Tohoku J. Exp. Med., 2020, **252**, 1-8

Expression of Ganglioside Disialosyl Globopentaosyl Ceramide in Prostate Biopsy Specimens as a Predictive Marker for Recurrence after Radical Prostatectomy

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Carbohydrate antigens are associated with carcinogenesis, cancer invasion, and metastasis and their expression reflect biological activities of various cancers. We previously reported that expression of disialosyl globopentaosyl ceramide (DSGb5), one of carbohydrate antigens, in radical prostatectomy specimens independently predicted biochemical recurrence (i.e., elevating serum prostate specific antigen without recurrent lesions in the image) after radical prostatectomy. However, it is important to evaluate the prognosis at the diagnosis. In this study we investigated DSGb5 expression in prostate biopsy specimens to develop a novel biomarker for providing appropriate management. Between 2005 and 2011, patients who underwent both prostate biopsy and radical prostatectomy in our institution were included. The median follow-up period was 88 months. DSGb5 expression was assessed by immunohistochemical staining and defined 116 patients as high DSGb5 expression (42 patients) or low DSGb5 expression (74 patients). High DSGb5 expression was significantly associated with lymphovascular invasion in radical prostatectomy specimens on both univariate and multivariable analyses (p = 0.028, 0.027). On multivariable analysis, Gleason Score in prostatectomy specimen, positive resection margin, and DSGb5 expression in the biopsy specimen were independently associated with biochemical recurrence-free survival following radical prostatectomy (p = 0.004, 0.008, 0.024). When targeting only patients with negative resection margin, DSGb5 expression was significantly associated with biochemical recurrence-free survival on both univariate and multivariable analyses (p = 0.006, 0.007). DSGb5 expression in prostate biopsy specimens is predictive of lymphovascular invasion and biochemical recurrence-free survival following radical prostatectomy. DSGb5 is a potential biomarker for preoperatively predicting oncological outcomes of prostate cancer.

Keywords: biochemical recurrence; ganglioside disialosyl globopentaosyl ceramide; immunohistochemical staining; lymphovascular invasion; prostate biopsy
Tohoku J. Exp. Med., 2020 September, **252** (1), 1-8.

Introduction

When treatment plans are decided for patients with localized prostate cancer, physicians and patients need to know the actual outcomes after radical treatment. In daily practice, most urologists often use the D'Amico risk classification (D'Amico et al. 1998), but this classification cannot always accurately reflect the condition of the disease. Recently, to address the problem of risk classification, several gene analyses of prostate cancer have become commercially available (Taylor et al. 2010). However, it is necessary to develop a more accurate risk classification or a new biomarker to provide appropriate management for each patient.

Carbohydrate antigens are involved with cell recognition, extracellular molecule recognition, generation, cell motion and cell growth, cell signaling, etc. Since carbohydrate antigens change with carcinogenesis and have rela-

Received May 1, 2020; revised and accepted July 22, 2020. Published online August 19, 2020; doi: 10.1620/tjem.252.1.
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tionships with cancer invasion and cancer metastasis, they have been a focus in the search for useful biomarkers (Hakomori and Kannagi 1983; Hakomori 1996).

