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Measurement of aberrant glycosylation of prostate specific antigen can improve specificity in early detection of prostate cancer

Tohru Yoneyama [a,1], Chikara Ohyama [a,*], Shingo Hatakeyama [a], Shintaro Narita [b], Tomonori Habuchi [b], Takuya Koie [a], Kazuyuki Mori [a], Kazuya I.P.J. Hidari [c,d], Maho Yamaguchi [d], Takashi Suzuki [d], Yuki Tobisawa [a,1]

[a] Department of Urology, Hirosaki University Graduate School of Medicine, 5-Zaifu-cho, Hirosaki 036-8562, Japan
[b] Department of Urology , Akita University School of Medicine, 1-1-1 Hondo, Akita 010-8543, Japan
[c] Department of Food and Nutrition, Junior College Division, University of Aizu, 1-1 Aza Kadota, Oaza Yahata, Ikki-machi, Aizuwakamatsu 965-0003, Fukushima, Japan
[d] Department of Biochemistry, University of Shizuoka, School of Pharmaceutical Sciences, 52-1 Yada, Suruga-ku, Shizuoka 442-8002, Shizuoka, Japan

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ABSTRACT

Introduction: We previously identified prostate cancer (PCa)-associated aberrant glycosylation of PSA, where α2,3-linked sialylation is an additional terminal *N*-glycan on free PSA (S2,3PSA). We then developed a new assay system measuring S2,3PSA using a magnetic microbead-based immunoassay. We compared the diagnostic accuracy of conventional PSA and percent-free PSA (%fPSA) tests.
Methods: We used MagPlex beads to measure serum S2,3PSA levels using anti-human fPSA monoclonal antibody (8A6) for capture and anti-α2,3-linked sialic acid monoclonal antibody (HYB4) for detection. We determined the cutoff values in a training test and measured serum S2,3PSA levels in 314 patients who underwent biopsy, including 138 PCa and 176 non-PCa patients with PSA of <10.0 ng/ml. Serum S2,3PSA levels were presented as mean fluorescence intensity (MFI). Receiver operating characteristic curves were used to evaluate the diagnostic accuracy of total PSA, %fPSA, and S2,3PSA.
Results: We determined an MFI cutoff value of 1130 with a sensitivity of 95.0% and specificity of 72.0% for the diagnosis of PCa in the training test. In the validation study, the area under the curve for the detection of PCa with S2,3PSA was 0.84, which was significantly higher than that with PSA or %fPSA.
Conclusions: Although the present study is small and preliminary, these results suggest that the measurement of serum S2,3PSA using a magnetic microbead-based immunoassay may improve the accuracy of early detection of PCa and reduce unnecessary prostate biopsy.

1. Introduction

Serum prostate specific antigen (PSA) is widely used as a powerful biomarker for the early detection of prostate cancer (PCa) [1,2]. Widespread use of the PSA test caused a stage migration of PCa [3]. However, the use of PSA in PCa screening is becoming controversial [4,5]. Particularly, in recent years, criticism against PCa screening using PSA has grown because of over-diagnosis and over-treatment [6]. In addition to complications associated with the treatment of PCa, such as urinary incontinence and erectile dysfunction that are associated with the treatment of PCa, harmful events associated with prostate biopsy are also a major concern for PSA-based PCa screening [7]. Therefore, the development of novel screening methods with improved specificity is of vital importance. Among the various molecular isoforms of PSA, proPSA is one of the most promising potential biomarkers [8,9]. Recently, the Food and Drug Administration of the United States of America approved the use of p2PSA and phi [10] in PCa screening. This innovative method for the early detection of PCa has had a significant impact on clinical practice. However, additional approaches focusing on the aberrant glycosylation of PSA are also promising [11,12].

Cancer-associated glycan alteration is observed frequently during carcinogenesis [13]. Majority of the tumor markers, such as alpha-fetoprotein (AFP; [14] and human chorionic gonadotropin (hCG; [15], are glycoproteins that have glycosylation sites in their amino acid sequences. More importantly, each glycan has specific cancer-associated carbohydrate alterations when compared with its normal counterpart, which can be detected using specific monoclonal antibodies or lectin.

