



# Narrative review of urinary glycan biomarkers in prostate cancer

Shingo Hatakeyama<sup>1</sup>^, Tohru Yoneyama<sup>2</sup>^, Yuki Tobisawa<sup>3</sup>^, Hayato Yamamoto<sup>3</sup>, Chikara Ohyama<sup>1,2,3</sup>^

<sup>1</sup>Department of Advanced Blood Purification Therapy, Hirosaki University Graduate School of Medicine, Hirosaki, Japan; <sup>2</sup>Department of Glycotechnology, Center for Advanced Medical Research, Hirosaki University Graduate School of Medicine, Hirosaki, Japan; <sup>3</sup>Department of Urology, Hirosaki University Graduate School of Medicine, Hirosaki, Japan

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*Correspondence to:* Shingo Hatakeyama, MD. Department of Urology, Hirosaki University Graduate School of Medicine, 5 Zaifu-cho, Hirosaki 036-8562, Japan. Email: shingoh@hirosaki-u.ac.jp.

**Abstract:** Prostate cancer (PC) is the second most common cancer in men worldwide. The application of the prostate-specific antigen (PSA) test has improved the diagnosis and treatment of PC. However, the PSA test has become associated with overdiagnosis and overtreatment. Therefore, there is an unmet need for novel diagnostic, prognostic, and predictive biomarkers of PC. Urinary glycoproteins and exosomes are a potential source of PC glycan biomarkers. Urinary glycan profiling can provide noninvasive monitoring of tumor heterogeneity and aggressiveness throughout a treatment course. However, urinary glycan profiling is not popular due to technical disadvantages, such as complicated structural analysis that requires specialized expertise. The technological development of glycan analysis is a rapidly advancing field. A lectin-based microarray can detect aberrant glycoproteins in urine, including PSA glycoforms and exosomes. Glycan enrichment beads can enrich the concentration of *N*-linked glycans specifically. Capillary electrophoresis, liquid chromatography-tandem mass spectrometry, and matrix-assisted laser desorption/ionization-time of flight mass spectrometry can detect glycans directory. Many studies suggest potential of urinary glycoproteins, exosomes, and glycosyltransferases as a biomarker of PC. Although further technological challenges remain, urinary glycan analysis is one of the promising approaches for cancer biomarker discovery.

**Keywords:** Prostate cancer (PC); urinary; biomarker; glycan; glycosylation

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## Introduction

Prostate cancer (PC) is the second most commonly diagnosed malignancy in men worldwide (1-6). The application of the prostate-specific antigen (PSA) test has greatly improved the diagnosis and treatment of PC (7-24). However, PSA has become associated with the

overdiagnosis of patients with the non-aggressive disease and displays limited usefulness in patients with castration-resistant PC (5,25-37). Therefore, there is an unmet need for novel diagnostic (detection of early-stage disease, and differentiation of benign from malignant disease), prognostic (prediction of disease outcome and monitoring

^ ORCID: Shingo Hatakeyama, 0000-0002-0026-4079; Tohru Yoneyama, 0000-0002-1098-407X; Yuki Tobisawa, 0000-0001-8026-9541; Chikara Ohyama, 0000-0003-1550-8379.

of disease recurrence), and predictive (monitoring of the response to therapeutics and aiding treatment decisions) biomarkers.

Many studies suggested the role of glycans as potential biomarkers for diseases (38-54). A broad range of glycan alterations have been observed in blood-based or tissue-based analysis, including aberrant PSA glycosylation, increased sialylation, core fucosylation, *O*-GlcNAcylation, and branched *N*-glycan formation (55-71). Of fluid-based biomarkers, urine is one of the most minimally invasive and promising sources for the discovery of new biomarkers of PC and contains cells, DNA, RNA, proteins, extracellular vesicles (exosomes), and glycans (72-80). However, not enough evidence currently exists to support the identification of urine-based glycan biomarkers of PC, due to methodological difficulties in analyzing complex glycan structure. However, the technological development of glycan analysis is rapidly advancing in association with the development of high-throughput platforms. In this review, we discuss the overview of glycan analysis and the potential of urinary glycans for diagnostic and prognostic biomarkers for PC. We present the following article in accordance with the Narrative Review reporting checklist (available at <http://dx.doi.org/10.21037/tau-20-964>).

## Overview of glycan analysis

### *Role of glycosylation and types of glycoproteins*

Glycans (saccharides) are known to have crucial roles in molecular communications and are essential for nearly every biological process (*Figure 1A*) (81). It is believed that over 50% of all proteins are glycosylated (40,82). Glycosylation is the most common posttranslational modification and is tightly controlled by specific glycosylation enzymes via one-to-one correspondence, the expression of glycosylation enzymes are regulated by epigenetic modification (41). There are two types of glycosylation; *O*-linked and *N*-linked glycosylation (*Figure 1B*) (40,82). *N*-linked glycosylation occurs at the consensus sequence of asparagine-X-serine/threonine (X is any amino acid except proline) and includes major three *N*-glycan structure types (high-mannose, complex, and hybrid glycans) (40). *O*-linked glycans are usually attached to serine or threonine residues and include eight *O*-GalNAc glycan core structures (cores 1 to 8) (82). The extent of glycosylation depends on the number of glycosylation sites in a protein and the expression of specific glycosylation enzymes within the cells. Dysfunction

of glycosylation can abnormally influence homeostasis (41,81,83-85). Therefore, cancer therapies targeting glycans may have the potential to improve diagnosis and treatment outcomes (86).

