

Sialylation and fucosylation of cancer-associated prostate specific antigen

M. M. Kosanović, M. M. Janković

Institute for the Application of Nuclear Energy – INEP, Beograd, Serbia and Montenegro

Summary

Purpose: To examine sialylation and fucosylation of prostate cancer-associated prostate-specific antigen (PCa PSA) from localized prostate cancer and from metastatic prostate cancer, as relevant indicators of tumour stage-dependent microheterogeneity of its oligosaccharide chain.

Materials and methods: Sera and urine were collected from a group of consenting patients and used as source of the examined PSA. Lectin-affinity chromatography was performed on the columns with immobilized sialic acid-specific lectins *Sambucus nigra* agglutinin (SNA) and *Maackia amurensis* lectin (MAA), and fucose-specific lectins *Aleuria aurantia* agglutinin (AAA) and *Ulex europaeus* agglutinin (UEA). PSA elution from the corresponding columns was monitored

by measuring immunoreactivity to free (f) PSA form.

Results: The results of lectin affinity chromatographies of PCa PSA indicated distinct and high microheterogeneity of its oligosaccharide chain in respect to the presence of glycoforms differing in the position and the type of linkage of sialic acid or fucose, as well as alterations related to the examined PSA source (localized or metastatic prostate cancer).

Conclusion: The observed decrease in SNA reactivity to metastatic PCa PSA in comparison with PCa PSA from localized prostate cancer may be of interest in the monitoring of response to therapy and scoring of its metastatic potential.

Key words: cancer, fucosylation, glycosylation, lectin, PSA, sialylation

Introduction

PSA is initially characterized as 33 kD glycoprotein having the biantennary N-linked oligosaccharide chain of N-acetylglucosamine type with a sialic acid group at the end of the two branches [1,2]. However, experimental evidence indicated that, besides this main form, the PSA molecule exists in various glycoforms, having also the monoantennary glycans as well as different outer chain moieties [3]. Microheterogeneity of the PSA molecule was observed in both normal and pathological conditions of prostate tissue [4-6]. The alterations in PSA sugar chain attract attention as

a potential marker for differential diagnosis between benign prostatic hyperplasia and cancer, both of them being associated with increase in serum PSA values in overlapping range of concentrations [7]. Based on chemical analysis and lectin-binding pattern, an inverse correlation between the amount of PSA glycan and its serum PSA concentration was suggested [8]. Our previous data obtained on cancer-associated urinary PSA isoforms as well as available data on tumor cell line-derived PSA suggested that sialylation and fucosylation may be of interest as relevant indicators of PSA glycosylation [4,5,9].

The purpose of this study was to further analyze PCa PSA in search for a difference between PSA from localized prostate cancer and PSA from metastatic prostate cancer.

Materials and methods

Materials

Vector Laboratories (Burlingame, USA) affinity columns with immobilized plant lectins were used.

Received 01-12-2004; Accepted 05-01-2005

Author and address for correspondence:

Miroslava Janković, PhD
Institute for the Application of Nuclear Energy – INEP
Banatska 31 b
11080 Zemun
Serbia and Montenegro
Tel: +381 11 199 949
Fax: +381 11 618 724
E-mail: miraj@inep.co.yu

Fucose and lactose were purchased from Sigma (St. Louis, USA). Mouse monoclonal anti-PSA antibodies clone 8311 (100% PSA and 76% PSA α 1-ACT reactivity) and clone 8A6, isotype IgG2a (100% fPSA epitope 1 reactivity) were purchased from Medix, Biochemica and HyTest, Pharmacity (Finland), respectively. All other chemicals were reagent grade.

Sample collection and PSA determination

This study was carried out on a group of consenting patients diagnosed at the Institute for Urology and Nephrology, Clinical Center of Serbia, SCG. Urine and blood samples were collected from patients with localized prostate cancer before initiation of any treatment, and from patients with metastatic prostate cancer, with no regard to any medical treatment. The samples were used immediately or stored at -20° C until processed.

Serum and urinary PSA concentration (total (t) or fPSA) was determined using a solid phase two-site immunoradiometric assay IRMA PSA/ IRMA fPSA (INEP, SCG). All probes were done in duplicate, and, when needed, samples were diluted in 0.05 M phosphate buffer saline (pH 7.4) containing 1% BSA.

Pooled urine from patients with localized prostate cancer ($n=4$) was chosen as a source of PSA, since its concentration was several times higher than the corresponding serum concentration, enabling its direct and reliable detection during subsequent chromatographic procedures. As for patients with metastatic prostate cancer ($n=8$), sera having highly elevated PSA (>1500 μ g/L fPSA) and alkaline phosphatase (>700 U/L) were used as a PSA source and were examined separately.