To date, we have reported the role of carbohydrate antigens and their clinical utilities in several urologic cancers. The increased expression of longer-chain gangliosides in primary renal cancer is one of the factors associated with the development of metastasis (Saito et al. 1991). Of these gangliosides, monosialosyl globopentaosyl ceramide (MSGb5), disialosyl globopentaosyl ceramide (DSGb5) (Saito et al. 1994), and N-acetyl galactosyl disialosyl lactotetraosyl ceramide (GalNAcDSLc4) (Ito et al. 2001b) were identified in renal cell carcinoma (RCC). Next, we produced the antibodies against these gangliosides (RM1 (Saito et al. 1994), 5F3 (Ito et al. 2001b), RM2 (Saito et al. 1994), identified the gene of glycosyltransferase (Senda et al. 2007), and reported some remarkable results related to RCC metastasis. Primary RCC with expression of MSGb5 or GalNAcDSLc4 had a high risk of metastasis based on immunostaining in fresh-frozen sections of RCC tissue (Saito et al. 1997; Maruyama et al. 2007). DSGb5 expression was associated with microvascular invasion in paraffin-embedded RCC tissues, and patients with high DSGb5 expression showed significantly lower recurrence-free survival rates (Itoh et al. 2017). From the results of in vitro experiments using small interfering RNA (siRNA) transfectant for DSGb5 synthase gene, it was found that DSGb5 expressed in RCC cells inhibits the cytotoxicity of natural killer (NK) cells through interactions between sialic acid-binding immunoglobulin-like lectin-7 (Siglec-7) on NK cells and DSGb5 on RCC cells (Ito et al. 2001a; Kawasaki et al. 2010). And high DSGb5 expression in RCC cells enhances the migration and infiltration of RCC cells (Kawasaki et al. 2015). These findings suggest that DSGb5 expression in RCC cells is correlated with the metastatic potential of RCC via promotion of cell motility and inhibition of the immune system.

In prostate cancer, we previously reported that GalNAcDSLc4 reflected a high Gleason Score and prostate specific antigen (PSA) recurrence-free survival following radical prostatectomy (Saito et al. 2005). PSA recurrence is equal to biochemical recurrence that is a condition in which serum PSA is elevated before the recurrent lesion is detected in the image. We also reported that DSGb5 expression in paraffin-embedded prostate cancer tissue obtained from radical prostatectomy was associated with lymphovascular invasion. In addition, patients with high DSGb5 expression had a significantly lower biochemical recurrence-free survival rate following radical prostatectomy, and DSGb5 expression is an independent predictor of biochemical recurrence-free survival (Shimada et al. 2014).

In the present study, the associations of DSGb5 expression in prostate biopsy specimens with pathological characteristics of radical prostatectomy specimens and biochemical recurrence after radical prostatectomy were investigated in order to develop a novel biomarker for providing appropriate management to each patient at the time of diagnosis of prostate cancer.

Materials and Methods

Patients' characteristics

This study was approved by the institutional review board at Tohoku University Hospital, and all patients gave their written, informed consent.

A total 121 consecutive patients who underwent both prostate biopsy and radical prostatectomy in our institution between January 2005 and December 2011 were included. One patient whose biopsy specimen could not be sliced, and four patients whose biopsy specimens did not include prostate cancer were excluded. The remaining 116 patients were evaluated.

Immunostaining

Anti-DSGb5 monoclonal antibody (5F3, mouse IgM) was established in our laboratory (Ito et al. 2001c).

Four-micrometer-thick sections were prepared from formalin-fixed paraffin-embedded prostate biopsy specimens, and hematoxylin and eosin (HE) staining and immunohistochemical staining with 5F3 were performed. For the diagnosis of prostate cancer, 12-core transrectal ultrasound prostate biopsy was performed in our hospital, as described previously (Orikasa et al. 2008). Pathological diagnosis was done by one uropathologist (M.W.) in our hospital.

In the present study, immunohistochemical staining of all cancer-positive cores of a patient was not performed. The prostate was divided into six regions (ventral apex of the right lobe, near apex of the right lobe, near base of the right lobe, ventral apex of the left lobe, near apex of the left lobe, near base of the left lobe), and the one representative specimen including the maximum length of prostate cancer in each region was chosen.