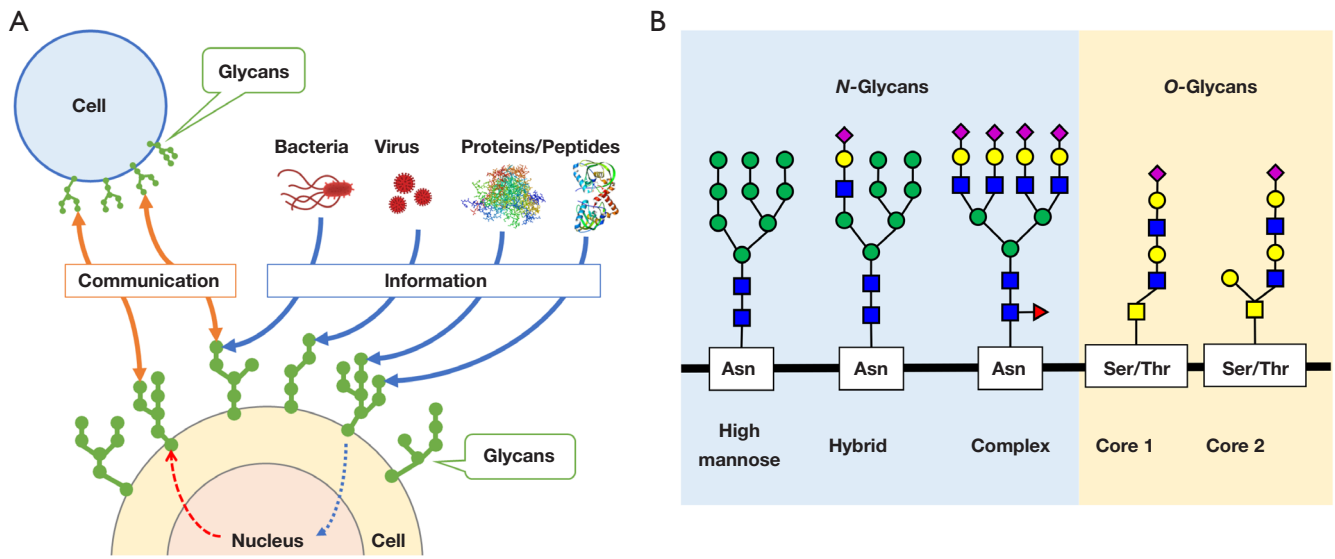
### *Potential biomarkers in urine*

The advantages of urine analyses include noninvasive and repeat sampling to identify cancers such as PC or urothelial carcinoma (*Figure 2*). Urine after prostate massage contains many potential biomarkers for PC, including cells, DNA, RNA, proteins, exosomes, bacteria (microbiome), viruses, and other small molecules (72,75,87-92). Several RNA biomarkers have been used clinically, such as in the urinary PC antigen 3 test (RNA-based urinary marker) (93) and the ExoDx Prostate test (detection of *PCA3*, *ERG*, and *SPDEF* genes in urinary exosomes) (94). However, not many glycan-based urinary biomarkers are available due to the technical difficulties of glycan analysis. A lectin-based microarray can detect aberrant glycoproteins in urine (95), including PSA glycoforms and exosomes (60). Glycan enrichment beads (Sweetblot) can specifically enrich the concentration of *N*-linked glycans (96). Capillary electrophoresis, liquid chromatography-tandem mass spectrometry (LC-MS) (97), and matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF/MS) can detect glycans directly (44). However, each methodology has its strengths and weaknesses for glycan analysis. The lectin-based assay can detect both glycans and core proteins but needs multiple lectins to detect the specific structure of glycans. Mass spectrometry is mandatory to detect the whole structure of glycans, but not easy to detect core proteins. Therefore, multiple steps are necessary to see an overall picture of glycans. This represents a bottleneck in the technique of glycan analysis.