PSA is a glycoprotein with one *N*-glycosylation site on its 45th amino acid from the *N*-terminus, asparagine (N) [16]. The amino acid sequence surrounding the glycosylated N is isoleucine (I),

* Corresponding author. Fax: +81 172 39 5092.
E-mail address: coyama@cc.hirosaki-u.ac.jp (C. Ohyama).
[1] These authors contributed equally to this work.

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arginine (R), N, and lysine (K), which is specific to PSA [16]. Therefore, in a prior study, we cleaved the PSA-specific sequence IRNK, which includes the glycosylated-N, and performed an intensive structural analysis of the glycan profile of PSA using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry [17]. Importantly, we identified that the terminal *N*-glycan structure of PSA from PCa patients was rich in sialic acid α2,3-linked to the galactose residue, whereas the terminal *N*-glycan structures of PSA from the seminal plasma were exclusively α2,6-linked [17] (Fig. 1A). These findings are consistent with our prior observations demonstrating that the binding of PCa-associated PSA to *Maackia amurensis* agglutinin (MAA) was remarkably stronger than that of non-PCa-associated PSA (18) because MAA lectin recognizes α2,3-linked sialic acid.

In the present study, we successfully developed a novel assay system using a magnetic microbead-based immunoassay for the detection of α2,3-linked sialylation as an additional terminal *N*-glycan on free PSA (S2,3PSA).

2. Materials and methods

2.1. Coupling the anti-free PSA (fPSA) monoclonal antibody (mAb) to magnetic microbeads

The coupling of anti-fPSA mAb (8A6, Abcam, Cambridge, UK) to magnetic beads (MagPlex beads, Luminex, TX, USA) was performed using the xMAP Antibody Coupling (AbC) Kit following the manufacturer's instructions. In brief, 1 ml (1.25×10^7) of MagPlex bead suspension was pelleted using a DynaMag™-2 magnetic separator for 2 min. The MagPlex beads were then resuspended in 500 μl of activation buffer using vortexing and sonication. The beads were pelleted, the supernatant was removed, and the washed microspheres were resuspended in 400 μl of activation buffer. Fifty microliters of *N*-hydroxysulfosuccinimide (sulfo-NHS) solution and 50 μl of EDC (10 μg/250 μl) were added to the microsphere suspension, which was mixed gently. The suspension was then incubated for 20 min at room temperature (RT) with gently vortexing. The activated beads were washed twice with 500 μl of activation buffer, and 62.5 μg of anti-fPSA mAb (8A6) diluted in 500 μl of activation buffer was then added. The suspension was then stirred for 2 h with gently vortexing. Finally, the antibody-conjugated beads were washed and resuspended in 2 ml of wash buffer (6250 beads/μl), and were then ready to use.

2.2. Quantification of serum S2,3PSA using the Luminex system

A schematic diagram demonstrating the quantification of S2,3PSA using the Luminex system [19] is shown in Fig. 1B. We used xMAP magnetic beads to measure serum S2,3PSA, using anti-human fPSA mAb (8A6) to coat the beads as the capture antibody, and anti-α2,3-linked sialic acid mAb (HYB4) (Wako, Osaka,

