REVIEW

The importance of determining the aggressiveness of prostate cancer using serum and tissue molecular markers

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Incidental prostate cancer (PCa) has been demonstrated at autopsy in about 80% of men aged 80 years and above and also in 10%-15% of younger men aged 30-50 years in the United States. These data imply a wide variation in aggressiveness of prostate cancer, from indolent tumors to aggressive cancers that kill the patients. The use of prostate specific antigen (PSA) in screening for PCa may detect even indolent disease for which radical prostatectomy may not be necessary. Currently available criteria such as histological grade, PSA level, stage of the disease do not always predict outcome. Furthermore, only about 80% of men with metastatic PCa will respond to first line hormone manipulation and once the patient develops hormone resistant prostate cancer (HRPCa), survival remains poor. Recent genomic and proteomic studies have provided many novel molecular markers that may help to redefine prognostic parameters. This paper is a review of studies using these novel markers in order to determine whether prostate cancer patients with the following characteristics have more aggressive cancer than those without: a) high serum levels of cathepsin B, survivin, Her – 2 / neu, IGFBP-2; b) low serum stefin A, IGFBP-3, c) positive immuno-staining of primary tumors for Her-2/neu, survivin and cathepsin B / stefin A ratio > 1 and d) gene expression of AMACR, HER-2/neu, high Bcl-2: Bax ratio and EZH2 in cancer cells. These markers have been chosen for review because they are among the most promising markers emerging currently.

Key Words: prostate cancer, aggressiveness, serum markers, molecular markers

Introduction

Prostate cancer (PCa) is a common malignancy in men and reports indicate that the incidence rate has increased markedly during the last two decades, whereas mortality has remained relatively stable. The apparent increase in the incidence may be a reflection of improved detection rates and possible overdiagnosis related to the use of serum prostate specific antigen (PSA).[1] The disease also has an extremely variable natural history, ranging from a noninvasive indolent form (so called "pussy cat") to a rapidly metastatic disease "tiger", which is fatal within a short time.[1] For example, about 80% of men aged 80 years old and above dying of disorders other than prostate cancer are often found to have incidental prostate cancer at autopsy. Similarly, autopsy findings in the United States revealed that 10%-15% of young men aged 30-50 years also had an incidental focus of prostate cancer. Furthermore, at the time of initial diagnosis of PCa, neoplastic cells may have already spread to extra prostatic organs in about 50% of patients.[1-4] In spite of advances in diagnosis and

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treatment of PCa in the last 50 years, our understanding of the biological behavior and clinical course of the cancer in patients remains unsatisfactory.[5,6] To resolve some of the complexities associated with this cancer, Gleason[7] proposed a histological grading system which is currently in routine use. The Gleason scoring system has been shown to be useful in determining prognosis of PCa.[8] The scoring system is defined into nine distinct histologic scores ranging from 2 to 10. The scores are grouped into three levels of differentiation, namely; well (2-4), moderately (5-7) and poorly (8-10) differentiated tumors.[7] In general, the lower the histologic score, the better the prognosis of the disease. There are numerous individuals, however, who do not follow the predicted course of this cancer using this system. Other parameters have been added to improve overall determination of prognosis of the disease. These include prostate volume, clinical stage of the disease, cancer cell nuclear morphometry and serum PSA to predict progression of the disease.[9-12] In spite of these additions, it is still not enough to predict the biological behavior or clinical progression of PCa in many cases.[5,6]

