Identification of prostate specific antigen (PSA) glycoforms in aggressive prostate cancer (PCa) patients

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Introduction and Objective

Prostate Cancer (PCa) is the most common cancer and the second cause of cancer death in men [1]. Serum levels of the glycoprotein Prostate-specific antigen (PSA) have been used in the diagnosis of PCa, however PSA levels may also rise in other prostate pathologies. Glycosylation is altered in cancer, indeed, changes in PSA glycosylation has been reported in PCa patients. In particular, an increase in the percentage of α2,3-linked sialic acid of PSA glycoforms are indicative of aggressive PCa [2]. However, the specific PSA glycoforms which are differently expressed either increased or decreased in aggressive PCa have not been characterised yet. Thus, the aim of this study is to determine by *N*-glycan sequencing the main PSA glycoforms of aggressive PCa patients from blood serum samples and compared them with those of standard PSA from healthy individuals' seminal plasma.

Experimental Approach

Select blood serum with high levels of PSA (> 300 ng/ml) from patients with aggressive PCa.

PSA purified from seminal plasma of healthy patients was used as a control (standard PSA)

→ To purify PSA from blood serum and separate α2,3/α2,6-sialic acid PSA glycoforms

PSA was immunoprecipitated using anti-tPSA antibody and α2,3/α2,6-sialic acid glycoforms were separated by SNA-agarose chromatography

→ The PSA collected fractions were immunoprecipitated using anti-fPSA antibody and resolved on SDS-PAGE

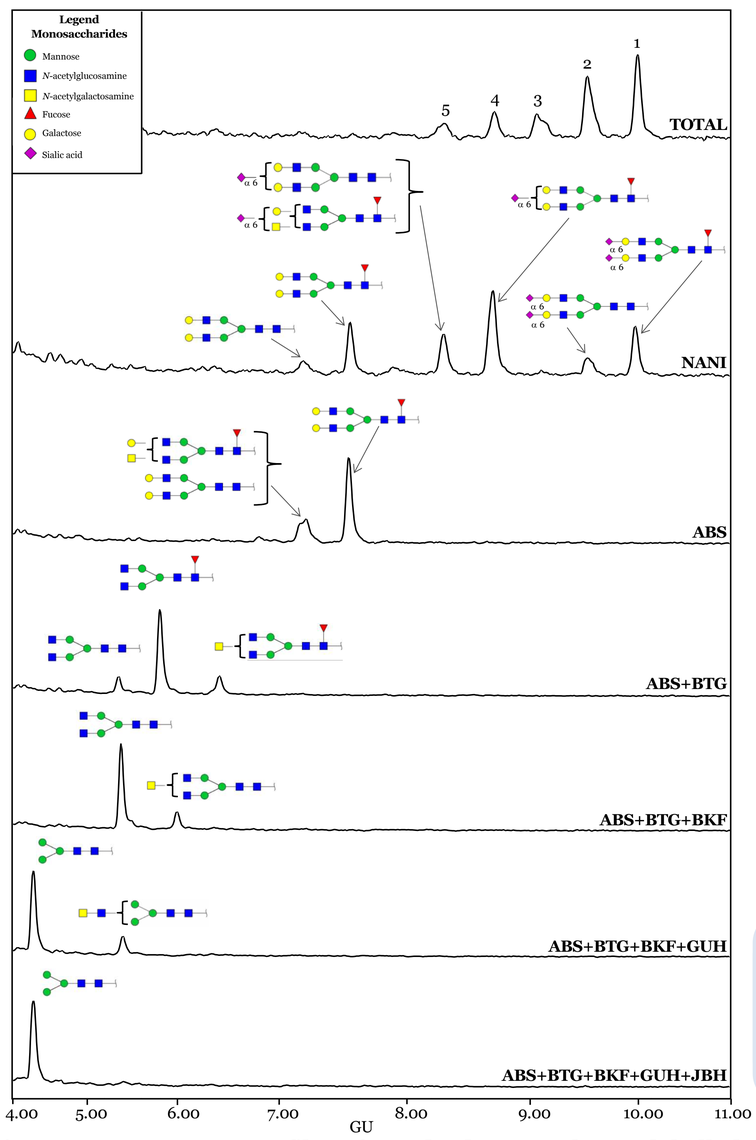
→ To perfom *N*-glycan sequencing to identify differences in PSA glycoforms between aggressive PCa and standard PSA