The methods of immunohistochemical staining were described in a previous report (Shimada et al. 2014). The immunoreactivities were evaluated by two different investigators without knowledge of the patient data. In this immunohistochemical staining, anti-DSGb5 monoclonal antibody (5F3) was used for prostate biopsy specimens. The immunoreactive DSGb5 was consistently detected in prostatic glandular cells of all specimens, but was undetectable in prostatic basal cells. Thus, prostatic glandular cells were used as positive controls, and prostatic basal cells were used as negative controls. The immunoreactivity of prostate cancer cells varied, depending on biopsy specimens (Fig. 1). Based on the immunoreactivity of prostatic glandular cells ranged from positive to strongly positive (Fig. 1), the immunoreactivity of prostate cancer cells was graded as negative, weak, positive, or strongly positive (0, 1+, 2+, or 3+). The immunoreactivity was defined as strongly positive (3+) when cancer cells showed the immunoreactivity similar to the strongly positive prostatic glandular cells (Fig. 1d); positive (2+), the similar immunoreactivity of the positive prostatic glandular cells (Fig. 1c); negative (0), the

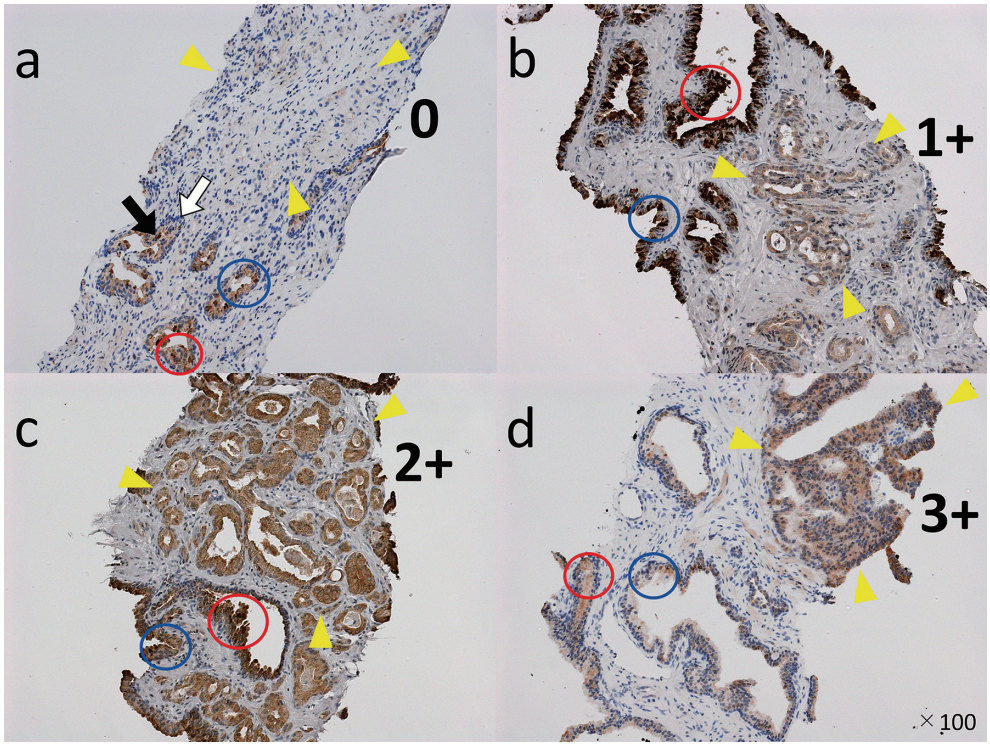

Fig. 1. Immunohistochemical staining of prostate biopsy specimens.
a, Prostate cancer cells graded as negative (0); b, weak (1+); c, positive (2+); and d, strongly positive (3+). Each biopsy specimen contained prostate cancer cells (yellow arrowhead) and adjacent prostate glandular cells (black arrow) and basal cells (white arrow). The immunoreactive DSGb5 was consistently detected in prostatic glandular cells of all specimens. Red circle indicates strongly positive part of prostatic glandular cells, and blue circle indicates positive part of glandular cells.

similar immunoreactivity of prostatic basal cells (Fig. 1a); and weak (1+), between negative and positive immunoreactivities (Fig. 1b). Positive (2+) or strongly positive (3+) immunoreactivity was defined as high DSGb5 expression, and negative (0) or weak (1+) immunoreactivity was defined as low DSGb5 expression. For the evaluation of the patients, the patients were divided into high and low DSGb5 expression groups according to the immunoreactivity of the biopsy specimen. If there were multiple specimens with cancer-positive cores, the strongest immunoreactivity among the biopsy specimens was considered the immunoreactivity of the patient.