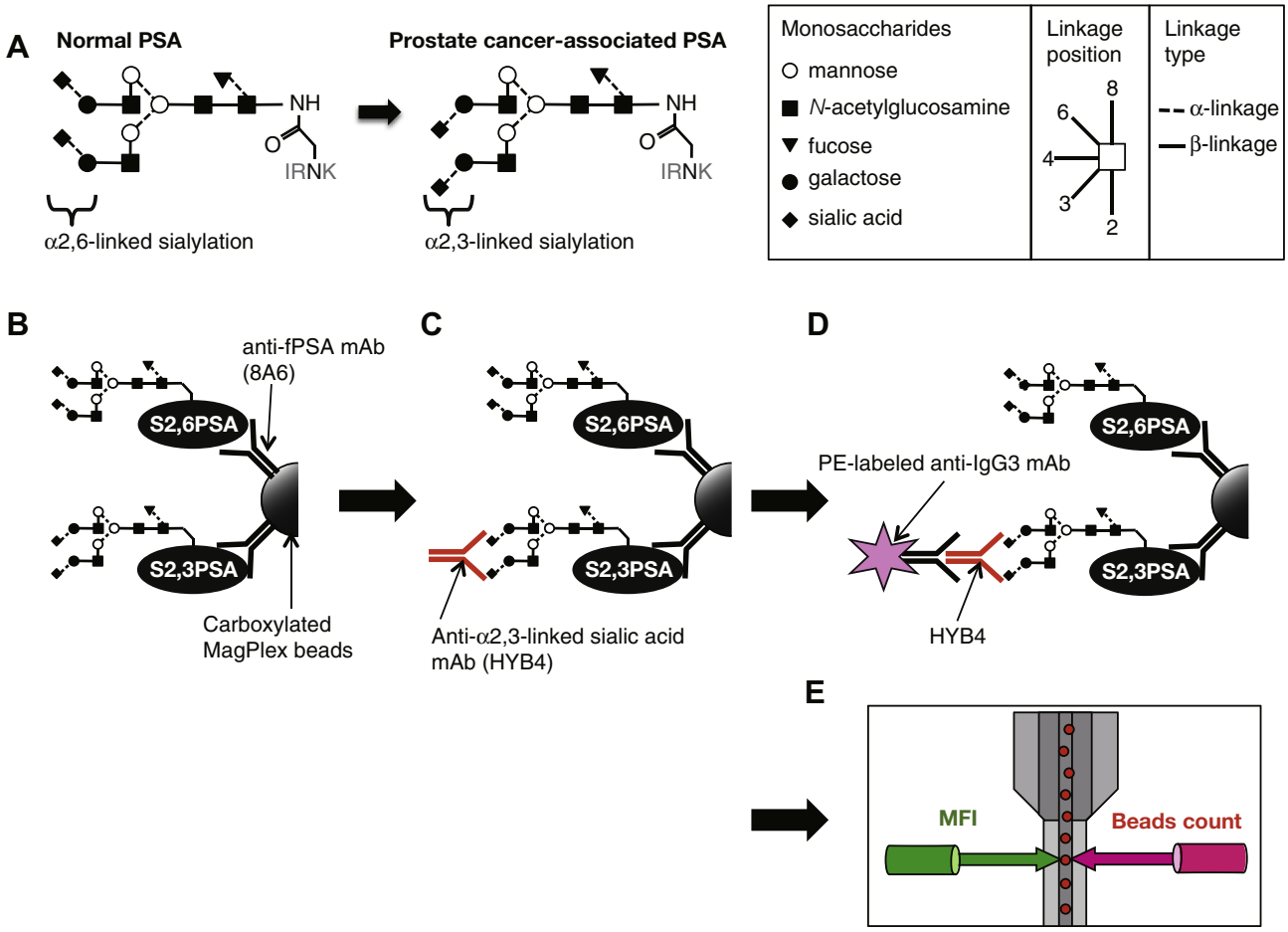

Fig. 1. Prostate cancer-associated aberrant glycosylation of *N*-glycan on PSA and schematic representation of the measurement of S2,3PSA. (A) In normal PSA, the terminal sialic acids link to galactose residues with an α2,6 linkage. In PCa-associated PSA, the linkage between the terminal sialic acid and galactose residues changes to an α2,3 linkage [14]. (B) Anti-fPSA mAb (8A6) was coupled to carboxylated MagPlex beads. Twenty microliters of serum was added to 8A6 mAb-conjugated bead to capture fPSA in the serum. (C) Anti-α2,3-linked sialic acid mAb (HYB4) was added to bind to S2,3PSA in the bead mixture, forming a bead–8A6 mAb–fPSA–HYB4 mAb complex. (D) PE-labeled anti-IgG3 mAb was added to the solution to couple with the bead complex. (E) The number of bead complexes were counted and expressed as MFI using Luminex100.