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Serial lectin affinity chromatography demonstrates altered asparagine-linked sugar-chain structures of prostate-specific antigen in human prostate carcinoma

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Abstract

Differences between prostate carcinoma (PCA) and benign prostatic hyperplasia (BPH) in asparagine (N)-linked sugar-chain structures of prostate-specific antigen (PSA) were investigated using serial lectin affinity chromatography. The amounts of PSA passing through columns of concanavalin A (Con A), phytohaemagglutinin E4 (PHA-E4) and PHA-L4 were significantly greater for PCA-derived PSA than BPH. We propose that the sugar moiety structure of PSA which is increased in PCA is a multiantennary complex type with branched *N*-acetylglucosamine β(1→4) mannose. We suggest that N-linked sugar chains in PSA are altered during oncogenesis in the human prostate and may serve as diagnostic tools for PCA.

Keywords: Prostate-specific antigen; Asparagine-linked sugar chain

1. Introduction

Serum prostate-specific antigen (PSA) is a sensitive assay for detection of early stage prostate cancer (PCA) and monitoring therapy of disease course [1,2]. The clinical utility of quantitative analysis of PSA, however, is limited because of a lack of specificity for PCA [3,4]. Recently, alterations in the sugar-chain structures of enzymes during oncogenesis have been reported in some human organs [5–8]. Although PSA is known to be a glycoprotein consisting of 237 amino acids and a single N-linked sugar moiety [9], few studies have discussed changes in PSA sugar-chain structures associated with malignant transformation. In the present study, we compared differences in these structures in benign prostatic hyperplasia (BPH) and PCA tissues using lectin affinity chromatography.

2. Materials and methods

2.1. Reagents

The following reagents were purchased: concanavalin A (*Canavalia ensiformis*, Con A)/sepharose 4B from Pharmacia Fine Chemicals (Uppsala, Sweden); phytohaemagglutinin E4 and L4 (*Phaseolus vulgaris*, PHA-E4 and PHA- L4)/agarose, pea lectin (*Pisum sativum*, PS)/agarose and

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wheat germ agglutinin (*Triticum vulgaris*, WGA)/ agarose from E.Y. Labs (San Mateo, CA). All other chemicals were of analytical grade and were obtained from Wako Pure Chemicals, Osaka, Japan.

2.2. Samples

PCA tissues were obtained from seven patients, aged 62–74 years, undergoing radical retropubic prostatectomy and pelvic lymphnode dissection. Samples were carefully dissected to exclude surrounding BPH or normal tissues. BPH tissues were obtained from eight patients, aged 65–77 years, undergoing suprapubic prostatectomy. All patients consented to the use of their tissues for the current study. After isolation of tissues, all samples were immediately frozen and kept at −80°C until use.

Microscopic sections were evaluated by a pathologist. One PCA specimen was well-differentiated, three were moderately differentiated and three were poorly differentiated adenocarcinomas. All BPH specimens were confirmed to be free of PCA.

2.3. PSA preparations

PCA and BPH tissues were weighed and cut into cubes measuring 1 mm^3 and homogenized in an ultradisperser with 10 volumes of 100 mmol l^{-1} phosphate buffer (pH 7.2) containing 0.1% Triton X-100, 150 mmol l^{-1} sodium chloride, 10 mmol l^{-1} magnesium chloride, 10 μmol l^{-1} zinc chloride and 0.02% bovine serum albumin (buffer A). The homogenate was further diluted by buffer A to a concentration of PSA of 1000 ng ml^{-1} and centrifuged at 105 000 *g* for 20 min at 4°C. The resulting supernatant was used as the PSA source for serial lectin affinity chromatography.

2.4. Serial lectin affinity chromatography

Columns (0.5×10 cm) of Con A, PS, WGA, PHA-E4 or PHA-L4, were equilibrated with buffer A. One ml containing 1000 ng of PSA was first applied to the Con A column and allowed to stand at room temperature for 2 h. The column was then washed with buffer A until PSA in the fraction was undetectable. Initial elution was carried out with 10 mmol l^{-1} α-methyl-D-mannoside (α-MM) in buffer A, and a subsequent elution was carried out with 500 mmol α-MM in buffer A. Three fractions were obtained: an unbound fraction (fraction A), a weakly bound fraction (fraction B) and a strongly bound fraction (fraction C). Fractions A, B and C were then applied to PHA-E4, PS and WGA columns, respectively. The unbound and bound fractions were separated on these columns using 500 mmol l^{-1} *N*-acetylglucosamine (GlcNAc), 500 mmol l^{-1} α-MM and 500 mmol l^{-1} GlcNAc in buffer A, respectively, as elution buffers. The unbound fraction on the PHA-E4 column was then applied to PHA-L4 and separated into unbound and bound fractions; the latter was eluted500 mmol l^{-1} GlcNAc in buffer A. The relative amount of PSA was calculated by dividing the PSA value in each fraction by the sum of PSA values in all fractions on each column.