Recently, genomic and proteomic studies have begun to provide novel tumor markers that may help to redefine prognostic parameters based on a better understanding of the biology of PCa progression at the molecular level. The molecular markers that are under intense evaluation in PCa include; proteases (e.g. cathepsin B), metalloprotease, or plasminogen activators and their endogenous inhibitors (e.g. cysteine protease inhibitors, cystatins or stefins, tissue inhibitor of matrix metalloproteins TIMPs or MMPIs) that are often involved in regulation of protein activities during tumor progression and in the development of metastatasis.[5,6,13-17] Other markers under investigation include human epidermal growth factor receptor – 2 (Her – 2/neu), a member of the human epidemal growth factor receptor (HER) family, which plays a key role in cooperation with other HER receptors via a complex signaling network to regulate cell growth, differentiation and survival.[18-20] Also under investigation are; serum level of survivin and its tissue expression in prostate cancer,[21] serum levels and tissue expression of interleukin – 6 and interleukin – 6 receptor α,[22-25] and lastly serum levels and tissue expression of insulin-like growth factor binding protein (IGFBP) IGFBP-2 and IGFBP-3.[26-28] The over-expression/amplification of certain molecular markers in prostate cancer tissue is said to assist in identifying patients with aggressive PCa. These molecular markers include, α-methylacyl-coenzyme A racemase (AMACR),[29,30] enhancer zeste homolog 2 (EZH2),[31,32] Bcl-2: Bax ratio[33,34] and HER-2/neu.[35,36]

Prostate carcinogenesis

Prostate cancer, as with other cancers, develops in the background of diverse genetic and environmental factors.[37] Multiple complex molecular events characterize prostate cancer initiation, unregulated growth, invasion and metastasis. Distinct sets of genes and proteins are believed to dictate progression from precursor lesion, to localized disease and finally to metastatic disease. Clinically localized prostate cancer can be effectively ablated using surgical or radiation treatment. Metastatic disease however, is invariably incurable and leads to death. Androgen ablation is the most common therapy for advanced prostate cancer, leading to massive apoptosis of androgen – dependent malignant cells and temporary tumor regression. In most cases, however the tumor reemerges and can proliferate independently of androgen signals.[6]

Prostate cancer detection

The advent of prostate specific antigen (PSA) screening has led to earlier detection of prostate cancer.[38] Coincident with increased serum PSA testing, there has been a dramatic increase in the number of prostate needle biopsies performed.[39] This has resulted in a surge of equivocal prostate needle biopsy reports and men at times worried unnecessarily about the threat of prostate cancer.[40] However, the stage shift associated with the advent of PSA screening may also be associated with diagnosis of a substantial number of prostate cancer cases that may have non aggressive clinical natural history, the so called "indolent" prostate cancer. Even before the advent of PSA screening, it was noted that 70% to 80% of Gleason score 6 cancers and as many as 20% of Gleason 7 cancers may have a non aggressive course with no cancer death if observed without intervention for up to 15years.[41] With the population of men greater than 65 years of age expected to increase due to improved health care, it is important to discern such indolent prostate cancers from aggressive cancers that require intervention.[6] Tissue/serum biomarkers can play a vital role in such clinical decision making, particularly because it has been shown that standard clinical parameters such as PSA, Gleason score and clinical stage, have limited utility in separating indolent from aggressive prostate cancers.[42] Genomic and proteomic approaches promise to accelerate identification of lethal biomarkers that can help distinguish clinically latent prostate cancer from aggressive prostate cancer. Conversely it is expected that some markers will discern aggressive cancers amenable

to local/regional therapy from those in which standard, regionally targeted therapy (e.g. prostatectomy or radiation therapy) would be inadequate and in which systemic therapy may be indicated, despite ostensible lack of metastases at presentation.

Nomogram based prediction of recurrence

Various prognostic models have been used in an attempt to objectively predict the risk of recurrence of prostate cancer after primary treatment.[43-45] These prognostic nomograms use pre and post treatment clinical and pathologic parameters such as PSA concentration, clinical stage, biopsy Gleason score, seminal vesicle status, prostatic capsular invasion, surgical margin status, or lymph node status to assess the risk of recurrence.[46-50] Although these nomograms currently provide physicians with the best available criteria for deciding treatment modalities, they are limited in their accuracy of outcome prediction and many parameters being relatively subjective are restricted in their universal applicability. In order to improve on (if not replace) the currently available nomograms, there is a need for better defined biochemical and molecular markers capable of objectively, independently and accurately predicting prostate cancer prognosis.[6]

There is a need to investigate the usefulness of some of these novel tissue and serum biomarkers in distinguishing benign prostatic diseases (BPH, BPH + acute/chronic prostatitis), causes of considerable diagnostic dilemma in our experience in Kuwait,[51] as well as indolent prostate cancer from those at risk of lethal progression or those likely to develop hormone resistance. The list of such markers is growing on a daily basis, in the urological literature.[6] We have chosen to review the literature on markers that are not only promising but those that are expressed in prostate cancer tissues and are also present in serum. Markers that are found to be prostate cancer specific will have a possible role in a more accurate determination of disease stage or progression.