Lectin affinity chromatography

Plant lectins capable of differential recognition of sialic acid and fucose were used as a tool for separating particular PSA glycoforms [10-12]. Affinity chromatography was performed on the columns with immobilized plant lectins, according to the manufacturers' instructions. The following lectins were used: SNA, MAA, UEA and AAA. The bed volume was 2mL for all columns used. Urine (0.3 mL; 730 μ g/L fPSA) or serum sample (0.3 mL; 1531 μ g/L fPSA) were loaded on each of the columns and after 3 h incubation at room temperature the fractions (0.5 mL) were collected. The unbound and the retarded fractions were eluted with binding buffer (0.1 M PBS, pH 7.2). The bound fraction was specifically eluted with addition of competitive sugars: 0.3 M lactose (for SNA), and 0.1 M fucose (for UEA and AAA). In the case of MAA no sugar was used for elution, since there was no competitive monosac-

charide. Finally, tightly bound fraction was eluted by the addition of low pH buffers: 0.1 M glycine-HCl, pH 3.0 (for AAA and UEA) or 0.3 M lactose in 0.2 M acetic acid (for SNA) and 0.2 M acetic acid (for MAA). The elution of PSA was monitored by recording immunoreactivity as follows: the aliquot of each fraction (100 μ L) was allowed to react simultaneously with capture anti-PSA immobilized on solid phase (polystyrene tube) and tracer iodinated anti-fPSA antibody (100 μ L) overnight at room temperature with constant shaking. The non-bound fPSA was aspirated, followed by three times of washing with 1 mL of 0.1 M PBS-Tween 20. The bound radioactivity (cpm) was counted on Wizard gamma counter (Perkin Elmer, USA).

Results

The elution profile of PCa PSA from the columns with immobilized sialic acid- and fucose-specific lectins is shown in Figure 1(a-d). Generally, low affinity interactions were observed with all lectins tested, resulting in the separation of PSA glycoforms retarded in elution. However, in the case of SNA, strong binding was also evident, especially to PSA from localized prostate cancer.

Thus, on the SNA column, PCa PSA from localized cancer was separated into 3 fractions, differing in their relative abundances (Figure 1a). Non-bound fraction appeared as one sharp peak, with no retardation in the trailing edge of the elution front. Bound fraction, elutable with lactose, comprised 70% of total PSA loaded and it was eluted as one broad peak. Its elution profile suggested the presence of at least 5 PSA molecular subpopulations. In addition to this, a small PSA fraction released by the addition of lactose in low pH solution, was also noticeable. The elution profile of metastatic PCa PSA from the SNA column revealed also 3 fractions (Figure 1a). The ratio of bound to non-bound PSA was significantly decreased in comparison to the examined PSA from localized prostate cancer, i.e. it passed through the column almost completely as non-bound.

PCa PSA from localized cancer interacted with immobilized MAA, and this was seen as strong retardation in its elution from the corresponding column (Figure 1b). In addition, one small broadened peak eluted as tightly bound fraction with low pH buffer was also observed. The retarded fraction (58% of total PSA) was partially separated from non-bound fractions. It appeared as one major broadened peak suggesting the presence of different PSA molecular subpopulations. A small tightly bound fraction eluted with low pH buffer was also observed. Metastatic PCa PSA showed similar

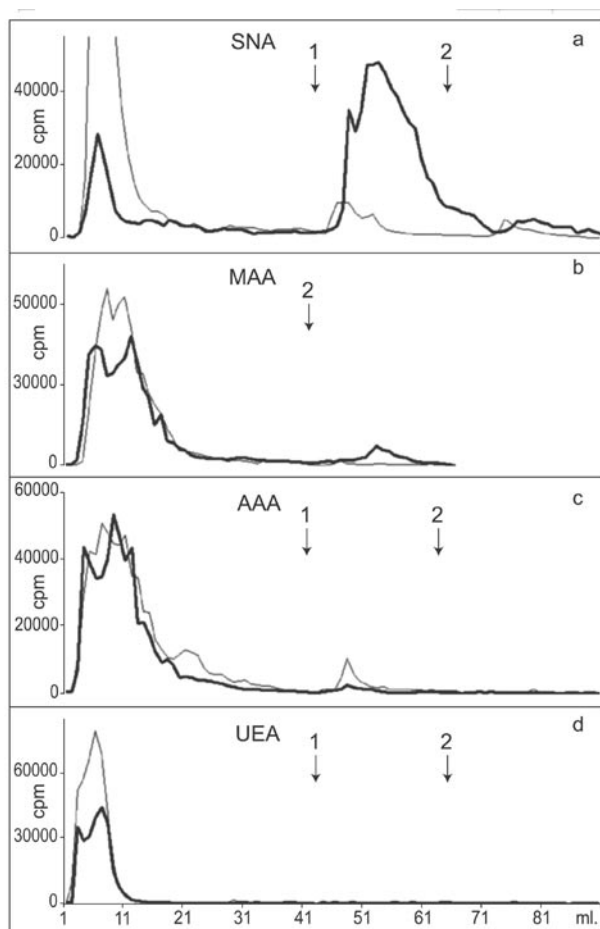


Figure 1. Lectin affinity chromatography of PCa PSA from localized prostate cancer (—) and metastatic prostate cancer (---). Arrows indicate the start of elution with: 1) sugar solution, 2) low pH solution, as described in Materials and Methods. The elution was monitored by free PSA -immunoreactivity.