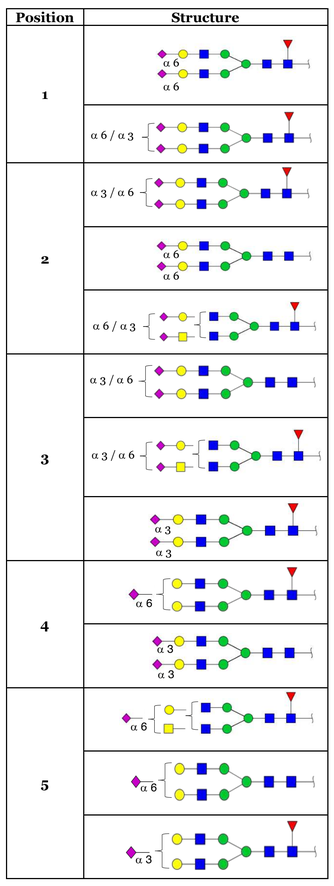
PSA bands were excised and digested with PNGase F.

→ The *N*-glycans were analysed by Hydrophilic Liquid Chromatography combined with exoglycosidase digestions

Results

1. Characterisation of the main PSA *N*-glycans of standard PSA (control)





Standard PSA *N*-glycans are 87% fucosylated and 17% of them contained *N*-acetylgalactosamine (ABS+BTG)

Figure 1. HILIC-UPLC profiles of standard PSA *N*-glycans labelled with 2AB. From top to bottom, chromatograms of consecutive panels correspond to the total profile and the subsequent digestions by the specified exoglycosidases. Profiles are standardised against a dextran hydrolysate with glucose units (GU).

Exoglycosidases specificity: NAN1 (digests α2-3 linked sialic acid residues), BTG (digests β1-3,4 galactose), BKF (digests α1-2,3,4,6 fucose), GUH (digests β-*N*-acetylglucosamine) and JBH (digests β *N*-acetylglucosamine and β1,2,3,4,6 *N*-acetylgalactosamine).

2. Purification of Unbound (UB)/Bound (B) sialylated PSA glycoforms

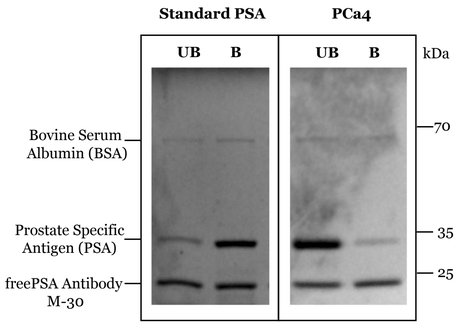


Figure 2. Representative gel electrophoresis of fPSA immunoprecipitated from unbound (UB) and bound (B) fractions of SNA affinity chromatography. Results of standard PSA and PSA from prostate cancer (PCa4) corresponding to two different gels are shown.

Table 1. Relative percentage of fPSA containing α2,3 sialylated glycoforms from the unbound (UB) fractions of PCa samples after SNA-lectin chromatography.