Tissue and serum biomarkers

Tissue and serum cathepsin B/stefin A ratio

Cathepsin B (CB) is involved in the degradation of extracellular matrix proteins and the progression of cancer cells in patients with prostate carcinoma and with other solid organ carcinomas.[52-56] Serum CB level is elevated in prostate carcinoma patients compared with levels in individuals with a normal prostate and in patients with benign prostatic hyperplasia (BPH).[52,53,57,58] Lysosomal and plasma membrane/endosome subcellular fraction associated CB activities are significantly higher in patients with malignancy than in those with BPH. This was further documented by immunogold electron microscopic localization of CB in these fractions.[53] The enzymatic function of CB is regulated by endogenous inhibitors, the stefins (cystatins), in patients with malignant and nonmalignant tumors[52,54] including patients with prostate carcinoma.[52,53,57] Therefore, it is important to evaluate CB and stefin A concurrently and to relate these to the Gleason histologic score. Sinha et al[59] found that within each Gleason score, the ratio of CB to stefin A can be subdivided into three groups: CB greater than stefin A, CB less than stefin A, and CB equal to stefin A. There is a need to determine if a specific ratio of cathepsin B to stefin A in prostatectomy tissue samples by quantitative immunohistochemistry could predict aggressiveness of prostate carcinoma as indicated by metastasis of cancer cells to the regional pelvic lymph nodes. The goal would be to relate these parameters to the phenotypes of aggressive prostate carcinomas with Gleason score, clinical stage, extraprostatic extension of cancer cells to the prostatic capsule, margin, and seminal vesicles, pre surgery and post surgery serum total PSA level, and/or mortality survival data of patients.

Prostate carcinomas with ratios of CB equal to stefin A or CB less than stefin A, regardless of Gleason score, have been postulated to have low metastatic potential,[59] and those with the ratio of CB greater than stefin A are thought to have increased lymph node metastases and higher mortality rates. Both increased lymph node metastases and higher mortality rates are indicative of the aggressiveness of prostate carcinoma in patients. These ratios may therefore be useful to identify clones of aggressive or less aggressive prostate carcinomas within a particular Gleason score of the tumor.[59] Recently, Hara et al[60] showed that elevated serum cathepsin D level in men with prostate cancer could be used as a new predictor of disease progression, but not of prognosis.

Tissue and serum human epidermal growth factor receptor-2 (Her-2/neu)

Human epidermal growth factor receptor-2 (Her-2/erbB-2) belongs to a family of four transmembrane receptors involved in signal transduction pathways that regulate cell growth and differentiation. Overexpression/amplification of Her-2/neu is associated with malignancy and a poor prognosis in breast cancer. Her-2/neu acts as a networking receptor that mediates signaling to cancer cells, causing them to proliferate. Her-2/neu receptors exist as

monomers but dimerize on ligand binding. HER ligands are bivalent growth factor molecules whose low affinity site binds to Her-2/neu. No Her-2/neu specific ligand has been identified but Her-2/neu is the preferred heterodimerization partner for other Her-2/neu receptors. Her-2/neu containing heterodimers are relatively long lived and potent.[1] HER3 has no inherent activity and is the major and most potent dimerization partner of Her-2/neu. Her-2/neu overexpression biases the formation of HER2 containing heterodimers, leading to enhanced responsiveness to stromal growth factor and oncogenic transformation. Removal of Her-2/neu from the cell surface or inhibition of its intrinsic enzymatic activity may reduce oncogenicity. Research suggests that the antitumor efficacy of Her-2/neu specific antibodies such as Herceptin relates to their ability to direct Her-2/neu to a Cbl dependent endocytosis and degradation pathway. The reported clinical therapeutic efficacy of anti Her-2/neu monoclonal antibodies in breast cancer highlights the importance of understanding the biology of Her-2/neu.[19]