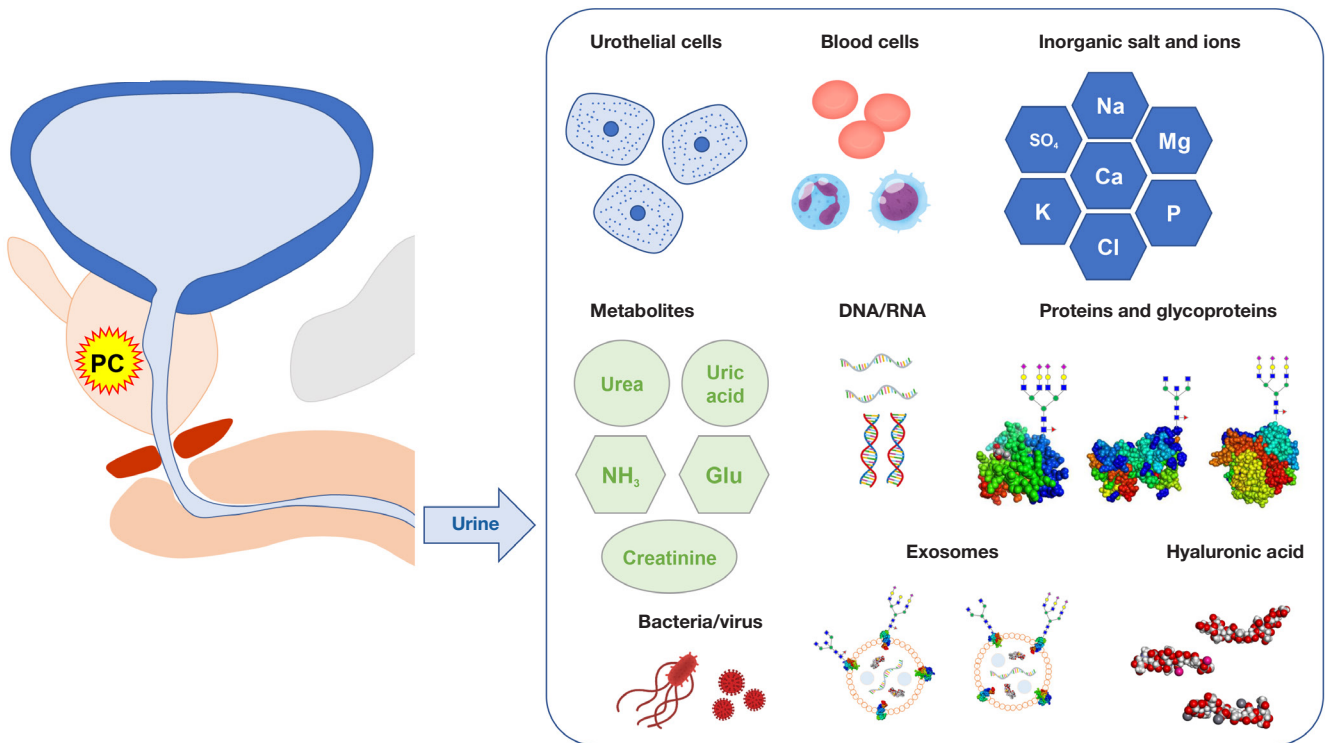
## Methods

### *Search methods for identification of studies*

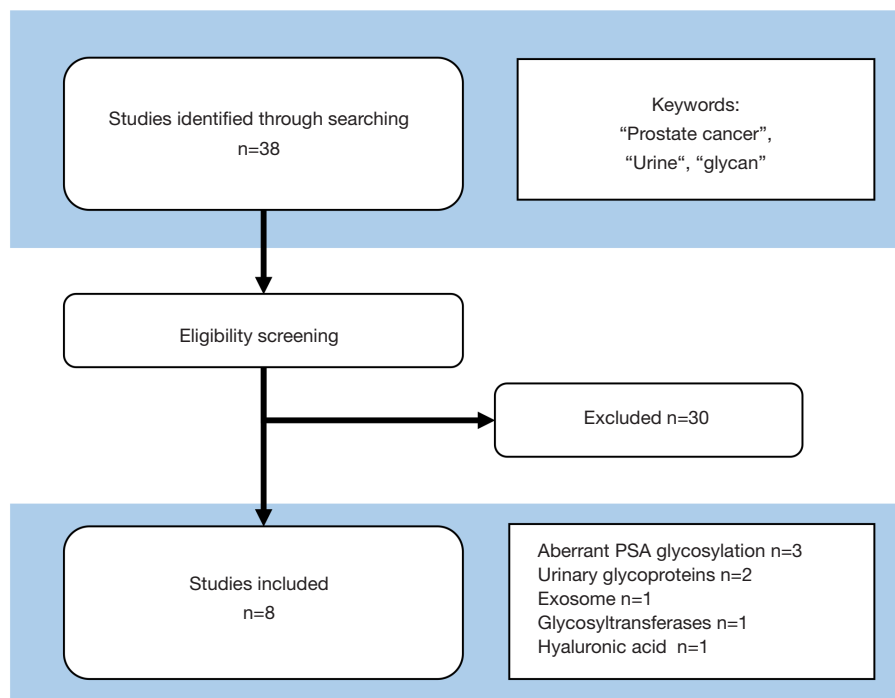
PubMed online database was accessed for research on Aug 10<sup>th</sup>, 2020. Searches were performed using the keywords: "prostate cancer", "urine", and "glycan". Each identified abstract was independently evaluated by two authors. All studies were independently evaluated and selected the consistent criteria such as independent cohort, a proper number of samples and controls, clinically meaningful outcomes, and promising diagnostic/prognostic



**Figure 1** Role and types of glycans. Role of glycans in cell-to-cell communications (A) and types of glycoproteins (B) are shown.



**Figure 2** Potential urinary biomarkers for prostate cancer. Urine after prostate massage contains many potential biomarkers for PC, including cells, DNA, RNA, proteins, exosomes, bacteria, virus, and other small molecules.



**Figure 3** Search methods for identification of studies. PubMed online database was accessed for research on Aug 10th, 2020. Searches were performed using the keywords: “prostate cancer”, “urine”, and “glycan”. Inclusion criteria were (I) independent cohort, (II) a proper number of samples and controls, (III) clinically meaningful outcomes, and (IV) promising diagnostic/prognostic performance.

performance. This study was performed according to the ethical standards of the Declaration of Helsinki and approved by the ethics review boards of the Hirosaki University School of Medicine (authorization number: 2019-001 and 2019-099).

### Result of study screening

We identified 38 studies and excluded 30 studies that did not meet the inclusion criteria. Finally, we included 8 studies in this narrative review (Figure 3). Studies were classified into 5 categories such as (I) aberrant PSA glycosylation, (II) urinary glycoproteins, (III) exosome, (IV) glycosyltransferases, and (V) hyaluronic acid. The number of studies for PC detection, aggressive disease, and both of them were 6 (80,97-101), 1 (77), and 1 (79), respectively.

