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Significance role of Biomarkers used in Prostate cancer

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Abstract: Human cancer is a complex disease caused by genetic instability and accumulation of multiple molecular alterations. Last several decades have witnessed the emergence of various therapeutic regimens to contain this fatal disease. However, survival rates for patients with cancers, especially those detected at an advanced stage remains discouragingly low. This correlates with the observation that at the time of clinical presentation, more than 60% of patients with breast, lung, colon, prostate, and ovarian cancer have hidden metastatic colonies. At this stage, therapeutic modalities are limited in their effectiveness, thereby warranting early disease detection using sensitive biomarkers. The present article is a brief review of the emerging trends in the development of biomarkers for early detection and precise evaluation of cancer disease.

Keywords: Biomarkers, Proteomics, Cancer, Prostate cancer, PSA

Introduction:

Cancer is a disease characterized by abnormal growth and development of normal cells beyond their natural boundaries. Despite of global efforts to limit the incident of this disease, cancer has become the leading cause of death in the last 50 years with breast cancer the most common malignancy in women and the second most common cause of cancer-related mortality and prostate cancer being the most common solid organ malignancy diagnosed in men in Europe and USA and the second most frequent cause of cancer-related death in men. The management of these high risk cancers requires diagnosis at

an early stage, which specifies the need for specific and sensitive biomarkers.[1, 2]

A biomarker is a quantifiable laboratory measure of a disease specific biologically relevant molecule that can act as an indicator of a current or future disease state. Sometimes, certain molecules are differentially expressed in cancer cells relative to their normal counterparts and their altered levels could be measured to establish a correlation with the diseased state. Alternatively, certain molecules are specifically present in tumors and are different from corresponding normal tissue and can be identified as biomarkers of tumorigenesis. Depending on their site of evaluation they may be tissue or circulating biomarkers.

Tissue markers include different categories such as membrane receptors, oncogenes, tumor suppressor genes, nuclear antigens, growth factors, components of degradome whereas circulating markers include the wide category of tumor-associated antigens (TAA). Tissue biomolecular markers, aside from being prognostic and predictor factors also play a central role in targeted therapies that are among the emerging directions of cancer therapeutics. Selective biomarkers may be able to define susceptibility risks and assist in tumor detection and diagnosis allowing timely therapeutic interventions for an effective treatment.[1]

Prostate cancer remains the most common malignancy and second-leading cause of cancer deaths among males in the United States, with an estimated 232,090 new cases in 2005, accounting for 33% of new male cancers.[3] Since the discovery of prostate-specific antigen (PSA) in 1970 by Ablin et al. routine PSA testing has become the mainstay of prostate cancer detection. Concurrent with the widespread use of PSA screening, prostate cancer incidence has increased, while mortality rates have decreased.[4-6] Since the advent of PSA screening, the improved detection of early disease has increased the lifetime risk of a diagnosis of prostate cancer to 16%, whereas the lifetime risk of death from prostate cancer is only 3.4% .[7]

Biomarkers for detection of prostate cancer:

1. Proteases as cancer biomarkers:

Cysteine proteases- Cysteine cathepsins (CCs) are a family of lysosomal proteases of papain family that are often upregulated in

various human cancers.[8, 9] This family has 11 members (cysteine cathepsin B, L, S, K, H, C, O, F, V, W and X) which share a conserved active site that is formed by a cysteine residue.[10]

Cysteine cathepsins are synthesized as 30–50 kDa preproenzymes which are directed into lysosomes where they serve their function of protein hydrolysis after cleavage to form active enzyme.[11] The best known CCs, cathepsins B, L,H, F, O, C, X are distributed ubiquitously in most tissues whereas cathepsins S, K, W and V are comprised to specific tissues.[12]

In normal cells, cysteine cathepsins are usually located in lysosomes, whereas during cancer progression they move to the cell surface, from where they can be secreted into the extracellular milieu to promote tumor invasion through several possible mechanisms. Altered levels of cysteine cathepsins have been found to be associated with several pathological states including cancer.[13, 14]