Biochemical recurrence

Biochemical recurrence after radical prostatectomy was defined as PSA > 0.2 ng/mL on two or more determinations. If the PSA level did not decrease to less than 0.2 ng/mL after radical prostatectomy, it was considered biochemical recurrence on the day of the surgery.

Statistical analysis

For statistical analysis, Pearson's chi-squared test and the Wilcoxon rank-sum test were used as appropriate to assess the differences between two groups. Comparison of biochemical recurrence-free survival curves between the two groups was performed using the Kaplan-Meier method with the log-rank test. Cox proportional hazards multivariable models were used to examine the associations of pathological characteristics and preoperative characteristics and DSGb5 expression with biochemical recurrence-free survival. All statistical analyses were performed with JMP 14.1 (SAS Institute, Cary, NC). A p value < 0.05 was considered significant.

Results

Immunohistochemical staining of prostate biopsy specimens is shown in Fig. 1. Prostate cancer cells showed a variety of immunoreactivities, ranged from negative to strongly positive.

All patients underwent open radical prostatectomy. The number of patients was 48 (41.4%) for non-lymphadenectomy, 60 (51.7%) for limited lymphadenectomy, and eight (6.9%) for extended lymphadenectomy, and one patient (0.9%) showed lymph node metastasis. The median follow-up period was 88 months (IQR 63-112.5 months). For the follow-up period, 17 patients showed a biochemical recurrence. Among these patients, nine had high DSGb5 expression and 8 had low DSGb5 expression in biopsy specimens.

Distributions of preoperative and pathological characteristics, operative methods, and median follow-up period by the levels of DSGb5 expression are shown in Table 1.

Table 1. Characteristics of 116 patients and DSGb5 expression levels.

Characteristics of 116 patients		High DSGb5 expression (n = 42)	Low DSGb5 expression (n = 74)	*p* value
Preoperative characteristics				
Median age (y) (IQR)	64.5 (59-68)	65 (59-70)	64 (58.8-67)	0.074
PSA (ng/mL) (IQR)	5.67 (4.48-8.53)	6.77 (4.75-9.35)	5.29 (4.18-7.34)	0.251
cT1c / cT2a / ≥ cT2b	82 / 26 / 8	30 / 9 / 3	52 / 17 / 5	0.981
Biopsy GS				0.815
3+3	38 (32.8%)	13 (31.0%)	25 (33.8%)	
3+4	48 (41.4%)	18 (42.9%)	30 (40.5%)	
4+3	22 (19.0%)	7 (16.7%)	15 (20.3%)	
≥ 8	8 (6.9%)	4 (9.5%)	4 (5.4%)	
% of cancer positive cores (%) (IQR)	25 (10-33)	25 (8-42)	24 (16-33)	0.175
Maximum cancer length (mm) (IQR)	4 (3-8)	5 (3-8)	4 (3-7)	0.231
D'Amico risk (low / intermediate / high)	32 / 69 / 15	10 / 26 / 6	22 / 43 / 9	0.779
Operative method				
Lymphadenectomy (non / limited / extended)	48 / 60 / 8	15 / 22 / 5	33 / 38 / 3	0.233
Nerve sparing (non / unilateral / bilateral)	10 / 43 / 63	3 / 14 / 25	7 / 29 / 38	0.689
Pathological characteristics				
pT2 / pT3	87 / 29	33 (78.6%) / 9 (21.4%)	54 (73.0%) / 20 (27.0%)	0.656
Prostatectomy GS ≤ 7 / ≥ 8	108 / 8	40 (95.2%) / 2 (4.8%)	68 (91.9%) / 6 (8.1%)	0.709
RM: + / –	32 / 84	8 (19.0%) / 34 (81.0%)	24 (32.4%) / 50 (67.6%)	0.136
pn: + / –	73 / 43	29 (69.0%) / 13 (31.0%)	44 (59.5%) / 30 (40.5%)	0.325
LVI: + / –	12 / 104	8 (19.0%) / 34 (81.0%)	4 (5.4%) / 70 (94.6%)	**0.028**
Cancer size (mm): ≥ 1.55 / < 1.55	58 / 58	24 (57.1%) / 18 (42.9%)	34 (45.9%) / 40 (54.1%)	0.334
Follow-up period (m) (IQR)	88 (63-112.5)	88 (60.8-110.8)	88 (60.8-114.3)	0.376