2.5. PSA measurement

PSA concentrations in the PSA preparations and in each fraction were determined by the Automated Chemiluminescence System (ACS II, Chyron, Tokyo, Japan).

2.6. Statistical analysis

Values were expressed as mean±SD. Statistical analyses were performed using the Mann-Whitney U test. A P value <0.05 was considered statistically significant.

3. Results

3.1. PSA concentration in BPH and PCA tissues

The mean PSA concentration in BPH tissue was 2.21±0.99 μg mg^{-1} of tissue (range 0.97–3.86) and in PCA tissue was 0.138±0.089 μg mg^{-1} (range 0.041–0.275). The PSA concentration in PCA was significantly lower than in BPH ($P=0.0001$) (Tables 1 and 2).

3.2. Serial lectin-binding studies

The seven fractions separated by serial lectin affinity were as follows: I, the fraction that passed

Table 1
Tumor differentiation, PSA concentration and relative amounts of seven types of sugar chains of PSA in prostate carcinoma

Case no.	Tumor differentiation	PSA concentration ($\mu g\ mg^{-1}$ of tissue)	Relative amounts (%)[a]						
			I	II	III	IV	V	VI	VII
1	Well	0.117	3.8	0	0	68.3	8.8	19.1	0
2	Moderately	0.275	5.7	0	0	68.2	10.6	15.5	0
3	Moderately	0.163	15.0	0	0	59.2	9.2	16.6	0
4	Moderately	0.193	25.4	0	0	51.1	12.4	11.1	0
5	Poorly	0.168	9.8	0	0	58.4	19.1	12.7	0
6	Poorly	0.041	14.1	0	0	57.4	15.7	12.8	0
7	Poorly	0.174	14.4	0	0	37.7	7.5	40.4	0
Mean±SD		0.138±0.089	12.6±7.2[a]			57.2±10.6	11.9±4.2[a]	18.3±10.1[a]	

[a] Mean values without common superscript 'a' differ significantly ($P<0.002$).

through all of the Con A, PHA-E4 and PHA-L4 columns; II, the fraction that passed through both Con A and the PHA-E4 but was bound to PHA-L4; III, the fraction that passed through Con A but was bound to the PHA-E4 column; IV, the fraction that was bound weakly to Con A and passed through PS; V, the fraction that was bound weakly to Con A and bound to PS; VI, the fraction that was bound strongly to Con A and passed through WGA; and VII, the fraction that was bound strongly to Con A and bound to WGA (Fig. 1). Total PSA recovered after separation by each column was >90% of the initial PSA content of the preparation added.

The relative amounts of PSA in the seven fractions in BPH and PCA tissues are shown in Tables 1 and 2. Fractions II, III and VII were undetectable in both BPH and PCA. In BPH tissues, the relative amounts of the other four fractions (I, IV–VI) were similar in each case, viz., fraction IV contained the largest amount, whereas fraction I the smallest. The relative amount of fraction IV was significantly greater than that of any other fraction ($P<0.001$), while the relative amount of fraction I was significantly less than that of any other fraction ($P<0.002$). By contrast, the relative amounts of fractions I, IV, V and VI in PCA varied among the cases: fraction IV was the largest in six of seven cases, fraction VI was higher than fraction IV in case 7; fraction I was the smallest in cases 1, 2 and 5, while fractions V or VI were the smallest in cases 3, 4, 6 and 7. However, fraction IV was significantly higher than any other fraction ($P<0.002$). Although the lowest relative amount was observed in fraction V, there were no significant differences between fractions V and I or VI.