Several studies have reported tissue specific enhanced expression, activity and mis-localization of cysteine cathepsins in human cancers.[12, 15-19] The upregulation of cysteine cathepsins has been reported in cancers of both epithelial and mesenchymal origin, including breast, brain, lung, gastrointestinal, colorectal cancer and melanoma among others. Due to their enhanced levels and tumor specific localization cysteine cathepsins have been implicated as significant biomarkers for the prognosis of cancer.[14]

Elevated cathepsin B expression correlates with good prognostic value in lung, breast, ovarian, brain, head and neck cancer and melanoma and in premalignant lesions situated within colon, thyroid, liver, and prostate.[18, 20] Similarly, increased cathepsin L activity has been observed in multiple tumor types and can be used as a prognostic indicator of shorter survival rates in patients with breast, colorectal and head and neck cancers.[11]It was also reported that both cathepsin B and L are also implicated in human pancreatic endocrine tumors.[21]

Serine proteases- Human kallikreins (hKs) are serine proteases widely expressed in diverse tissues and implicated in a range of normal physiological functions. Dysregulated enzyme expression is associated with multiple diseases including cancer. Their role has been suggested in

cancer metastasis and invasion. This has made them promising diagnostic biomarkers for several cancer types, including ovarian, breast, and prostate.[22, 23]

The most important kallikrein is prostate specific antigen (PSA) or hK3 having high diagnostic significance in prostate cancer (to be discussed in detail in the succeeding section). However, several other kallikreins are emerging as novel biomarkers for various cancers such as ovarian. These include hK1-2 and hK4-15 expressed in a myriad of tissues. All 15 kallikrein genes are differentially expressed in cancer with overexpression of kallikreins 4, 5, 6, 7, 8, 10, 11, 13, 14, and 15 observed in ovarian carcinoma tissues and serum.24,25In particular, KLK4 and KLK5 mRNAs have been shown to be overexpressed and are indicators of poor prognostic outcome in grade 1 and grade 2 tumors, suggesting that these genes are associated with more aggressive forms of ovarian cancer.[24, 25]

The KLK10 gene is downregulated in acute lymphblastic leukemia.[26]It is also noted that hK11and hK14 have emerged as complimentary biomarkers for prostate cancer along with hK3 (PSA).[27, 28]These have been shown to be overexpressed in prostate malignancy and shows an incremental expression pattern as the disease progresses from earlier stage (stage I) to late stage (stage II).[29] Furthermore, the differential expression of KLK10 and KLK14, along with KLK13 splice variants in testicular cancer tissues, have been reported to be favorable markers, showing reduced expression in malignant forms of the disease than in healthy individuals.[30-32] Table 1 summarizes the status of various proteases of diagnostic significance in cancer.[11, 23]

Table 1:

Altered levels of proteases as biomarkers of cancer diagnosis and prognosis.

Cancer	**Cysteine protease (cathepsins)**	**Expression**	**Localization in tissues/ fluids**
Breast cancer	CatB, L	↓	Cancer tissues and cell lines
	CatH	↑	Cancer tissues and

			serum
Ovarian cancer	CatB	↑	Cancer tissues
	CatB	↑	Serum
	CatL	↑	Serum
Uterine cervix cancer	CatB	↑	Serum and cancer tissues
Prostate cancer	CatB, H, S	↑	Cancer tissues and cell lines
	CatB, L	↑	Cancer tissues
	CatB, L, H	↓	Cancer cell cultures
Bladder cancer	CatB	↑	Plasma and urine
	CatL	↑	urine
Colorectal cancer	CatB	↑	Cancer tissues
	CatH, L	↑	Cancer tissues
Gastric cancer	CatB, L	↑	Cancer tissues
Pancreatic cancer	CatB	↑	Cancer tissues
	CatH	↑	SK-PC-1 pancreas
Lung cancer	CatB, L	↑	Cancer tissues
	CatH		Cancer tissues
	CatH	↑	Serum
	CatS	↑	Cancer tissues
Brain tumor	CatB, L, H	↑	Cancer tissues
Head and neck cancer	CatB, L	↑	Cancer tissues
	CatH	↓	Cancer tissues
Melanoma	CatB, H	↑	Serum
	CatB		Cancer tissues