GS, Gleason Score; IQR, Interquartile Range; LVI, lymphovascular invasion; pn, perineural invasion; PSA, prostate specific antigen; RM, resection margin.

Table 2. Associations between preoperative characteristics and lymphovascular invasion.

	Univariate analysis		Multivariable analysis		Multivariable analysis (Stepwise)	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Age (y) (≥ 65 vs. < 65)	3.4 (0.86-13)	0.125	2.9 (0.70-12)	0.124	2.9 (0.70-12)	0.123
PSA (ng/mL) (≥ 10 vs. < 10)	1.1 (0.22-5.5)	1.000	1.0 (0.19-5.7)	0.968		
cT stage (≥ T2 vs. ≤ T1c)	1.2 (0.35-4.4)	0.745	1.4 (0.35-5.6)	0.635		
Biopsy GS (≥ 8 vs. ≤ 7)	3.3 (0.58-18)	0.193	2.2 (0.34-14)	0.434		
Percentage of cancer positive cores (≥ 25% vs. < 25%)	0.96 (0.29-3.2)	1.000	1.7 (0.38-7.6)	0.485		
Maximum cancer length (mm) (≥ 4 vs. < 4)	0.55 (0.17-1.8)	0.357	0.33 (0.073-1.5)	0.152		
DSGb5 expression in biopsy specimens (high vs. low)	4.1 (1.2-15)	**0.028**	4.3 (1.1-16)	**0.027**	3.7 (1.0-13)	**0.040**

GS, Gleason Score; PSA, prostate specific antigen.

Of 116 patients, 42 patients had high DSGb5 expression, and 74 patients had low DSGb5 expression in prostate biopsy specimens. The rate of high DSGb5 expression in prostate biopsy specimens was 20-30% regardless of the biopsy Gleason Score. The rate of high DSGb5 expression in patients was 34.2% for biopsy Gleason Score 3+3, 37.5% for 3+4, 31.8% for 4+3, and 50.0% for 8, therefore showing the same tendency as a previous report (Shimada et al. 2014). For the associations between DSGb5 expression level and preoperative characteristics, pathological charac-

Table 3. Associations between preoperative characteristics or pathological characteristics and biochemical recurrence after radical prostatectomy.