#### *Urinary glycan analysis detecting aberrant PSA glycosylation*

PSA is a glycoprotein that has been used widely as a

biomarker for PC. However, it has been associated with overdiagnosis and overtreatment of non-aggressive cancers (21,25,35,102). There is an unmet clinical need to identify aggressive PC requiring intensive treatment. Several studies have identified specific cancer-associated glycan structures and PSA glycosylation (63,65,103). Several studies suggest the importance of fucosylation associated with cancer and inflammation (55,104). Fujita *et al.* investigated the association of urinary fucosylated PSA levels with the detection of aggressive PC (79). They investigated Lewis-type or core-type fucosylated PSA (PSA-AAL) and core-type fucosylated PSA (PSA-PhoSL) in from urine in 69 patients who suspected PC (20 patients without PC and 49 patients with PC) and found urinary fucosylated PSA was significantly decreased in the men with PC compared with the men without PC ( $P=0.026$  and  $P<0.001$ , respectively). Also, both PSA-AAL and PSA-PhoSL were significantly associated with the Gleason scores of the biopsy specimens ( $P=0.001$ , and  $P<0.001$ , respectively). The area under the receiver-operator characteristic curve (AUC) value for the prediction of cancers of Gleason score  $\geq 7$  was 0.69

**Table 1** Summary of urinary glycan biomarkers the information of the Food and Drug Administration (FDA) approved biomarkers and/or those commercially availability

First author, year	Samples, n	Glycans	Clinical utility	AUC	Sensitivity	Specificity	FDA approval
Fujita, 2016	Ctrl, 20 vs. PC, 49	Serum PSA	PC detection	0.59	N/A	N/A	Yes
		Urinary PSA	PC detection	0.58	N/A	N/A	No
		Urinary PSA-AAL	PC detection	0.69	N/A	N/A	No
		Urinary PSA-PhoSL	PC detection	0.72	N/A	N/A	No
		Urinary PSA-AAL	Aggressive disease (GS ≥8)	0.69	N/A	N/A	No
		Urinary PSA-PhoSL	Aggressive disease (GS ≥8)	0.77	N/A	N/A	No
		Urinary PSA-AAL and PSA-PhoSL levels	Aggressive disease (GS ≥7)	0.82	74.1%	81.5%	No
Hisao CJ, 2016	Ctrl, 61 vs. PC, 38	Urinary H5N4S1F1, monosialylated, sialylated, and unfucosylated glycoforms	PC detection	0.72	87.5%	60.0%	No
Barrabés, 2017	Ctrl, 18 vs. PC, 35	Urinary S2,6PSA	PC detection	N/A	N/A	N/A	No
Vermassen, 2013	BPH, 62 vs. PC, 42	Urinary glycoprofile marker	PC detection (gray zone)	0.77	90%	47%	No
Vermassen, 2015	BPH, 93 vs. PC, 74	Urinary glycoprofile marker	PC detection (gray zone)	0.81	58%	91%	No
Nyalwidhe, 2013	Ctrl pool, 10 vs. PC pool, 48	Urinary exosome	PC detection	N/A	N/A	N/A	No
Kojima, 2015	Prostatectomy, 35	Glycosyltransferases (GCNT1)	Disease localization and tumor expansion	0.7614	N/A	N/A	No
Skarmoutsos, 2018	Ctrl, 52 vs. PC, 66 (PIN 26)	Hyaluronidase	PC detection	0.69	–	–	No
		Hyaluronic acid	PC detection	0.65	–	–	No

PIN, prostatic intraepithelial neoplasia ratio of nonfucosylated bi-, tri-, and tetra-antennary glycan structures on total of triantennary glycan structures; GCNT1, Core2  $\beta$ -1,6-N-acetylglucosaminyltransferase-1; N/A, not applicable.

( $P=0.0064$ ) for urinary PSA-AAL and 0.72 ( $P=0.0014$ ) for urinary PSA-PhoSL. They developed an optimum logistic regression model to predict the probability of detecting cancers with a  $GS \geq 7$  in biopsy was obtained as  $P = [1 + \exp(1.247 + 4.56 \times PSAD - 0.00448 \times PSA-AAL - 0.0493 \times PSA-PhoSL)]^{-1}$ . Using this model, the AUC value for the prediction was 0.82 (95% CI 0.72–0.92,  $P < 0.0001$ ) with the sensitivity and specificity of the model at the best cutoff value were 74.1% and 81.5%, respectively (Table 1). Although the biological mechanism leading to decreased

urinary fucosylated PSA level in urine remains unclear, decreased urinary fucosylated PSA level may be a potential marker for aggressive PC.

It is possible to analyze urine PSA glycoforms using LC-MS with ion accumulation. Hsiao *et al.* reported that monosialylated, sialylated, and unfucosylated glycoforms of PSA were significantly different between PC and control samples (97). They investigated 61 benign prostate hyperplasia (BPH) urine samples and 38 PC urine samples. After the immunoprecipitation and in-gel protein

digestions, the peptides and *N*-glycopeptides generated from the chymotrypsin digestion were analyzed with an LC-MS. The normalized Hex5HexNAc4NeuAc1dHex1 (H5N4S1F1), monosialylated, sialylated, and unfucosylated glycoforms showed significant differences between BPH and PC. The ROC curve and the AUC of those glycoforms showed significant differences in PC detection with sensitivity and specificity of 87.5% and 60%, respectively (Table 1). This result suggests the unfucosylated glycoforms of PSA were potential urinary glycan biomarkers in PC, in opposition to the results from Fujita *et al.* (79). One reason for this discrepancy might be the methodological differences between the lectin-antibody ELISA detection and LC-MS detection. Furthermore, the preparation of urine samples greatly influences the outcomes of downstream analyses. For example, urinary Tamm-Horsfall Protein (uromodulin) interferes with urinary assays and forms contaminant precipitates in the urine. Therefore, urinary aberrant PSA glycosylation needs further study to apply the clinical practice.