	CatL	↑	Cancer tissues
	CatB, L, H	↑	Cancer tissues
	CatH	↑	Cancer tissues
Kidney cancer	CatB, C, H, L, S	↓	Cancer tissues
Uterus cancer	CatL	↓	Cancer tissues
Thyroid cancer	CatB	↑	Cancer tissues
Serine protease (kallikreins)			
Acute lymphoblastic leukemia (all)	KLK10	↓	Cancer cells
Brain	KLK6	↓	Cancer cells
Breast	KLK1, 14	↓	Cancer cells
	KLK5, 6, 7, 8,10,12,13	↓	Serum
Cervical	KLK7, 8	↑	Cancer cells
Colon	KLK1	↓	Cancer tissues
	KLK6, 8, 10	↑	Cancer tissues
Colorectal	KLK6	↑	Cancer cells
Esophageal	KLK6	↑	Cancer cells
Gastric	KLK6, 10	↑	Cancer cells
Lung	KLK5, 10, 11	↑	Cancer cells
Ovarian	KLK2, 4,7,9,11,15	↑	Cancer cells
	KLK5, 13	↑	Serum Atscites
	KLK6, 8, 10	↑	Cancer cells and serum
	KLK14	↓	Cancer tissue
Pancreatic	KLK6, 10	↑	Cancer cells

Prostate	KLK2, 5, 6, 10, 13	↓	Cancer cells
	KLK4, 11, 14, 15	↑	Cancer cells

(↑) Enhanced expression of protease in diseased as compared to normal material.

(↓) Lowered expression of protease in diseased as compared to normal material.

2. Nucleic acid based biomarkers:

Methylated DNA as biomarker- The regulation of gene expression by aberrant methylation has been well established in tumor biology.[33] The epigenetic phenomenon of hypermethylation in tumor-related genes has been implicated in cancer development and progression.[34, 35]Tumor-related free methylated DNA in blood of cancer patients have been assessed for their clinical utility. Circulating tumor cells are considered to be the source of floating DNA which is released into the circulation upon the death of the tumor cells.[36, 37]Studies have reported the presence of tumor specific hypermethylated DNA at tumor-related gene promoter regions in patients with metastatic tumors.[38, 39]Methylation-specific PCR (MSP) is a sensitive and specific assay for tumor-related DNA methylation in serum/plasma, urine and other fluids.[40]

Various studies have reported the diagnostic potential of circulating tumor- related methylated DNA in serum for detection of cancer.[41] Lofton-Day et al. have identified three markers, TMEFF2 (transmembrane protein with EGF-like and two follistatin-like domains 2), NGFR [nerve growth factor receptor), and SEPT9 (septin 9), all having a colorectal cancer (CRC)-specific methylated pattern in plasma.[42] However, SEPT9 predicted the presence of CRC significantly more accurately than TMEFF2 ($P < 0.001$) or NGFR ($P < 0.01$). A cutoff of 0.011 lg/L of methylated SEPT9 DNA was shown to produce a specificity of 95% and a sensitivity of 52%. Similar panels of cancer specific methylated markers for prostate and bladder cancer have also been evaluated in urine samples.[43, 44]

MicroRNAs as cancer biomarker- MicroRNAs (miRNA) are naturally occurring and highly conserved small non coding RNAs, 18–25 nucleotides in length, that regulate mRNA expression by binding to the 30 untranslated region (3' UTR) of mRNA leading to the impairment in the synthesis of the corresponding protein.[45] A

general downregulation of miRNAs has been observed in tumors compared with normal tissues.[46] 110 This is consistent with the earlier findings related to the functional aspects of first identified miRNAs, the products of the C. elegans genes lin-4 and let-7.[47]

3. Prostate-specific antigen (PSA):

Description: 33-kDa single-chain glycoprotein secreted by prostatic epithelium.

