpoint region or with p53 protein accumulation. Mod Pathol 9:41–47
105. Gioeli D, Mandell JW, Petroni GR, Frierson HF Jr, Weber MJ (1999) Activation of mitogen-activated protein kinase associated with prostate cancer progression. Cancer Res 59:279–284
106. Yoshida BA, Dubauskas Z, Chekmareva MA, Christiano TR, Stadler WM, Rinker-Schaeffer CW (1999) Mitogen-activated protein kinase kinase 4/stress-activated protein/Erkkinase 1 (MKK4/SEK1), a prostate cancer metastasis suppressor gene encoded by human chromosome 17. Cancer Res 59:5483–5487
107. Razani B, Schlegel A, Liu J, Lisanti MP (2001) Caveolin-1, a putative tomor suppressor gene. Biochem Soc Trans 29:494–499
108. Li L, Yang G, Ebara S, Satoh T, Nasu Y, Timme TL, Ren C, Wang J, Tahir SA, Thompson TC (2001) Caveolin-1 mediates testosterone-stimulated survival/clonal growth and promotes metastatic activities in prostate cancer cells. Cancer Res 61:4386–4392
109. Cui J, Rohr LR, Swanson G, Speights VO, Maxwell T, Brothman AR (2001) Hypermethylation of the caveolin-1 gene promoter in prostate cancer. Prostate 46:249–256
110. Varambally S, Dhanasekaran SM, Zhou M, Barrette TR, Kumar-Sinha C, Sanda MG, Ghosh D, Pienta KJ, Sewalt RG, Otte AP, Rubin MA, Chinnaiyan AM (2002) The polycomb group protein EZH2 is involved in progression of prostate cancer. Nature 419:624–629
111. Yang G, Truong LD, Wheeler TM, Thompson TC (1999) Caveolin-1 expression in clinically confined human prostate cancer: a novel prognostic marker. Cancer Res 59:5719–5723
112. Joensuu H, Anttonen A, Eriksson M, Makitaro R, Alfthan H, Kinnula V, Leppa S (2002) Soluble syndecan-1 and serum basic fibroblast growth factor are new prognostic factors in lung cancer. Cancer Res 62:5210–5217
113. Anttonen A, Heikkila P, Kajanti M, Jalkanen M, Joensuu H (2001) High syndecan-1 expression is associated with favorable outcome in squamous cell lung carcinoma treated with radical surgery. Lung Cancer 32:297–305
114. Wiksten JP, Lundin J, Nordling S, Kokkola A, Haglund CA (2000) prognostic value of syndecan-1 in gastric cancer. Anticancer Res 20:4905–4907
115. Seidel C, Sundan A, Hjorth M, Turesson I, Dahl IM, Abildgaard N, Waage A, Borset M (2000) Serum syndecan-1: a new independent prognostic marker in multiple myeloma. Blood 95:388–392
116. Chakravarti A, Winter K, Wu C-L, Kaufman D, Hammond E, Parliament M, Tester W, Hagan M, Grignon D, Heney N, Sandler H, Shipley W (2002) Expression of the epidermal growth factor receptor (EGFR) is associated with improved outcome in muscle-invading bladder cancer. Proc ASCO, abstract 713
117. Anttonen A, Kajanti M, Heikkila P, Jalkanen M, Joensuu H (1999) Syndecan-1 expression has prognostic significance in head and neck carcinoma. Br J Cancer 79:558–564
118. Watanabe H, Hori A, Seno M, Kozai Y, Igarashi K, Ichimori Y, Kondo K (1991) A sensitive enzyme immunoassay for human basic fibroblast growth factor. Biochem Biophys Res Commun 175:229–235
119. Battistini B, D'Orleans-Juste P, Sirois P (1993) Endothelins: circulating plasma levels and presence in other biologic fluids. Lab Invest 68:600–628
120. Nelson JB, Hedican SP, George DJ, Reddi AH, Piantadosi S, Eisenberger MA, Simons JW (1995) Identification of endothelin-1 in the pathophysiology of metastatic adenocarcinoma of the prostate. Nature Med 1:944–949
121. Kopetz ES, Nelson JB, Carducci MA (2002) Endothelin-1 as a target for therapeutic intervention in prostate cancer. Invest New Drugs 20:173–182
122. Zellweger T, Ninck C, Mirlacher M, Annefeld M, Glass AG, Gasser TC, Mihatsch MJ, Gelmann EP, Bubendorf L (2003) Tissue microarray analysis reveals prognostic significance of syndecan-1 expression in prostate cancer. Prostate 55:20–29