behaviour on the MAA column, demonstrably interacting with this lectin, except that no heterogeneity related to PSA subpopulations was so noticeable (Figure 1b). No tightly bound fraction eluted with low pH buffer was observed as in PCa PSA from localized cancer.

PCa PSA from localized cancer was separated on the AAA column into 2 main fractions corresponding to the non-bound and retarded fraction, whereas sugar elutable fractions were present in trace amounts (Figure 1c). The retarded fraction was predominant and had a profile indicating one main retarded subpopulation and 3-4 partially separated subpopulations in the trailing edge of the elution front. No significant differences were observed when metastatic PCa PSA was subjected to lectin affinity chromatography on AAA, except for relative abundance of sugar elutable fraction which was even less noticeable.

Affinity chromatography on the UEA column

resulted in comparable PCa PSA elution profile of both examined samples (Figure 1d). Addition of binding buffer partially separated non-bound and retarded fraction, but the presence of PSA molecular subpopulations in the latter was not noticeable as on the AAA column. Also, in both samples, only trace amounts of fucose-elutable PSA fraction were observed.

Discussion

The results of lectin affinity chromatographies of PCa PSA indicated distinct and high microheterogeneity of its oligosaccharide chain in respect to the presence of glycoforms differing in the position and type of linkage of sialic acid or fucose, as well as alterations related to the examined PSA source (localized or metastatic prostate cancer). The discrimination of the microheterogeneity of oligosaccharide chain which was related to PSA itself from that of PSA-ACT complex, being influenced by glycosylation of ACT, was achieved by recording immunoreactivity to fPSA.

Elution profiles from the columns with sialic acid-specific lectins suggested the existence of cancer-associated glycoforms differing in the extent of sialylation and having both α 2,6 linked sialic acid (recognized by SNA) and α 2,3 linked sialic acid (exclusively recognized by MAA) at the terminal position of the PSA oligosaccharide chain. SNA reactivity was significantly decreased in metastatic PCa PSA in comparison with PCa PSA from localized prostate cancer. MAA reactivity was not so changed in metastatic PCa PSA, except for the loss of distinct molecular subpopulations being evident in the elution front of PSA from localized cancer.

The current proposed structure of PSA oligosaccharide chain refers to α 2,6 linked sialic acid at the terminal position, but recent evidence based on chemical analysis as well as lectin-binding pattern speaks in favour of the existence of α 2,3 linked sialic acid in both normal and PCa PSA, too [4,13]. Specifically, decrease in sialic acid content of PSA from serum and urine, or even total absence of sialylation of PSA from the metastatic cell line LNCaP were reported [4]. The results obtained in this study were in general agreement with data indicating decrease in PSA glycosylation accompanying malignant transformation, whereas the distinct discrepancies in the observed extent of sialylation could be probably related to the examined sources of PSA.

In addition to sialylation we also examined the fucosylation of PCa PSA. The approach was based on the use of two fucose-specific lectins, AAA and UEA, which recognize α 1,6 linked fucose (core) and α 1,2 linked fu-

cose (outer) residue, respectively [11,12]. It is reported that 80% of PSA molecules from normal seminal fluid are core-fucosylated (Fuc α 1,6 linked to the innermost GalNAc) and that Fuc α 1,2Gal, i.e. H2 epitope, can be found on PSA isolated from LNCaP cell line [1,4].

Our results point to the presence of both α 1,6 linked and α 1,2 linked fucose in PSA oligosaccharide chain, with no significant differences seen between PSA from localized or metastatic prostate cancer. PCa PSA microheterogeneity was more pronounced in respect to AAA reactivity.

It is well known that the glyco-phenotype of different cells and tissues can change profoundly during carcinogenesis [14-17]. One of the possible reasons is shown to be related to the alterations in the activity and/or expression of particular enzymes involved in glycosylation pathways [17,18]. Thus, abnormal production of sialyl- and fucosyl-transferases resulting in the appearance of distinct carbohydrate epitopes in genitourinary malignancies including prostate cancer has been reported [4,7,19,20]. The results obtained in this study suggested that such changes could also have affected PSA oligosaccharide chain i.e. its microheterogeneity. In clinical terms, the observed differences between PCa PSA from localized and metastatic prostate cancer may be of importance in the monitoring of response to therapy and scoring of its metastatic potential.