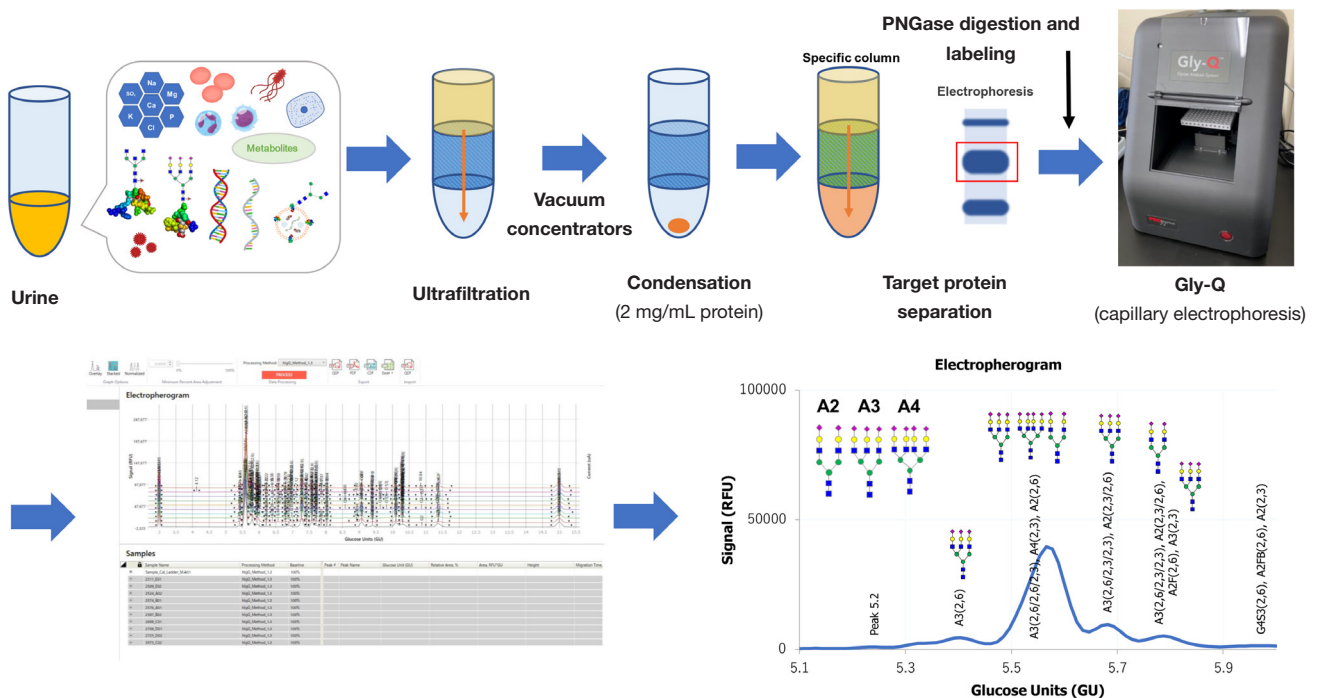
PSA has a single *N*-glycosylation site at asparagine-69 (103). Multiple studies have confirmed the ratio of complex biantennary glycans of  $\alpha$ -2,3-sialic acid (S2,3PSA) and  $\alpha$ -2,6-sialic acid (S2,6PSA) in serum have been closely linked to aggressive PC (56,58,105,106) in over 50 glycoforms of serum PSA. However, this was not replicated in the urinary PSA. A previous study evaluated the clinical utility of S2,6PSA from urine after prostate massage in 35 patients diagnosed with PC and in 18 controls (98). They found no significant difference in S2,6PSA levels between the biopsy negative patients and PC patients with Gleason score 6 ( $P=0.364$ ), between the biopsy negative patients and PC patients with Gleason score 7 ( $P=0.116$ ), and between the biopsy negative patients and PC patients with Gleason score 8 or more ( $P=0.276$ ). Also, they found no relationship was found between S2,6PSA and PC aggressiveness. These results may suggest the limited utility of S2,6PSA alone in urine to detect PC. The ratio of S2,3PSA and S2,6PSA needs to be investigated because these 2 glycoforms are associated with each other during the PC progression. Therefore, this finding needs to be interpreted with caution because of the small sample size and limitation measurement of PSA glycoforms. Currently, urinary fucosylated PSA levels are a promising biomarker for PC detection and aggressiveness among the aberrant PSA glycosylation.

### Capillary electrophoresis of urinary glycoproteins

Capillary electrophoresis is a technique that separates molecules via an electric field according to size and charge. Several capillary electrophoresis-based systems for urinary glycan analysis are available, such as the Gly-Q system (Figure 4) and the multibacillary electrophoresis-based ABI3130 sequencer. Vermassen *et al.* (99) evaluated urinary *N*-glycosylation profiles in post-prostate massage urine using capillary electrophoresis and demonstrated differences between patients with PC and benign prostate hyperplasia. Also, they developed a urinary glycoprofile marker (ratio of non-fucosylated bi-, tri-, and tetra-antennary glycan structures on total triantennary glycan structures divided by the prostate volume), and showed the potential to differentiate benign prostate hyperplasia from PC with the AUC, sensitivity, and specificity of 0.77, 90%, and 47%, respectively (Table 1). The updated analysis showed similar performance of the urinary glycoprofile marker in the patients with a gray zone (Table 1). The predictive accuracy of the urinary glycoprofile marker was significantly better than that of serum PSA ( $P<0.001$ ) (80). A Capillary electrophoresis system can analyze glycoprotein in urine; however, limited evidence is currently available. Also, we need to combine some glycans (such as urinary glycoprofile marker) to detect PC. Further large-scale studies are necessary to address the use of capillary electrophoresis-based analysis to identify urinary glycan PC biomarkers.

