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Invited critical review

Glycosylation of prostate specific antigen and its potential diagnostic applications

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ABSTRACT

Prostate specific antigen (PSA) assays are widely used for early detection of prostate cancer. However, those analyses are associated with considerable sensitivity and specificity problems. Several approaches have been developed to tackle this issue. PSA is a glycoprotein, which is primarily produced by the prostatic epithelial cells. Aberrant glycosylation modification of proteins is a fundamental characteristic of tumorigenesis. Study of PSA glycoforms offers interesting diagnostic perspectives. Modern technology allows us to analyze PSA glycoforms in a variety of clinical samples (serum or plasma, urine, seminal fluid, tissue). A number of novel techniques, such as lectin-based detection methods, mass spectrometry, 2-dimensional electrophoresis and capillary electrophoresis have been developed to analyze PSA glycosylation. This article reviews the technical and diagnostic aspects of PSA glycoforms.

Contents

Abbreviations: 2-DE, two-dimensional electrophoresis; AA(A), *Aleuria aurantia* agglutinin; AMACR, α-methylacyl-CoA racemase; BPH, benign prostatic hyperplasia; BPSA, benign prostatic hyperplasia associated PSA; Con A, Concanavalin A; CE, capillary electrophoresis; ELLA, enzyme-linked immunosorbent lectin assay; EPCA, early prostate cancer antigen; (G)LIA, (glycoprotein) lectin-based immunosorbent assay; GSTP1, glutathione S-transferase-π gene; HPLC, high-performance liquid chromatography; fPSA, free PSA; hK2, human kallikrein-related peptidase 2; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LIF, laser induced fluorescence; MAL, *Maackia amurensis* lectin; Mgat5b, β1,6-Nacetylglucosaminyltransferase-5b; MS, mass spectrometry; MS/MS, tandem mass spectrometry; PCA3, prostate cancer antigen 3; PCI, protein C inhibitor; PHA-E, *Phaseolus vulgaris* erythroagglutinin; PHA-L, *Phaseolus vulgaris* leukoagglutinin; pI, isoelectric point; proPSA, inactive proenzyme PSA; PSA, prostate specific antigen; PSMA, prostate-specific membrane antigen; qRT-PCR, quantitative real-time-polymerase chain reaction; ROC, receiver operating characteristic; SEMG, semenogelin; SLex, Sialyl Lewis X; SNA, *Sambucus nigra* agglutinin; SPEG, solid-phase extraction of N-linked glycoprotein/peptide; SRM, selected reaction monitoring; TJA-II, *Trichosanthes japonica* agglutinin-II; tPSA, total PSA; UEA, *Ulex europaeus*; UPAR, urokinase-type plasminogen activator receptor.

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1. Introduction

Prostate cancer is the most common malignancy in men. Prostate specific antigen (PSA) assays are widely used for early detection of prostate cancer. However, those analyses are associated with considerable sensitivity and specificity problems (especially in the diagnostic gray zone with a PSA concentration of 2.5–10 ng/mL), complicating the distinction of the various forms of prostate disease [1]. The majority of patients (65–75%) who undergo a prostate biopsy due to a moderate PSA elevation have no evidence of cancer and a quarter of the prostate cancer patients are known with normal PSA levels [2–5].

Immunoreactive PSA can occur in serum as a free, unbound molecule (fPSA) [10–30% of total serum PSA (tPSA)] or as a complex with protease inhibitors, such as α_1-antichymotrypsin and α_2-macroglobulin [6]. In prostate disease, the basement membrane can be disrupted, and PSA can access the peripheral circulation. Prostate cancer patients have less fPSA than those with benign prostatic hyperplasia (BPH) and the serum ratio of non-complexed fPSA to tPSA is used for the diagnosis of or to better differentiate BPH and prostate cancer. However this calculation is not without problems as the levels of fPSA vary with age [6–10]. In order to increase the diagnostic accuracy, multiple immunoassays for different molecular forms of PSA [proPSA, intact PSA and BPSA (benign prostate hyperplasia associated PSA)] have been proposed without a substantial improvement in the ability to distinguish between benign and malignant disease [2,11,12]. Only the percentage of PSA circulating in the free form (%fPSA) has been widely accepted as a diagnostic tool in patients with low tPSA levels [13]. In urine, PSA is only detected in its free form (fPSA) with concentrations largely exceeding the values observed in serum or plasma [8].

Some new biomarkers have recently emerged, such as kallikrein 2, urokinase-type plasminogen activator receptor (UPAR), prostate-specific membrane antigen (PSMA), early prostate cancer antigen (EPCA), prostate cancer antigen 3 (PCA3), α-methylacyl-CoA racemase (AMACR) and glutathione S-transferase-π gene (GSTP1) hypermethylation, but only a few of them have shown clinical value [14]. Given that PSA is a glycoprotein, efforts for improvement of its diagnostic potential have focused on searching for cancer-specific PSA forms in both the amino-acid and carbohydrate portions. Aberrant glycosylation of proteins occurs in approximately half of all serum proteins and as this process is a fundamental characteristic of tumorigenesis and aggressive clinical behavior, variation of PSA glycosylation has been studied [15]. This common posttranslational modification affects protein–protein interactions, cell-cell recognition, adhesion, and motility [16]. Characterization of protein glycosylation patterns from disease tissues may identify changes specific to the disease development and improve diagnostic performance. In the present paper, the knowledge about glycosylation of PSA will be reviewed, as well as its potential diagnostic implications.