Acknowledgements

This work was supported by the Ministry of Science and Environment Protection of the Republic of Serbia, project code 1504: Glycobiological aspects of physiological and pathophysiological processes.

References

1. Belanger A, Halbeek H, Graves HC et al. Molecular mass and carbohydrate structure of prostate-specific antigen: studies for establishment of an international PSA standard. *Prostate* 1995; 27: 187-197.
2. Okada T, Sato Y, Kobayashi N et al. Structural characteristics of the N-glycans of two isoforms of prostate-specific antigens purified from human seminal fluid. *Biochem Biophys Acta* 2001; 1525: 149-160.
3. Sumi S, Arai K, Yoshida K. Separation methods applicable to prostate cancer diagnosis and monitoring therapy. *J Chromatogr B Biomed Sci Appl* 2001; 764: 445-455.
4. Peracaula R, Tabares G, Royle L et al. Altered glycosylation pattern allows the distinction between prostate-specific antigen (PSA) from normal and tumor origins. *Glycobiology* 2003; 13: 457-470.
5. Prakash S, Robbins PW. Glycotyping of prostate specific antigen. *Glycobiology* 2000; 10: 173-176.
6. Sumi S, Arai K, Kitahara S, Yoshida K. Serial affinity chromatography demonstrates altered asparagine linked sugar chain structures of prostate-specific antigen in human prostate carcinoma. *J Chromatogr B Biomed Sci Appl* 1999; 727: 9-14.
7. Stephan C, Jung K, Diamandis E, Rittenhouse HG, Lein M, Loening SA. Prostate-specific antigen, its molecular forms, and other kallikrein markers for detection of prostate cancer. *Urology* 2002; 59: 2-8.
8. Samadi AA, Davidson SD, Mordente JA et al. Differential glycosylation of cellular prostate specific antigen and the regulatory mechanism of its secretion. *Mol Urol* 1999; 3: 147-152.
9. Jankovic MM, Kosanovic MM. Glycosylation of urinary prostate-specific antigen in benign hyperplasia and cancer: assessment by lectin-binding patterns. *Clin Biochem* 2005; 38: 58-65.
10. Wang WC, Cummings RD. The immobilized leucoagglutinin from the seeds of *Maackia amurensis* binds with high affinity to complex-type Asn-linked oligosaccharides containing terminal sialic acid-linked alpha 2,3 to penultimate galactose residues. *J Biol Chem* 1988; 263: 4576-4585.
11. Yamashita K, Kochibe N, Ohkura T, Ueda I, Kobata A. Fractionation of L-fucose-containing oligosaccharide on immobilized *Aleuria aurantia* lectin. *J Biol Chem* 1985; 8: 4688-4693.
12. Cummings RD. Use of lectins in analysis of glycoconjugates. *Methods Enzymol* 1994; 230: 66-86.
13. Ohyama C, Hosono M, Nitta K et al. Carbohydrate structure and differential binding of prostate specific antigen to *Maackia amurensis* lectin between prostate cancer and benign prostate hypertrophy. *Glycobiology* 2004; 14: 671-679.
14. Brockhausen I, Kuhns W. Cancer of the urinary and reproductive tract. In : Brockhausen I, Kuhns W (eds): *Glycoproteins and human disease*. Austin: R.G. Landes Co, 1997, pp 202-206.
15. Yamashita K, Totani K, Iwaki Y et al. Comparative study of the sugar chains of gamma-glutamyltranspeptidases purified from human hepatocellular carcinoma and from human liver. *J Biochem* 1989; 105: 728-735.
16. Koyama I, Miura H, Matsuzaki H, Sakagishi Y, Komoda T. Sugar-chain heterogeneity of human alkaline phosphatases: differences between normal and tumour-associated isozymes. *J Chromatog* 1987; 413: 65-78.
17. Fernandes B, Sagman UA, Demetrio M, Dennis JW. Beta 1-6 branched oligosaccharides as a marker of tumor progression in human breast and colon neoplasia. *Cancer Res* 1991; 51: 718-723.
18. Yao M, Zhou DP, Jiang SM et al. Elevated activity of N-acetylglucosaminyltransferase V in human hepatocellular carcinoma. *J Cancer Res Clin Oncol* 1998; 124: 27-30.
19. Chandrasekaran EV, Chawda R, Locke RD, Piskorz CF, Matta KL. Biosynthesis of the carbohydrate antigenic determinants, Globo H, blood group H, and Lewis b: a role of prostate cancer cell alpha1,2-L-fucosyltransferase. *Glycobiology* 2002; 12: 153-162.
20. Arenas MI, Romo E, De Gaspar I et al. A lectin histochemistry comparative study in human normal prostate, benign prostatic hyperplasia and cancer. *Glycoconj J* 1999; 16: 375-382.