### Exosomes

Exosomes are extracellular vesicles with a diameter of 30–200 nm that are secreted from most cell types. Urinary exosomes contain not only RNA and proteins, but also glycoproteins (75,107). However, glycan profiles in exosomes are largely unexplored in patients with PC. Urinary exosomes in PC may be promising sources of novel biomarker discovery, as urinary glycoproteins can be analyzed by lectin-based ELISA, lectin-based microarray, capillary electrophoresis, and mass spectrometry. Nyalwidhe *et al.* (101) reported *N*-glycan profiling of urinary exosomes after prostate massage using lectin and MALDI-TOF-based profiling techniques. They demonstrated a decline of larger branched triantennary and tetraantennary *N*-glycans in exosomes using pooled samples (Table 1) (101). However, the major limitation of exosome analysis might be the



**Figure 4** Schematic protocol of direct glycan analysis using capillary electrophoresis (Gly-Q). Capillary electrophoresis-LED-induced fluorescence-based Gly-Q *N*-glycan analysis system (Prozyme, Inc., CA, USA) combined with Gly-X rapid *N*-glycan preparation method can measure the amount of glycans under controlled automated Sweetblot™ (System Instruments, Tokyo, Japan) machinery. Briefly, 1 mg/mL of target protein from the urine and 2  $\mu$ L of Gly-X denaturant was mixed. Then, 2  $\mu$ L of *N*-glycanase working solution was added to the denatured samples. After deglycosylation, 5  $\mu$ L of InstantPC dye solution was added to the deglycosylated samples. The InstantPC Dye and deglycosylated sample mixture was then loaded onto pretwetted Gly-X cleanup plate and applied vacuum to <5 inHg. Then, 100  $\mu$ L of Gly-X InstantPC eluent added to each well and collected InstanPC-labeled glycan samples into the Collection Plate using vacuum. Finally, InstantQ is a charged *N*-glycan dye that facilitates separation of labeled *N*-glycans on the Gly-Q CE system. Composition and structures of the glycans were analyzed using the Gly-Q Manager software performing automated peak analysis (Relative Fluorescence Unit: RFU and Glucose Unit: GU) and glycan assignments from the glycan library.

time-consuming methods of purification and small yield of exosomes from urine. Exosome preparation requires several sessions of ultracentrifugation (e.g., 25,000  $\times$ g for 30 min, followed by supernatant centrifugation at 100,000  $\times$ g for 4 h) and the expression of exosome protein markers (CD63 and CD9) needs to be verified. In some cases, pooled samples (from three patients) are needed due to small numbers of exosomes. Therefore, this protocol may not be feasible for the analysis of individual patients. Further methodological advancement for exosome enrichment is necessary for clinical application.

### Upregulation of glycosyltransferases

Aberrant glycosylations are caused by the over- or under-expression of glycosyltransferases in cancer cells (35,108). Several reports suggest positive links between aberrant glycosyltransferases and disease progression (57,59,62,77,104,109-112) through the androgen receptor regulation. Of these, *N*-acetylgalactosaminyltransferase 7 (GALNT7) and Core2  $\beta$ -1,6-*N*-acetylglucosaminyltransferase-1 (GCNT1) are associated with androgen receptor splice variant-7 (AR-V7) (113). Taken together, these observations suggest the importance

of glycosyltransferases for PC progression. However, only a few studies investigate the clinical utility of urinary glycosyltransferases in PC. Kojima *et al.* (77) have reported the detection of GCNT1 in post-massage urine by immunoblotting can predict the extracapsular extension of PC after radical prostatectomy. They investigated post-digital rectal examination urine from 35 patients before underwent radical prostatectomy and detected GCNT1 by an anti-GCNT1 monoclonal antibody, followed by a horseradish peroxidase (HRP)-conjugated antibody. The GCNT1 expression ( $P=0.006$ ) was highly correlated to the extracapsular extension of PC in a logistic regression analysis with the AUC value of 0.7614 (*Table 1*). Of urinary glycan markers, GCNT1 may be a potential predictive marker for tumor recurrence after radical prostatectomy. However, the association of these glycosyltransferases with specific final products (glycans) remains unclear, as overexpression of individual glycosyltransferases does not always lead to the overexpression of specific glycans. Therefore, further studies are necessary before the use of these glycosyltransferases as diagnostic and prognostic biomarkers for PC.

#### ***Hyaluronic acid and hyaluronidase***

Hyaluronic acid and hyaluronidase may represent potential novel urine biomarkers for the diagnosis of PC. Skarmoutsos *et al.* (100) investigated post-prostate massage urine from 118 high-risk PC patients, and hyaluronic acid and hyaluronidase were detected via enzyme-linked immunosorbent assay. Their results suggested that hyaluronic acid and hyaluronidase were independently associated with PC and that higher levels of hyaluronic acid and hyaluronidase were associated with a higher incidence of PC (100). ROC analysis for hyaluronic acid and hyaluronidase had a significant predictive ability for PC with AUC of 0.65 (70% sensitivity and 55.2% specificity) and 0.69 (65% sensitivity and 53.9% specificity), respectively (*Table 1*). However, limitations of this study included the lack of molecular size analysis of hyaluronic acid and the determination of specific hyaluronidases. The molecular size of hyaluronic acid has been hypothesized to play a role in tumor aggressiveness (114,115), and several types of hyaluronidases are associated with the digestion of hyaluronic acid (116,117). Additionally, no information is available to correlate tumor aggressiveness with levels of hyaluronic acid and hyaluronidase. Therefore, these findings may suggest a role for hyaluronic acid and hyaluronidase, as yet understudied potential biomarkers for PC.