When BPH and PCA tissues were compared for the relative amount of each fraction, fraction I of PCA was significantly higher than fraction I of BPH

Table 2
PSA concentration and relative amounts of seven types of sugar chains of PSA in benign prostatic hyperplasia

Case no.	PSA concentration ($\mu g\ mg^{-1}$ of tissue)	Relative amounts (%)[a]						
		I	II	III	IV	V	VI	VII
1	3.86	2.2	0	0	75.3	11.6	10.9	0
2	1.60	2.4	0	0	67.6	14.8	15.1	0
3	2.42	2.6	0	0	66.1	17.6	13.7	0
4	3.09	3.1	0	0	68.5	18.3	10.1	0
5	0.97	3.3	0	0	66.5	8.3	21.9	0
6	1.60	3.3	0	0	62.7	22.2	11.8	0
7	2.79	3.8	0	0	42.9	33.5	19.8	0
8	1.30	3.8	0	0	74.8	9.2	12.2	0
Mean±SD	2.21±0.99	3.1±0.6			65.6±10.1	16.9±8.2[a]	14.4±4.3[a]	

[a] Mean values without common superscript 'a' differ significantly ($P<0.002$).

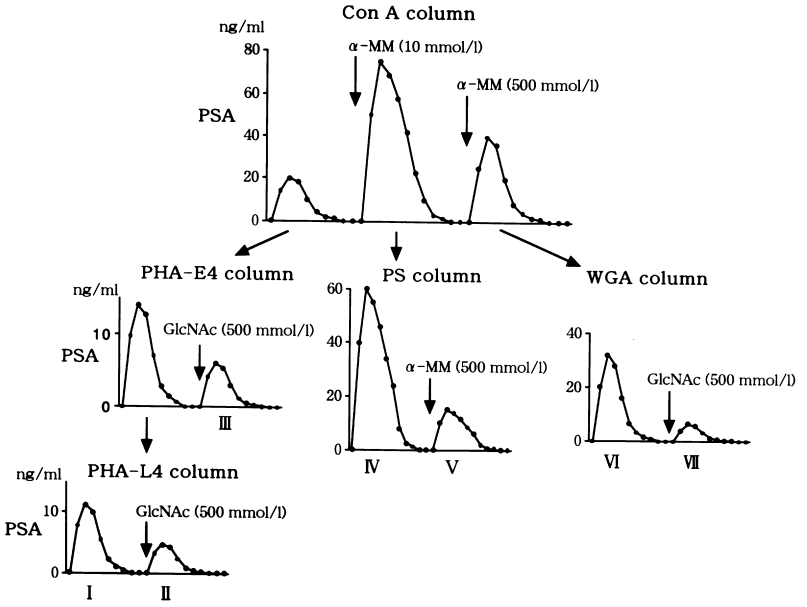

Fig. 1. Separation of PSA preparations into seven fractions by serial lectin affinity chromatography. Fractions A, B and C obtained on the Con A column were subsequently applied to PHA-E4, PS and WGA columns, respectively. The unbound fraction on the PHA-E4 column was further applied to the PHA-L4 column. α-MM, α-Methyl-D-mannoside; GlcNAc, *N*-acetylglucosamine. Arrows indicate the starting points of elution.

(12.6±7.2% vs. 3.1±0.6%; $P=0.0024$) (Fig. 2). For the other fractions, no significant differences were observed. However, the sum of fractions IV and V, which bound weakly to Con A, was significantly lower in PCA than BPH (69.1±11.9% vs. 82.5±4.5%; $P=0.011$).

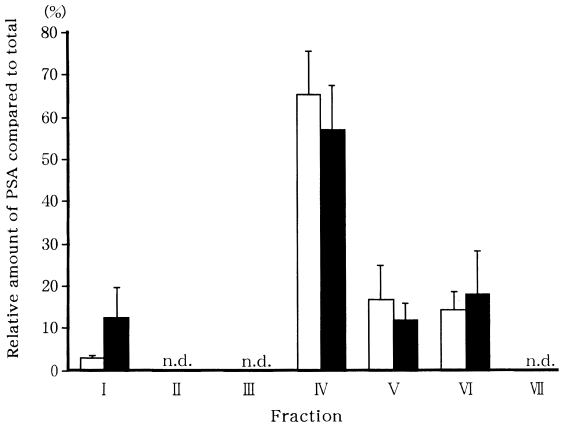

Fig. 2. Comparison between BPH and PCA tissues of relative amounts of seven types of sugar chains of PSA. Columns and bars represent means and standard deviations, respectively. Open column, BPH tissue; closed column, PCA. The relative amount of fraction I was significantly increased in PCA tissue ($P=0.0024$). n.d.=Not detectable.