	Univariate analysis		Multivariable analysis		Multivariable analysis (Stepwise)	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Age (y) (≥ 65 vs. < 65)	0.91 (0.35-2.4)	0.850	1.1 (0.30-3.8)	0.919		
PSA (ng/mL) (≥ 10 vs. < 10)	2.4 (0.83-6.7)	0.133	1.1 (0.30-3.7)	0.939		
D'Amico (high vs intermediate & low risk)	2.1 (0.67-6.4)	0.236	0.42 (0.069-2.6)	0.316		
DSGb5 expression in biopsy specimen (high vs. low)	2.3 (0.88-6.2)	0.088	3.9 (1.1-13)	**0.024**	4.0 (1.4-12)	**0.012**
Prostatectomy GS (≥ 8 vs. ≤ 7)	7.8 (2.7-22)	**0.001**	16 (2.6-101)	**0.004**	8.5 (2.8-26)	**< 0.001**
pT stage (≥ pT3 vs. ≤ pT2)	2.2 (0.83-5.7)	0.127	0.99 (0.26-3.8)	0.991		
RM (+ vs. –)	3.7 (1.4-9.8)	**0.008**	5.3 (1.5-19)	**0.008**	4.5 (1.6-12)	**0.005**
pn (+ vs. –)	2.0 (0.64-6.0)	0.212	1.2 (0.30-4.6)	0.814		
LVI (+ vs. –)	2.9 (0.95-9.0)	0.092	1.8 (0.49-6.3)	0.394		
Cancer size (cm) (≥ 1.55 vs. < 1.55)	2.6 (0.91-7.3)	0.062	0.67 (0.15-3.0)	0.603		

GS, Gleason Score; LVI, lymphovascular invasion; pn, perineural invasion; PSA, prostate specific antigen; RM, resection margin.

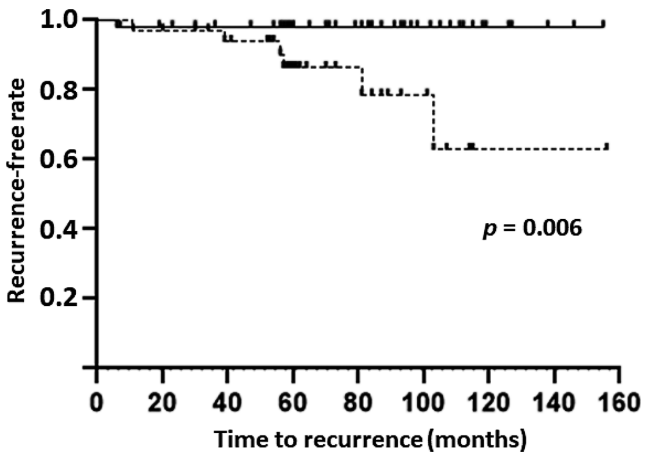

Fig.2. Biochemical recurrence-free survival following radical prostatectomy.
Shown is the biochemical recurrence-free survival following radical prostatectomy among the patients with negative resection margins (34 patients with high DSGb5 expression and 50 patients with low DSGb5 expression). Solid line, low DSGb5 expression in biopsy specimens; broken line, high DSGb5 expression in biopsy specimens.

teristics, operation methods, or median follow-up period, no significant differences were observed for them except for lymphovascular invasion. The pathological characteristics of biopsy specimens (i.e., Gleason Score, % of cancer positive cores, maximum cancer length, D'Amico risk classification) and prostatectomy specimen (i.e., pathological T stage, Gleason Score, resection margin, perineural invasion, cancer size) are almost similar between DSGb5 expression levels. There was a significant difference in lymphovascular invasion between high DSGb5 expression and low DSGb5 expression ($p = 0.028$). Table 2 shows the associations between preoperative characteristics and lymphovascular invasion. There was a significant difference in DSGb5 expression levels on univariate and multivariable analyses ($p = 0.028$, $p = 0.027$).

Table 3 shows the associations between preoperative characteristics or pathological characteristics and biochemical recurrence after radical prostatectomy. Univariate analysis showed that prostatectomy Gleason Score ≥ 8 and positive resection margin were significantly associated with biochemical recurrence ($p = 0.001$, $p = 0.008$). There was no significant relationship between high DSGb5 expression and biochemical recurrence on univariate analysis. However, on multivariable analysis, not only prostatectomy Gleason Score ≥ 8 and positive resection margin, but also high DSGb5 expression was significantly associated with biochemical recurrence ($p = 0.004$, $p = 0.008$, $p = 0.024$). Next, only patients with negative resection margins were investigated for the associations between preoperative characteristics and biochemical recurrence after prostatectomy. Of the several factors described above, the patients with high DSGb5 expression in biopsy specimens showed a significantly lower biochemical recurrence-free survival rate following radical prostatectomy (Fig. 2, $p = 0.006$). On univariate and multivariable analyses, only high DSGb5 expression was significantly associated with biochemical recurrence ($p = 0.006$, $p = 0.007$) (Table 4).