Taneja et al,[20] recently showed that, serum Her-2/neu level predicts disease stage and outcome in men with prostate cancer. Her-2/neu overexpression predicts for progression to advanced disease in breast cancer patients. A small percentage of patients with localized prostate cancer have been found to demonstrate Her-2/neu overexpression upon immunohistochemical staining.[20] They concluded that, serum Her-2/neu levels are a reproducible means of identifying prostate cancer patients with Her-2/neu overexpression, serum levels correlate with disease stage and the presence of metastatic disease and in hormone refractory disease. Taneja et al[20] reported that elevation of serum Her-2/neu predicts a shortened time to death. Further clinical follow-up will be required in order to identify the progression rates of men with increased Her-2/neu levels at earlier stages of disease. It is also necessary to characterize the levels and clinical significance of shed Her-2/neu antigen in the serum of men with prostate cancer as well as its expression in prostate cancer tissue.

Tissue and serum insulin-like growth factor binding protein genes

Complementary DNA microarray based gene expression profiling of hormone refractory CWR22R prostate cancer xenograft compared with a xenograft of the parental, hormone sensitive CWR22 strain leads to the identification of insulin like growth factor binding protein 2 (IGFBP-2) as the most consistently overexpressed gene in the hormone refractory tumors.[6,61,62] Further, immunohistochemical analysis of tissue microarrays demonstrated high expression of IGFBP2 protein in 100% of the hormone refractory clinical tumors, in 36% of the primary tumors and in 0% of the benign prostatic specimens ($p = 0.0001$).[61] Subsequent immunohistochemical study of 193 radical prostatectomy specimens from patients with localized prostate adenocarcinoma revealed significant overexpression of IGFBP2 in all cases of high grade PIN and in > 90% of cancers, regardless of the grade.[63]

Analyses of circulating levels of IGFBP2/3 revealed that plasma IGFBP2 level was elevated in patients with prostate cancer as compared with healthy subjects, whereas among patients with localized prostate cancer, IGFBP2 was inversely associated with tumor volume, Gleason score, and seminal vesicle involvement. A higher IGFBP2 level was a significant predictor of organ confined disease, and a lower preoperative IGFBP2 level was significantly associated with an increased probability of disease progression.[27] In contrast, plasma IGFBP3 levels are highest in healthy subjects, followed by patients with localized prostate cancer, and in lymph node metastasis; levels are lowest in bone metastases.[27] In a more recent report, Miyata et al[28] examined prognostic potential of IGFBP3 levels and IGFBP3 to PSA ratios in patients with prostate cancer and they too observed that IGFBP3 levels were lower in patients with localized tumor or BPH. In addition the IGFBP3 to PSA ratio was found to be correlated significantly with relapse free survival of patients with advanced prostate cancer treated with hormonal therapy and it served as an independent predictor of cause specific survival.[28] These observations have also been confirmed recently by Inman et al[64] who showed that, IGFBP-2 is an independent predictor of prostate cancer survival. They further showed that high preoperative IGFBP-2 is a predictor of post-radical prostatectomy biochemical relapse-free survival that is independent of clinical stage, biopsy Gleason score and preoperative PSA. They also showed that high IGFBP-2 levels were associated with better survival in patients who had received neoadjuvant androgen deprivation therapy (ADT) but worse survival in those who did not. Thus experimental and epidemiologic evidence suggest that the insulin like growth factor (IGF) family is important in prostate cancer. Some members of this family appear to have prognostic significance in prostate cancer. It is therefore necessary to evaluate the usefulness of serum levels and tissue immunostaining intensity of IGFBP-2 and -3 in predicting the extent of disease as well as disease progression in patients with prostate cancer.