4. Discussion

It has been previously demonstrated by both amino acid and cDNA sequencing techniques that PSA contains 237 amino acids as a single polypeptide [10]. Baffa et al. compared cDNA sequences between benign and malignant prostate tissues and found a 100% match with no mutations [11]. PSA is also known to contain 7–12% carbohydrate and recent studies have shown that the protein contains only one N-linked chain at asparagine 45 of the molecule [12]. With regard to this sugar moiety, a biantennary complex type with fucose linkages to the innermost GlcNAc was proposed as the major sugar-chain structure of PSA purified from seminal plasma by NMR spectroscopy [9]. However, the presence of four or five isoforms of PSA with different isoelec-

tric points (p*I*) in BPH tissue and seminal plasma has been suggested by Wang et al. [13]. They also have shown that treatment with neuraminidase converts some isomers to isomers with a higher p*I*, suggesting that the variations in the p*I* value reflect, at least in part, a difference in sialic acid content.

Alterations in sugar-chain structures of glycoproteins during oncogenesis have been reported in some human carcinomas [5–8]. To our knowledge, however, few studies have been done on changes in the sugar-chain structures of PSA associated with malignant transformation. Although in 1989 Barak et al. reported significantly different PSA-Con A binding ratios in the sera of patients with PCA compared to those with BPH [14], other investigators have found considerable overlap between the two groups, suggesting that the measurement of this ratio lacks clinical utility [15–17]. PSA in the serum exists predominantly in a complex with α1-antichymotrypsin (ACT), which is itself extensively glycosylated; therefore, the lectin-binding properties determined in those studies using total serum PSA may reflect the sugar-chain structures not only of PSA but also of ACT. In contrast, PSA observed in extracts of prostatic tissue, either benign or malignant, has been shown to be noncomplexed [13,18]. For that reason, we compared PSA derived from BPH tissue with that derived from PCA tissue for lectin-binding characteristics to determine whether any structural alterations take place in the sugar chains of PSA during oncogenesis of the human prostate. In the present study, we also have confirmed that PSA in the PSA source is only of noncomplexed type, molecular weight of which is approximately 30 kDa, by gel filtration chromatography.

Based on reports of serial lectin affinity chromatographic studies [6,19], the sugar-chain structures of the seven fractions are proposed as follows: I, multiantennary complex type with branched GlcNAc β(1→4) mannose; II, multiantennary complex type with branched GlcNAc β(1→6) mannose; III, complex type with bisecting GlcNAc; IV, biantennary complex type without fucose linkages to the innermost GlcNAc; V, biantennary complex type with fucose linkages to the innermost GlcNAc; VI, high mannose type or hybrid type with fucose linkages to the innermost GlcNAc; and VII, hybrid type without fucose linkages to the innermost GlcNAc. In both BPH and PCA tissues, no significant amounts were found in fractions II, III or VII. In BPH tissues, the relative amounts of the remaining fractions were similar in each case: fraction IV was the largest and fraction I the smallest . The relative amounts of the four fractions in PCA varied, suggesting that sugar-chain structures of PSA in PCA differ from those in BPH and also from each other. When the seven types of sugar chains were compared between the two groups, the relative amount of fraction I [multiantennary complex type with branched GlcNAc β(1→4) mannose] was significantly increased in PCA. Furthermore, the sum of fractions IV and V [biantennary complex type without/with fucose linkages to the innermost GlcNAc] was significantly decreased in PCA compared to BPH. These findings suggest that *N*-acetylglucosaminyl-transferase IV, which adds GlcNAc to sugar chains by β1→4 binding and converts biantennary to multiantennary types, is expressed at higher levels in PCA. Although our sample size was too small to find an association between tumor differentiation and the percentage of fraction I, case 1 with well-differentiated adenocarcinoma had the smallest fraction I of all cases.

PSA concentrations in PCA tissue have been reported to be significantly lower than that in BPH [20,21]; this finding was confirmed in the present study. Buffer A was modified from previous studies [7,8,22], to increase recovery of PSA.

Several studies have investigated maximizing the clinical utility of PSA by improving its specificity and yet preserving its sensitivity for PCA. These new techniques include PSA density, PSA velocity, age- and race-specific reference ranges and percent free PSA [23–26]. However, diagnostic biopsies continue to be necessary. Our results suggest that post-translational glycosylation of PSA is altered during malignant transformation of the human prostate. Detailed studies of qualitative differences in sugar-chain structures of PSA may lead to the development of a useful diagnostic tool for PCA.