#### ***The potential methodology of detection of urinary glycan biomarkers***

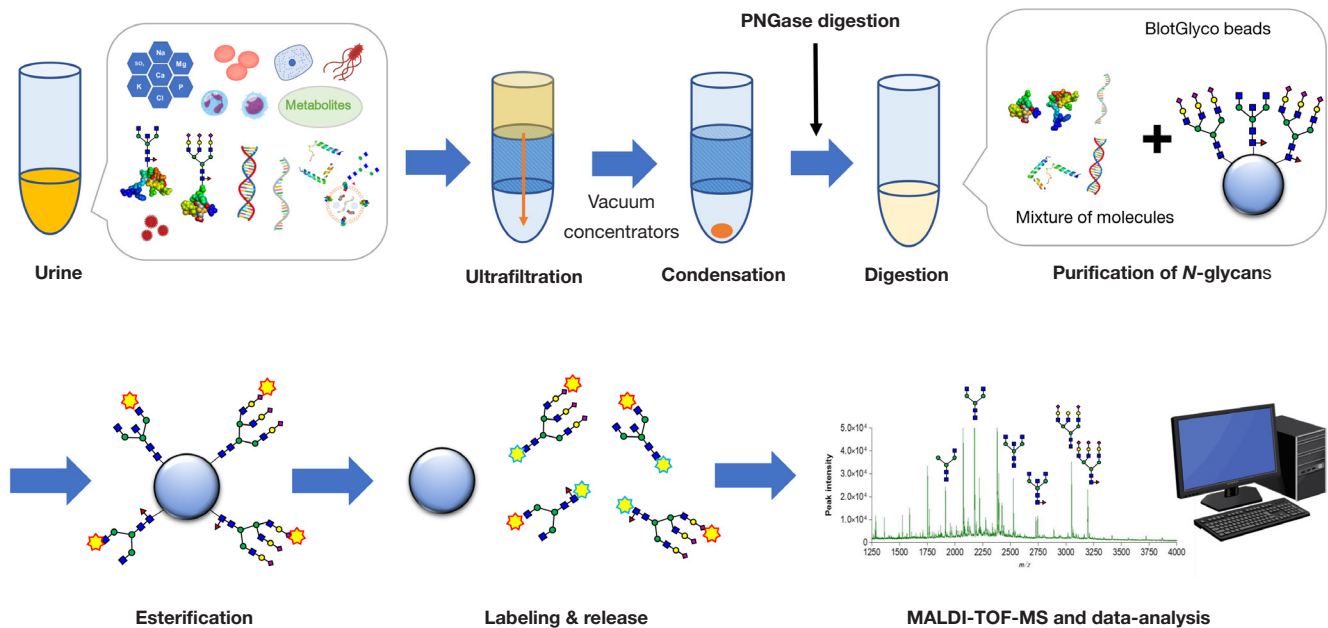
##### **Direct measurement of urinary glycans using mass spectrometry**

Advances in mass spectrometry has led to the direct detection of glycans from fluid-based samples. With this methodology, it is extremely important to purify glycans from contaminants despite small sample sizes. To achieve this, a new technology for glycan-specific enrichment, called “a glycoblotting method,” was developed (96). A combination of glycoblotting and MALDI-TOF/MS enabled a high-throughput and quantitative glycomic analysis of various biological samples that included a large number of impurities (*Figure 5*) (44-46,49,51,60,118). However, the removal of impurities and adjustment of protein concentration is challenging for urinary analysis compared to a blood-based platform. Also, *O*-glycan analysis requires specialized techniques and processes to separate the *O*-glycans from proteins, such as a chemical reaction with ammonia salts. Although *N*-glycans are able to be released from proteins by peptide *N*-glycanase-F or -A (PNGases), no enzyme can specifically release *O*-glycans from proteins. No study has evaluated *N*- and *O*-glycans in urine from patients with PC, and only one study has evaluated urinary *O*-glycans in bladder cancer (44). The high cost of mass spectrometry is one of the major concerns in using this system for clinical applications. Further technological advancement is necessary for the direct measurement of urinary glycans.

##### **Lectin-based microarray analysis for glycoproteins**

Another methodology for the analysis of protein glycosylation is the lectin array (*Figure 6*). Lectins are glycan-binding proteins that selectively recognize free carbohydrates or glycoprotein epitopes. Lectin-based microarray systems have been developed to analyze both glycan profiles and glycoproteins (60,95) and can analyze both serum and urine. Matsumoto *et al.* (60) reported the use of lectin-based microarrays to identify serum  $\alpha$ -1-acid glycoprotein in patients with metastatic castration-resistant PC (CRPC). They found terminal  $\alpha$ -2,3-sialylated glycan,  $\alpha$ -2,6-sialylated glycan, and terminal galactose were significantly increased in the CRPC patients (60). Anan *et al.* (95) reported the use of lectin-based microarrays to identify urinary osteopontin, and found that the glycosylation profile of osteopontin was significantly different in patients with urolithiasis (95). One limitation of





**Figure 5** Schematic protocol of direct glycan analysis using SweetBlot and MALDI-TOF/MS. Fluid samples are applied to the SweetBlot for glycoblotting. After enzymatic cleavage from serum protein, total serum N-glycans released into the digestion mixture are directly mixed with BlotGlyco H beads to capture N-glycans. After the beads are separated from other molecules by washing, sialic acid is methyl-esterified. These processed N-glycans are then labeled with benzyloxyamine (BOA) and released from BlotGlyco H beads. Mass spectra of BOA-labeled N-glycans are acquired using an Ultraflex III instrument.

this system is the requirement for protein concentration in urine samples. Concentrated urine (2 mg/mL protein) was applied to a lectin-based microarray after ultrafiltration and vacuum concentration. As the density of urine varies in each sample, urinary protein concentrate needs to be adjusted and normalized for downstream analysis. Lectin-based microarray systems are promising methods of novel urinary biomarker discovery. However, no study has yet reported the use of lectin-based microarrays for urinary biomarkers in PC.