Tissue and serum interleukin–6 and interleukin–6 receptor–α

Localized prostate cancer tissue has been shown to over express interleukin–6 and interleukin–6 receptor as compared with normal prostate tissue.[25] Serum interleukin–6 levels have been found to be significantly elevated in hormone refractory patients with prostate cancer[22] and they are associated with prostate cancer progression, decreased survival and extent of disease, making serum interleukin–6 level a promising prognostic marker.[25] In another study, interleukin–6 and the interleukin 6 soluble receptor levels in the plasma of men with metastatic prostate cancer were reportedly highly increased, and independently predicted biochemical progression after radical prostatectomy.[24] In a review by Canto et al,[65] the investigators surmise that, based on a correlation of their levels with tumor volume, interleukin-6 and the interleukin-6 receptor are produced by the cancer cells. It remains to be seen if these observations and subsequent studies would lead to the emergence of interleukin-6 and interleukin-6 receptor as prognostic markers of tumor recurrence.[6]

Tissue and serum survivin

Survivin, first described in 1997 as a structurally unique inhibitor of apoptosis protein (IAP) is present during fetal development but is generally absent in fully differentiated adult tissue.[66] Currently, there are two small exceptions; survivin is expressed in normal human endometrium during the proliferative phase and in prostatic neuroendocrine cells.[67] Survivin is expressed at the G2/M regulatory point in the cell cycle, and is thought to associate with microtubules of the mitotic spindle in a specific and saturable reaction that is regulated by microtubule dynamics. Thus, survivin may play a role in maintaining cell viability at mitosis via the coupling of apoptosis control with cell division.

Survivin is over expressed in a number of common human cancers in vivo, including those of lung, colon, pancreas, prostate, and breast. In colorectal cancer, breast cancer, non small cell lung cancer diffuse large B-cell lymphoma esophageal cancer and neuroblastoma survivin expression correlates with unfavorable prognosis and poor survival.[68,69] Survivin expression also has been shown to have negative correlation with response to conventional chemotherapy. A recent in vitro study has targeted Survivin with an antisense oligonucleotide prior to chemotherapy, with encouraging results in malignant pre-B cell lines.[70]

Recently, Shariat et al[21] reported that survivin expression is associated with features of biologically aggressive prostate cancer. They stated that, the expression of survivin gradually increased from normal prostate tissue, to low grade primary carcinoma, to high grade primary carcinoma and was highest in lymph node metastases. Furthermore, they found that survivin expression was associated with alteration of the TGF beta pathway and with overall and aggressive biochemical progression after radical prostatectomy.

Molecular markers of disease aggressiveness and progression

α-methylacyl – coenzyme A racemase (AMACR)

AMACR is an enzyme involved in the β-oxidation of branched fatty acids. Protein levels of AMACR were found to be elevated in prostate carcinomas and premalignant lesions, thus rendering AMACR an important PCa diagnostic marker.[29,71] A variant of the AMACR gene has been genetically linked with cellular differentiation in PCa and was found to co-segregate with the PCa phenotype in hereditary prostate cancer.[72] Although AMACR has been correlated with cell differentiation in PCa cell lines, no correlation has been established yet for the expression of AMACR between indolent and aggressive cancers.[30] Most of the detection methods used to study the expression of AMACR were looking at its protein levels.

Enhancer zeste homolog 2 (EZH2)

EZH2 is a transcription factor that belongs to the polycomb protein family (PcG). It is a repressor protein, which is mostly active during embryogenesis and declines after differentiation.[73,74] Both tissue and cDNA Microarray analyses showed that EZH2 was over-expressed in hormone-refractory, metastatic and localized prostate cancer.[74] Over expression of EZH2 was also found to be associated with poor prognosis of PCa thus suggesting its involvement in the progression of PCa.[75] This finding led to the hypothesis that the suppressor EZH2 could be blocking prostate cellular proliferation and further differentiation thus promoting PCa development. EZH2 is considered to be one of the important markers currently used to distinguish between indolent and aggressive PCa.[32]