2. General characteristics

PSA is a 28.4 kDa single chain glycoprotein (237 amino acids) with isoenzymes in the isoelectric point (pI) range of 6.2–7.5 [17,18]. It is made up of five interchain disulphide bonds and 8.3% carbohydrate in the form of an N-linked oligosaccharide side chain at Asn-45 [19]. This serine protease is a member of the kallikrein family produced primarily by the prostatic epithelial cells and is secreted as an inactive proenzyme (proPSA) into the seminal plasma fluid, where it can be activated by kallikrein-related peptidase 2 (hK2) and other endopeptidases of the prostate [20–22]. Due to its restricted chymotrypsin-like endoproteolytic activity, PSA is able to cleave semenogelin 1 and 2 (SEMG1, SEMG2), fibronectin, laminin and gelatin [23–25]. PSA is present at relatively large concentrations (0.5–3 mg/mL) in seminal fluid. Only a minor fraction is inactivated due to internal cleavages or complex formation with the protein C inhibitor (PCI, SERPINA5), released from the seminal vesicles. However in serum, PSA reference values are much lower. A considerable structural heterogeneity of fPSA has been reported in serum, seminal plasma, and hyperplastic or cancerous tissue [26–31]. Glycosylation analysis of PSA from normal seminal fluid and from the prostate cell line LNCaP revealed major differences in their glycan structures, reflecting the changes in glycosylation pattern that usually take place on the tumor cells surface. Significant changes mostly affect the outer ends of the oligosaccharide chains. PSA glycans from normal and tumor sources are both complex biantennary structures that differ in their content of GalNAc, sialic acid, and fucose, giving rise to distinct carbohydrate epitopes [32,33]. Sialic acid is a negatively charged carbohydrate. An increase or decrease in the content of this monosaccharide on a PSA N-glycan chain can modify the PSA's pI [34].

3. Techniques used to demonstrate PSA glycosylation

3.1. Lectin-based detection formats

Lectins are plant and animal proteins with a natural carbohydrate binding functionality. Several lectin-based glycan detection methods have been developed, such as lectin microarray, lectin column chromatography, lectin affinity electrophoresis and enzyme-linked lectin assay (ELLA) or (glycoprotein) lectin-based immunosorbent assays ((G)LIA) [35–37] (Table 1). An interesting method to study glycan structures is the ELLA or (G)LIA, which is a modified enzyme-linked immunosorbent assay (ELISA) using lectins instead of specific antibodies [38]. In (G)LIA, the glycoprotein is first captured from a complex clinical sample onto antibody coated plates and afterwards the glycosylation of the target glycoprotein is directly detected using lectins [16]. Lectins recognize specific mono- or oligosaccharides through complimentary sugar-binding sites on proteins and at least in the case of plant lectins no interaction with protein backbones has been observed [39].

Sambucus nigra agglutinin (SNA) has been used to investigate PSA sialylation by its binding to the disaccharide structure of sialic acid in an α2,6-linkage to galactose. *Maackia amurensis lectin I* (MAL I) binds to the trisaccharide structure of sialic acid in an α2,3-linkage to galactose which is then in a β1,4-linkage to N-acetylglucosamine [40]. *Maackia amurensis lectin II* (MAL II) appears to bind only particular carbohydrate structures that contain α2,3-linked sialic acid [41]. In a small study of patients with biopsy-confirmed prostate cancer ($n=26$) and patients with biopsy-confirmed non-cancer ($n=26$) prior to biopsy, Meany et al. investigated the clinical performance of these assays. ROC analysis showed that %free PSA (AUC 0.85) was superior to ELLA (AUC 0.53–0.63) in all 52 subjects. However, an improved performance trend ($p=0.27$) of the total SNA assay (AUC = 0.71) in comparison with %free PSA (AUC = 0.54) was observed in patients with %free PSA of 10–20% (diagnostic gray zone) [42].

Concanavalin A (Con A) is a lectin with an affinity towards branched α-mannosidic structures, high-mannose type, hybrid type and biantennary complex type N-glycans. Using a quantitative precipitin method, the interaction of Con A with serum PSA has been explored for differentiation between BPH and prostate cancer. An altered posttranslational glycosylation or less glycosylation of PSA during malignant transformation of the human prostate has been proposed to explain the difference in Con A binding pattern with PSA. Although an important overlap has been observed, it has been suggested that a cut off value of <3.0 μg/mL carbohydrate content in the precipitate for the interaction between Con A and serum PSA is a strong indication of malignant process [43–48].

The lectin from *Ulex europaeus* (UEA-1) binds avidly α1,2-linked fucose structures. fPSA from prostate cancer patients showed a significant increase in fucosylation compared with fPSA from patients with BPH. An ELLA with UEA-1 used for differentiation between BPH and prostate cancer was developed with a specificity of 92% and a sensitivity of 69%. fPSA measurement was 70% specific and 56% sensitive