Function: Serine protease involved in the liquefaction of seminal fluids.

Method of detection: Serum immunoassays used to measure free PSA, complexed PSA, and total PSA.

PSA is a serine protease that is secreted by the prostatic epithelium, as well as the epithelial lining of the periurethral glands, and functions in the liquefaction of seminal fluids. Because the role of PSA in the detection and prognosis of prostate cancer has been well described elsewhere in multiple publications, this review of PSA will be brief.[48] 12 Using the generally accepted cutpoint of 4.0 ng/ml to prompt prostate biopsies, PSA has a reported positive predictive value of 37% and a negative predictive value of 91% when averaged across 11 series [13].[49] In other words, a man with a normal digital rectal examination and PSA value between 4.0 and 10.0 ng/ml has approximately a 25% chance of being diagnosed with prostate cancer .[50]

4. prostate-specific membrane antigen (PSMA)

Description: 100-kDa type 2 integral membrane glycoprotein overexpressed by prostate cancer (PCA) epithelial cells.

Function: Cell surface peptidase, possibly involved in hydrolyzing peptides in prostatic fluids, signal transduction, cell migration, and nutrient uptake. Also potential receptor function.

Method of detection: Measured in serum, seminal fluid and urine by polymerase chain reaction (PCR), mass spectroscopy, or monoclonal antibody assays. In vivo imaging detects PSMA expressing tumor cells through immunoscintigraphy.

Prostate-specific membrane antigen (PSMA) is a type II integral membrane glycoprotein, originally identified from an antibody to the LNCaP prostate cancer cell line, and recognized by Horoszewicz et al. in 1987 as being overexpressed in the epithelial cells and serum of patients with prostate cancer.[51] A complementary DNA encoding for PSMA was subsequently cloned, and the gene was mapped to chromosome 11.[52, 53] Since that time, PSMA has been evaluated in multiple studies as a potential diagnostic and prognostic marker for prostate cancer with mixed results.[51, 54-57]

In particular, multiple studies have suggested that PSMA correlates well with Gleason score and/or the stage of disease, and can be used to monitor posttreatment disease progression.[58, 59, 60-63]

5. Prostate stem cell antigen (PSCA):

Description: 123 amino acid glycosyl phosphatidylinositol-anchored glycoprotein expressed on the cell surface of prostate basal cells.

Function: Unknown. May play a role in progenitor cell function, tumorigenesis, and clinical progression.

Method of detection: Detected in tissues immunohistochemically and by mRNA in situ hybridization. mRNA detected in peripheral blood through reverse transcriptase polymerase chain reaction (RT-PCR).

Prostate stem cell antigen (PSCA) is a prostate-specific glycosyl phosphatidylinositol-anchored glycoprotein that is expressed on the cell surface and is encoded by a gene on chromosome 8.[64] Several studies have shown a correlation between increased PSCA expression and the presence of prostate cancer, as well as with Gleason score, stage, progression, and the presence of metastasis. In addition to identifying PSCA protein in prostate cancer tissues immunohistochemically[65-68] investigators have successfully detected PSCA RNA in peripheral blood through the use of reverse transcriptase polymerase chain reaction (RT-PCR).[69]

6. Early prostate cancer antigen (EPCA):

Description: PCA-associated nuclear structural protein.

Function: Unknown. Possibly involved in early prostate carcinogenesis.

Method of detection: Detectable in tissues immunohistochemically and in serum using an enzyme-linked immunosorbent assay (ELISA).

Early prostate cancer antigen (EPCA) is a prostate cancer-associated nuclear structural protein, originally identified through protein profiling of rat prostate tissue.[70] Subsequent studies of immunohistochemical assays for EPCA have shown sensitivities and specificities ranging from 84% to 94% and 85% to 100%, respectively, for the detection of prostate cancer.[71, 72]

7. Chromogranin A:

Description: Pro-hormone peptide released by neuroendocrine cells.