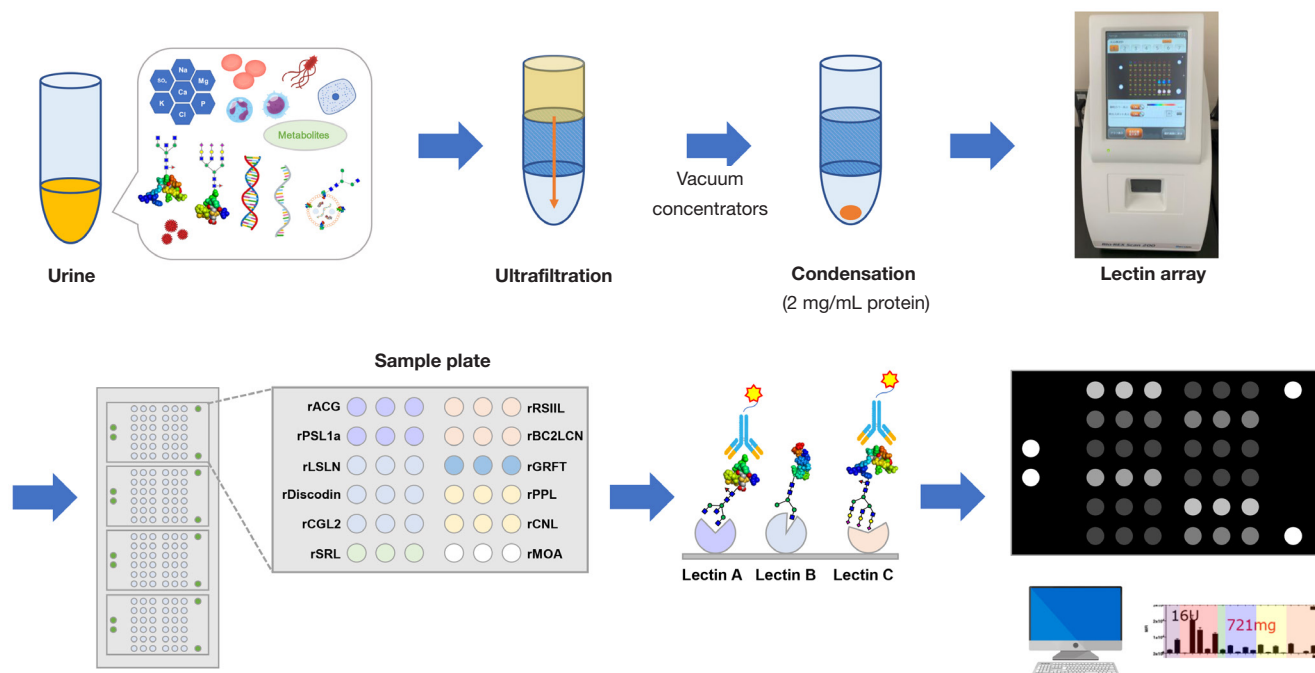
### Summary of urinary glycan biomarkers and the information of the Food and Drug Administration (FDA) approved biomarkers and/or those commercial availabilities

In this narrative review, we showed potential urinary glycan biomarkers for PC detection and aggressive disease (Table 1). Of those, urinary fucosylated PSA levels are a promising biomarker for PC detection and aggressiveness among the aberrant PSA glycosylation. Urinary CGNT1

in the post-massage urine can be useful for the prediction of the extracapsular extension after radical prostatectomy. However, no FDA approved urinary glycan biomarker is available. Also, urinary glycan biomarker analyses were carried out using a custom technique, tools, and machines, while those are commercially available. Therefore, there is a significant hurdle between the urinary glycan analysis and clinical implementation. Therefore, urinary glycan analysis is far from clinical implementation. Further studies and methodological improvements are necessary to overcome these limitations.

### Conclusions

Urinary glycan profiling exhibits high clinical potential as a noninvasive assay to monitor tumor heterogeneity and aggressiveness and may lead to personalized cancer therapies. Currently, urinary fucosylated PSA levels and urinary CGNT1 in the post-massage urine are a promising biomarker for PC detection and aggressiveness. Although several challenges remain, the technological development



**Figure 6** Schematic protocol of glycoprotein analysis using lectin-based microarray system. Glycoproteins are added to the wells of the recombinant lectin array chip. After reactions occur, the chip is scanned utilizing evanescent-wave fluorescence microscopy. Briefly, 20  $\mu\text{L}$  of sample was diluted by 80  $\mu\text{L}$  of probing buffer. Diluted sample was added to the well of recombinant lectin array chip (Rexxam Co. Ltd., Osaka, Japan). After 70 mins incubation and washing twice, 100  $\mu\text{L}$  of probing buffer containing 1  $\mu\text{g}/\text{mL}$  biotinylated specific antibody for the target protein was added to well. After 60 mins incubation and washing twice, 100  $\mu\text{L}$  of probing buffer containing 1  $\mu\text{g}/\text{mL}$  Cy3 labeled streptavidin (GE-Healthcare, Buckinghamshire, UK) was added to the well. After 30 mins incubation and washing twice, the chip was scanned by utilizing Bio-REX Scan 200 evanescent fluorescence scanner (Rexxam Co., Ltd.). Mean fluorescence intensity of lectin reactive glycan carrying Igs was normalized by total Igs level.

of glycan analysis is rapidly advancing. Urinary glycan analysis is one of the most promising approaches for cancer biomarker discovery.

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### Footnote

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“Urinary Biomarkers of Urothelial Malignancies” published in *Translational Andrology and Urology*. The article has undergone external peer review.

**Reporting Checklist:** The authors have completed the NARRATIVE REVIEW reporting checklist. Available at <http://dx.doi.org/10.21037/tau-20-964>

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**Conflicts of Interest:** All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tau-20-964>). The series “Urinary Biomarkers of Urothelial Malignancies” was commissioned by the editorial office without any funding or sponsorship. The authors have no other conflicts of interest to declare.

**Ethical Statement:** the authors are accountable for all

aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was performed according to the ethical standards of the Declaration of Helsinki and approved by the ethics review boards of the Hirosaki University School of Medicine (authorization number: 2019-001 and 2019-099).

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