Bcl-2: Bax ratio

Bcl-2 and Bax are well known markers of cell death by apoptosis. While Bcl-2 belongs to the anti-apoptotic protein family, Bax belongs to the pro-apoptotic family.[76] Bcl-2 and Bax exert their regulatory effect via hetero-dimerization in order to induce

apoptotic cell death as a response to external cellular stress.[77] The ratio of Bcl-2: Bax is thought of as a fine cellular balance that regulates cellular viability. It has been reported that the cellular expression of these two proteins is inversely proportional in PCa. Over expression of Bcl-2 has been associated with decreased levels of Bax and poor clinical treatment outcome and radiation resistance in PCa patients.[78] Recurrent tumors and radiotherapy were found to induce the expression of Bcl-2, which led workers to argue that Bcl-2 could be used as a marker for aggressive tumors.[79] Few studies have shown the usefulness of Bax expression as a pretreatment predictor of outcome after radiotherapy in patients with PCa.[80]

Her-2/neu

Real – time (RT) PCR has also been used to determine the expression of Her-2/neu from prostate tissue. Preliminary findings are as for those using serum and as discussed above under Her-2/neu serum markers.

Hypotheses

1) From the foregoing, it is possible to theorize that aggressive prostate cancer:
   - demonstrates the following immunohistochemical patterns; Her-2/neu +ve, survivin +ve, cathepsin B / stefin A ratio > 1
   - is associated with higher serum concentrations of cathepsin B, IGFBP-2, survivin, Her-2/neu and low serum concentration of stefin A and IGFBP-3
   - is associated with tissue overexpression of AMACR, EHZ2, HER-2/neu, and high Bcl : Bax ratio at the mRNA level.
2) Patients with BPH, acute or chronic prostatitis have normal serum cathepsin B (4.5 ng/ml $\pm$ 0.5 ng/ml) stefin A (6.1ng/ml + 0.4 ng/ml), Her-2neu (< 15 ng/ml), survivin (< 4 ng/ml), IGFBP-2 and IGFBP-3 (900 ng/ml-4000 ng/ml).
3) Serum levels of these markers are not affected by prostatic inflammation, infarction or prostatic manipulations e.g. prostatic biopsy.

Conclusions

Most of the studies cited above are preliminary, and have been performed in Caucasian populations with a high incidence of prostate cancer. It will be interesting to find out whether these observations in Caucasians can be replicated in populations with low incidence of the disease as well as possible difference in clinical manifestation and course of the disease. Overall, exploration of prognostic molecular markers is expected to help attain the grander objectives of 1) elucidation of molecular determinants of prostate cancer progression, 2) better stratification of disease stages, 3) prediction and assessment of response to radical prostatectomy, radiation therapy, or neoadjuvant therapies and 4) better understanding of the mechanism of acquisition of androgen insensitivity as well as the capacity for metastasis, including occult metastatic cells that may be present in some cases of localized prostate cancer.

It is not a hyperbole to state that not much advance has been made in terms of effective therapy of hormone responsive prostate cancer since the landmark observations of Huggins and Hodges in the early 1940's.[81] For androgen independent metastatic prostate cancers the prognosis remained dismal until early this century when docetaxel based chemotherapeutic regimens were shown to be associated with significantly longer median survival.[82] It is our hope that using molecular biology, in the years ahead, our understanding of the aggressiveness of each prostate cancer can be further refined. Consequently and hopefully surgical, chemo-hormonal therapy or chemotherapeutic regimens can become more individualized. For example, this may lead to a change in the current practice where all men with localized prostate cancer are subject to radical prostatectomy. Clearly, the field of molecular tissue and serum biomarkers in prostate cancer is an ever expanding one with new markers being discovered on a regular basis.[83] For this field to mature from an area of interesting research to one of clinical utility, there is a need for the process to change from one of discovery to a phased development approach. The process of sorting out the winners needs to begin in earnest for the benefit of mankind. □

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