Sample ID	% of fPSA containing α2,3-SA (UB fraction)
Standard PSA	**22.6**
PCa1	**32.4**
PCa2	**44.9**
PCa3	**61.0**
PCa4	**90.2**
PCa5	**35.1**
PCa6	**41.1**

α2,3 sialylated PSA glycans increase in aggressive PCa

3. Characterisation of the main PSA N-glycans of Unbound fractions (α2,3- sialic acid PSA)

SNA-chromatography UB fractions of PCa samples present heterogeneity in their main PSA glycoforms

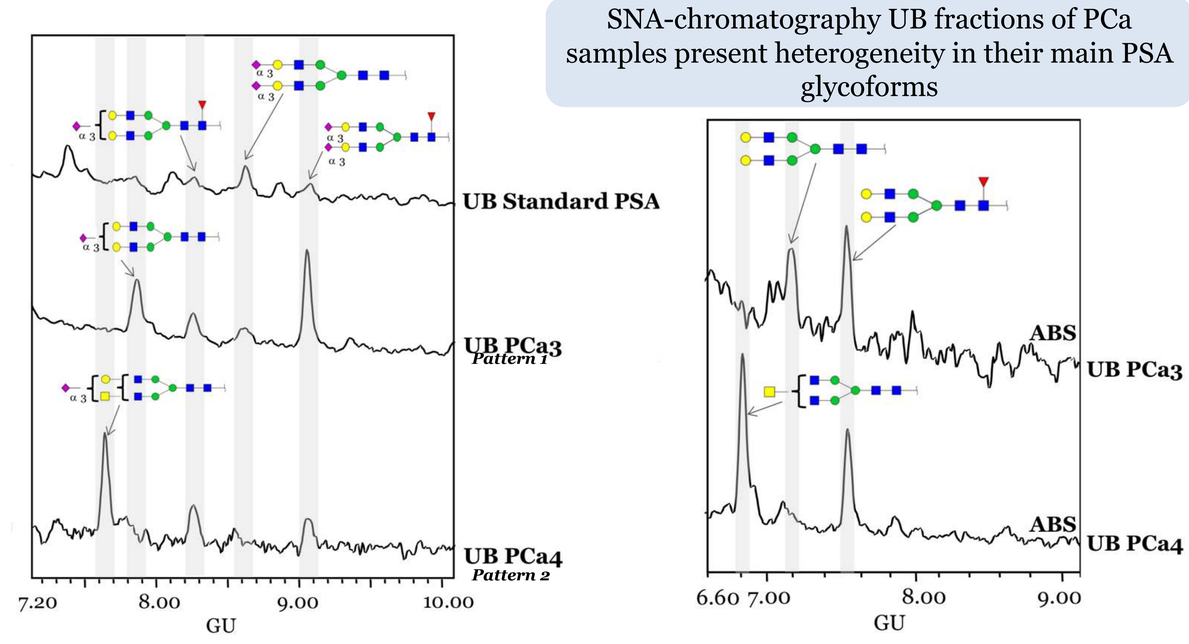


Figure 3. HILIC-UPLC profiles of PSA *N*-glycans from unbound (UB) fractions. (a) total UB fractions of standard PSA (top panel) and PSA from aggressive prostate cancer (PCa3-PCa4) (middle and bottom panel) and (b) ABS digested *N*-glycans from PSA from UB fractions of PCa3 (top panel) and PCa4 (bottom panel). Profiles are standardised against a dextran hydrolysate with glucose units (GU).

4. Characterisation of the main PSA N-glycans of Bound fractions (α2,6- sialic acid PSA)

In B fractions, the two main changes in PSA glycoforms between standard and PCa samples are the increase of *N*-acetylgalactosamine structures (9.0 GU) and the absence of the main standard PSA glycoforms (10.0 GU)