References

[1] J.E. Oesterling, J. Urol. 145 (1991) 907.
[2] A.J. Patrin, J.E. Oesterling, J. Urol. 152 (1994) 1358.
[3] D.A. Armbruster, Clin. Chem. 39 (1993) 181.

[4] R.B. McCormack, T.J. Wang, H.G. Rittenhouse, R.L. Wolfert, J.A. Finlay, H. Lilja, R.L. Sokoloff, J.E. Oesterling, Urology 45 (1995) 729.
[5] K. Yamashita, K. Totani, Y. Iwaki, I. Takamizawa, N. Tateishi, T. Higashi, Y. Sakamoto, A. Kobata, J. Biochem. 105 (1989) 728.
[6] I. Koyama, M. Miura, H. Matsuzaki, Y. Sakagishi, T. Komoda, J. Chromatogr. 413 (1987) 65.
[7] K.-I. Yoshida, S. Sumi, M. Honda, Y. Hosoya, M. Yano, K. Arai, Y. Ueda, J. Chromatogr. B. 672 (1995) 45.
[8] K.-I. Yoshida, M. Honda, K. Arai, Y. Hosoya, H. Moriguchi, S. Sumi, Y. Ueda, S. Kitahara, J. Chromatogr. B. 695 (1997) 439.
[9] A. Belanger, H. Van Halbeek, H.C.B. Graves, K. Grandbois, T.A. Stamey, L. Huang, I. Poppe, F. Labrie, Prostate 27 (1995) 187.
[10] P.H.J. Riegman, P. Klaassen, J.A.G.M. Korput, J.C. Aomijn, J. Trapman, Biochem. Biophys. Res. Commun. 155 (1988) 181.
[11] R. Baffa, J.G. Moreno, M. Monne, M.L. Veronese, L.G. Gomella, Urology 47 (1996) 795.
[12] H. van Halbeek, G.J. Gerwig, J.F.G. Vliegenthart, R. Tsuda, M. Hara, K. Akiyama, K. Schmid, Biochem. Biophys. Res. Commun. 131 (1985) 507.
[13] M.C. Wang, M. Kuriyama, L.D. Papsidero, R.M. Loor, L.A. Valenzuela, G.P. Murphy, T.M. Chu, Meth. Cancer Res. 19 (1982) 179.
[14] M. Barak, Y. Mecz, A. Lurie, N. Gruener, Oncology 46 (1989) 375.
[15] D.W. Chan, Y.M. Gao, Clin. Chem. 37 (1991) 1133.
[16] J. Marrink, H. Klip, R. De Jong, The Lancet 339 (1992) 619.
[17] M.P. van Dieijen-Visser, J. van Pelt, K.P.J. Delaere, Eur. J. Clin. Chem. Clin. Biochem. 32 (1994) 473.
[18] S. Sumi, H. Umeda, M. Yano, F. Koga, T. Arai, Y. Hosoya, S. Sakamoto, M. Honda, H. Moriguchi, K.-I. Yoshida, Jpn. J. Urol. Surg. 10 (1997) 127.
[19] K. Arai, K.-I. Yoshida, T. Komoda, N. Kobayashi, H. Saitoh, Y. Sakagishi, Clin. Chim. Acta 184 (1989) 75.
[20] M.C. Wang, L.A. Valenzuela, G.P. Murphy, T.M. Chu, Invest. Urol. 17 (1979) 159.
[21] L.D. Papsidero, M. Kuriyama, M.C. Wang, J.S. Horoszewicz, S.S. Leong, L.A. Valenzuela, G.P. Murphy, T.M. Chu, J. Natl. Cancer. Inst. 66 (1981) 37.
[22] K. Arai, S. Sumi, K.-I. Yoshida, T. Komoda, Biochimica et Biophysica Acta 1253 (1995) 33.
[23] M.C. Benson, I.C. Whang, A. Pantuck, K. Ring, S.A. Kaplan, C.A. Olsson, W.H. Cooner, J. Urol. 147 (1992) 815.
[24] H.B. Carter, J.D. Pearson, E.J. Metter, L.J. Brant, D.W. Chan, R. Andres, J.L. Fozard, P.C. Walsh, JAMA 267 (1992) 2215.
[25] R.J. Babaian, H. Miyashita, R.B. Evans, E.I. Ramirez, J. Urol. 147 (1992) 837.
[26] A. Christensson, T. Bjork, O. Nilsson, U. Dahlen, M.T. Matikainen, A.T. Cockett, P.-A. Abrahamsson, H. Lilja, J. Urol. 150 (1993) 100.