Discussion

In this study, DSGb5 expression in prostate biopsy specimens was significantly associated with lymphovascular invasion in prostatectomy specimens and biochemical recurrence after radical prostatectomy. Of the preoperative clinical characteristics, only DSGb5 expression in biopsy specimens could predict lymphovascular invasion in radical prostatectomy specimens at the time of diagnosis of prostate cancer. As preoperative predictors of adverse pathology in prostatectomy specimens, several materials or parameters have been reported, e.g., gene expression (Klein

Table 4. Associations between preoperative characteristics and biochemical recurrence in only patients with negative resection margins.

	Univariate analysis		Multivariable analysis		Multivariable analysis (Stepwise)	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Age (y) (≥ 65 vs. < 65)	1.6 (0.37-7.4)	0.514	1.5 (0.27-8.1)	0.655		
PSA (ng/mL) (≥ 10 vs. < 10)	1.3 (0.16-11)	0.815	1.5 (0.12-18)	0.753		
cT stage (≥ T2 vs. ≤ T1c)	0.36 (0.043-3.0)	0.292	0.31 (0.028-3.3)	0.282		
Biopsy GS (≥ 8 vs. ≤ 7)	2.3 (0.27-19)	0.488	2.8 (0.27-30)	0.425		
Percentage of cancer positive cores (≥ 25% vs. < 25%)	1.1 (0.24-4.7)	0.944	3.3 (0.43-25)	0.249		
Maximum cancer length (mm) (≥ 4 vs. < 4)	0.28 (0.054-1.5)	0.113	0.15 (0.016-1.4)	0.077	0.29 (0.053-1.6)	0.127
DSGb5 expression in biopsy specimen (high vs. low)	11 (1.3-92)	**0.006**	11 (1.3-95)	**0.007**	11 (1.3-87)	**0.007**

GS, Gleason Score; PSA, prostate specific antigen.

et al. 2014), PSA, the number of cancer-positive cores (Park et al. 2018), and total cancer length of Gleason Pattern 4 on prostate biopsy (Dean et al. 2019). However, to the best of our knowledge, the items that could preoperatively predict lymphovascular invasion in prostatectomy specimens have not yet been established. Therefore, ganglioside DSGb5 expression was thought to be most promising as a biomarker for preoperatively predicting pathological and clinical outcomes of prostate cancer.

On univariate analysis of biochemical recurrence after radical prostatectomy, not DSGb5 expression in biopsy specimens, but prostatectomy Gleason Score ≥ 8 and positive resection margin were significantly associated with biochemical recurrence. However, on multivariable analysis, high DSGb5 expression was also significantly associated with biochemical recurrence. The discrepancy in the results between univariate and multivariable analyses was observed because the rate of patients with a positive resection margin was higher in low DSGb5 expression patients, although there was no significant difference. In the present study, the number of patients with a positive resection margin was 32, and most positive surgical margins were caused by operative procedures; of margin-positive patients, 21 (66%) were pT2 on the prostatectomy specimens. To remove the effect of operation technique that could not be taken into consideration preoperatively, DSGb5 expression was investigated in prostate biopsy specimens targeting only patients with negative surgical resection margins. For these patients, of the many preoperative characteristics, only high DSGb5 expression was significantly associated with biochemical recurrence after radical prostatectomy.