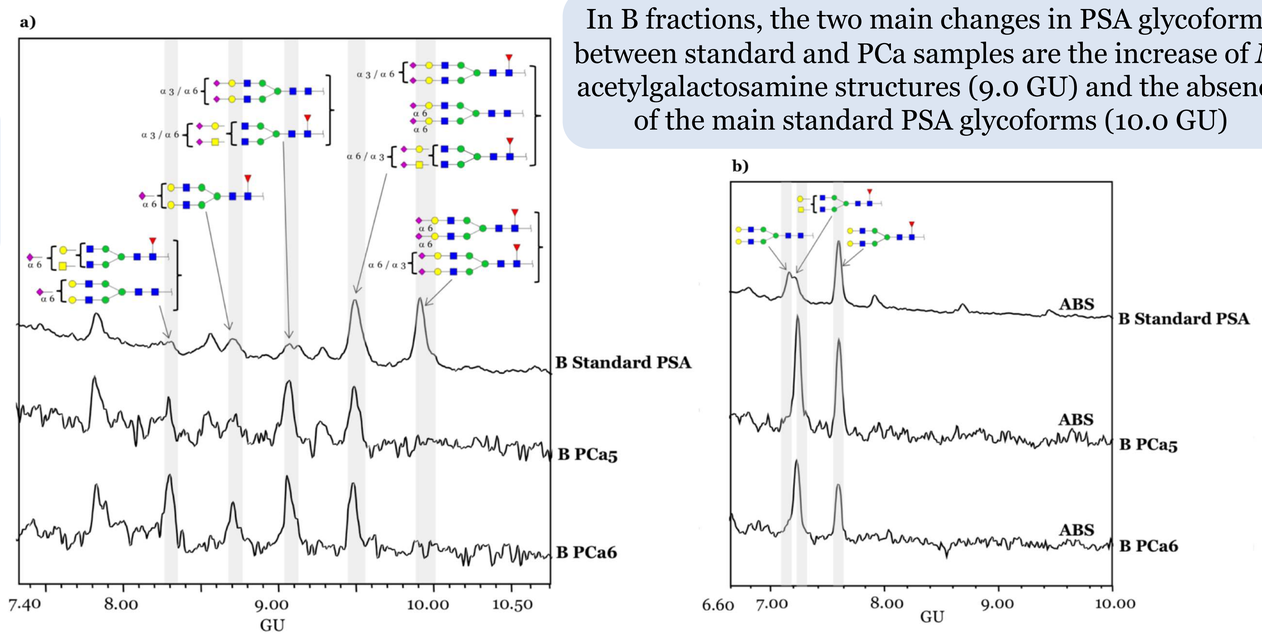


Figure 4. HILIC-UPLC profiles of PSA *N*-glycans from bound (B) fractions. (a) total B fractions of standard PSA (top panel) and PSA from aggressive prostate cancer (PCa5-PCa6) (middle and bottom panel) and (b) ABS digested *N*-glycans of B fraction from standard PSA (top panel), PCa5 and PCa6 (middle and bottom panel). Profiles are standardised against a dextran hydrolysate with glucose units (GU).

5. Summary of the differentially expressed PSA glycoforms in aggressive PCa samples

UB PSA glycoforms

Structure	GUs	% change (range)
α3 α3	9.1	↑ 7.0 - 22.7
α3	7.9	↑ 3.0 -10.8
α3	7.7	↑ 37.1- 41.5

B PSA glycoforms

Structure	GUs	% change (range)
α6 α6	10.0	↓ 41.4[c]
α6/α3		
α3/α6	9.1	↑ 11.5 - 28.9
α6	8.3	↑ 9.3 - 29.3

[a] Changes in this PSA glycoform were only observed in group 1 pattern of the unbound PSA fractions of aggressive PCa samples.
[b] These PSA glycoforms were only present in group 2 pattern of the unbound PSA fractions of aggressive PCa samples.
[c] These PSA glycoforms were not present in any bound PSA fraction of aggressive PCa samples.

Conclusions

- The main PSA glycoform in standard PSA at 10.0 GU decreased noteworthy, and was not detected in any of the aggressive PCa samples.
- Specific PSA glycoforms containing *N*-acetylgalactosamine increased in all α2,6-sialic acid glycoforms and in some of the α2,3-sialic acid ones.
- The identification of these particular PSA glycoforms that are increased or decreased in aggressive PCa patients paves the way to develop high throughput methodologies for screening them in diagnostic set ups.

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