Function: Unknown. Likely has paracrine and/or autocrine functions related to its calcium-binding properties.

Method of detection: Detectable in serum using a quantitative sandwich immunoassay, immunoradiometric assay, or ELISA.

Chromogranin A (CgA) is the peptide most frequently produced by neuroendocrine cells and is, therefore, commonly used as tissue or serum marker to detect neuroendocrine features.[73] Although the exact function of chromogranin A is unknown, it is thought to participate in the regulation of protein secretion, including insulin, packaging of secretory granules, and peptide hormone processing.[74]

8. α-methylacyl-CoA racemase (AMACR):

Description: 382 amino acid (approximately 44 kDa) peroxisomal and mitochondrial enzyme up regulated in PCA.

Function: Involved in bile acid synthesis, peroxisomal -oxidation of branched-chain fatty acids and conversion of branchedchain fatty acids from Rstereo- isomers to S-stereoisomers.

Method of detection: Detected in tissues immunohistochemically and through RT-PCR. Detected in urine by Western blot analysis and in prostatic secretions by RT-PCR. Autoantibodies to AMACR are detectable in serum.

Through the use of high-throughput molecular profiling, the gene for α-methylacyl-CoA racemase (AMACR) has

been overexpressed in prostate cancer tissues.[75-77] AMACR is an enzyme that catalyzes the conversion of branched-chain fatty acids from R-stereoisomers to S-stereoisomers and also participates in the peroxisomal β-oxidation of branched-chain fatty acids.[78-80] Immunohistochemical staining using monoclonal antibodies to AMACR is now commonly used in many institutions to assist in the pathologic diagnosis of prostate cancer. More recently, detection of AMACR in prostatic secretions and urine has also shown promise for the diagnosis of prostate cancer.[81, 82]

9. Autoantibody signatures:

Description: Panel of peptides derived from PCA tissue to which patients with PCA have autoantibodies commonly form.

Function: Identity and function of peptides currently under investigation.

Method of detection: Autoantibodies to select PCA associated peptides are detectable in serum using phage-peptide microarrays.

The detection of autoantibodies has long been the mainstay of diagnosis for autoimmune and infectious diseases, such as systemic lupus erythematosus and hepatitis. Multiple studies have also shown that patients with cancer produce detectable autoantibodies to tumor-associated antigens overexpressed in neoplastic cells and recognized as "foreign" by the immune system.[83-86]

10. Gene fusion proteins:

Description: Fusion of the 5' untranslated region of the androgen regulated gene transmembrane protease, serine 2 (TMPRSS2) to transcription factors ETS related gene (ERG) or ETS related variant gene 1 (ETV1).

Function: Unknown. Potential protein products are currently under investigation.

Method of detection: Gene fusions detectable in tissue using RT-PCR or FISH. Detection of the gene fusions in serum and urine is currently under investigation.

Most of the biomarkers described thus far were identified through assays designed to identify proteins that are highly differentially expressed between 2 classes of samples, such as benign and cancerous prostate tissue. However, the genetic events, often chromosomal rearrangements,

underlying the development of cancer in specific cases are frequently heterogeneous. For example, both chromosomal inversion, which results in CBFb:MYH11 fusion proteins and translocation (8;21), which results in AML1:ETO fusion proteins, contribute to the development of acute myeloid leukemia.[87-89]

Conclusion:

Biomarkers have the potential to be used clinically to screen for, diagnose, or monitor the activity of diseases and to guide molecular targeted therapy or assess therapeutic response. Prostate-specific antigen (PSA) levels in blood are widely used in prostate cancer for the management of this disease at every stage of progression. Especially in men with no additional risk factors, PSA alone provides an appropriate marker up to 30 yr into the future. After assessment of an early PSA test, the screening frequency may be determined based on individualized risk. It may rather be that a constellation of markers will have more predictive power. There is no doubt that progress will continue based on the collaboration of basic researchers, clinicians, and biomedical firms.

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