Once again, the associations between preoperative characteristics and biochemical recurrence were investigated in the patients with negative resection margins. The results showed that high DSGb5 expression was significantly associated with biochemical recurrence. Thus, before radical prostatectomy, one can predict biochemical recurrence after the procedure. As predictors of biochemical recurrence after radical prostatectomy, several of the preoperative characteristics have been reported, including Ki-67 labeling index in biopsy specimens (Bubendorf et al. 1998), Core 2 β-1, 6-N-acetyl glucosaminyl transferase-1 (GCNT1) expression (Sato et al. 2016), tumor vascularity in prostate core biopsies (Khatami et al. 2005), and Clusterin (Pins et al. 2004). GCNT1 is a glycosyltransferase that synthesizes core 2 branched O-glycans from core 1 O-glycan (Sato et al. 2016), and Clusterin is a ubiquitous secretory sulfated glycoprotein that plays an antiapoptotic role in prostate cancer cells. Ganglioside DSGb5 is also one of the carbohydrate antigens, and these results indicate that the expression of carbohydrate antigens in prostate cancer indicates the aggressiveness of prostate cancer. For esophageal cancer (Makino et al. 2001) or colon cancer (Yang et al. 2011), it was reported that high levels of CA19-9 and CA125, which are also carbohydrate antigens, were significantly associated with postoperative recurrence. Carbohydrate antigens that are closely associated with carcinogenesis, cancer invasion, and metastasis are clinically useful as tumor markers. In addition, carbohydrate antigens have a role as differentiation antigens. In our report, we found that prostate cancer cells showed a variety of immunoreactivities and normal glandular cells showed high immunoreactivity. As we reported previously (Shimada et al. 2014), DSGb5 may have a role as a differentiation antigen in prostate glands and cancer cells. From now on, detection of ganglioside DSGb5 from minute samples such as blood or urine will be applied as a useful tumor marker for prostate cancer.

There are many reports that lymphovascular invasion is associated with recurrence after radical prostatectomy. Mitsuzuka et al. (2015) reported that lymphovascular invasion was significantly associated with biochemical recurrence after radical prostatectomy, even in patients with pT2N0 and a negative resection margin. However, the association is still controversial (Ng et al. 2012). In the present study, high DSGb5 expression in prostate biopsy specimens preoperatively predicted lymphovascular invasion in prostatectomy specimens and biochemical recur-

rence-free survival following radical prostatectomy independently. However, lymphovascular invasion of prostatectomy specimens was not associated with biochemical recurrence after radical prostatectomy. It is considered that cancer invasion to vessels does not always lead to biochemical recurrence, and multiple steps of metastasis through vessels also lead to biochemical recurrence. We previously reported that DSGb5 enhanced the migration of RCC cells (Kawasaki et al. 2015) and inhibited the cytotoxicity of NK cells through interactions between Siglec-7 and DSGb5 (Kawasaki et al. 2010). These findings suggest that DSGb5 is correlated with migration of cancer cells and inhibition of the immune system. The mechanism of cancer metastasis involves multiple steps. In prostate cancer metastasis, not only lymphovascular invasion, but also another mechanism, i.e. inhibition of the immune system, is necessary for metastasis.

This study has some limitations. First, it was a retrospective study, and the sample size was limited. Second, the tumor found in prostate biopsy specimens may not be the index cancer that determines the prognosis for the patient.

In the present study, high ganglioside DSGb5 expression in prostate biopsy specimens could preoperatively predict lymphovascular invasion in prostatectomy specimens and biochemical recurrence after radical prostatectomy. However, many steps were needed to obtain the results of immunohistochemistry, therefore, it is necessary to develop a new, simpler method to detect DSGb5 for daily medical practice. We are now engaged in developing a new method that detects ganglioside DSGb5 from a patient's blood or urine. We hope that ganglioside DSGb5 will be a novel biomarker reflecting the therapeutic strategy and postoperative recurrence.

Acknowledgments

We thank Yayoi Aoyama for her great efforts in preparing sections. This study was supported in part by Grants-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science and Technology (No. 17K11120 to AI).

Conflict of Interest

The authors declare no conflict of interest.

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