

RESEARCH ARTICLE

ST6Gal-I Predicts Postoperative Clinical Outcome for Patients with Localized Clear-cell Renal Cell Carcinoma

Hai-Ou Liu¹, Qian Wu¹, Wei-Si Liu¹, Yi-Dong Liu¹, Qiang Fu¹, Wei-Juan Zhang², Le Xu³, Jie-Jie Xu^{1*}

Abstract

Hyperactivated α 2-6-sialylation on N-glycans due to overexpression of the Golgi enzyme β -galactoside: α 2-6-sialyltransferase (ST6Gal-I) often correlates with cancer progression, metastasis, and poor prognosis. This study was aimed to determine the association between ST6Gal-I expression and the risk of recurrence and survival of patients with localized clear-cell renal cell carcinoma (ccRCC) following surgery. We retrospectively enrolled 391 patients (265 in training cohort and 126 in validation cohort) with localized ccRCC underwent nephrectomy at a single center. Tissue microarrays were constructed for immunostaining of ST6Gal-I. Prognostic value and clinical outcomes were evaluated. High ST6Gal-I expression was associated with Fuhrman grade ($p < 0.001$ and $p = 0.016$, respectively) and the University of California Los-Angeles Integrated Staging System (UISS) score ($p = 0.004$ and $p = 0.017$, respectively) in both cohorts. Patients with high ST6Gal-I expression had significantly worse overall survival (OS) ($p < 0.001$ and $p < 0.001$, respectively) and recurrence free survival (RFS) ($p < 0.001$ and $p = 0.002$, respectively) than those with low expression in both cohorts. On multivariate analysis, ST6Gal-I expression remained associated with OS and RFS even after adjusting for the UISS score. Stratified analysis suggested that the association is more pronounced among patients with low and intermediate-risk disease defined by the UISS score. High ST6Gal-I expression is a potential independent adverse predictor of survival and recurrence in ccRCC patients, and the prognostic value is most prominent in those with low and intermediate-risk disease defined by the UISS score.

Keywords: Clear-cell renal cell carcinoma - ST6Gal-I - prognostic biomarker - overall survival - recurrence free survival

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Introduction

Renal cell carcinoma (RCC) accounts for approximately 90% of all kidney malignancies, and 85% of RCC are clear cell types (Motzer et al., 2009). The incidence of RCC has increased of about 2% over the past two decades worldwide, contributing to a steadily increasing mortality rate per unit population (Escudier et al., 2012). Despite an increased detection of low-risk ccRCC with cross-sectional imaging following subsequent removal with surgery, about 20-40% ccRCC develop metastasis and remain largely incurable (Escudier and Kataja, 2010). Taken together, these trends underscore the need for biomarkers indicative of metastatic disease to help stratify patients toward targeted therapy, particularly among the growing number of individuals diagnosed with low-risk ccRCC.

Renal cell carcinoma of clear-cell type is characterized by frequent inactivation of the VHL gene and chromatin remodeling enzymes (polybromo-1, SET domain containing 2 and BRCA1-associated protein-1)

(Brugarolas, 2014). These commonly mutated genes, VHL, PBRM1, SETD2 and BRAP1, could have far-reaching effects on metabolic shift, involving downregulation of genes involved in the TCA cycle, upregulation of the pentose phosphate pathway and the glutamine transporter genes (Network, 2013). By altering their metabolism to favour aerobic glycolysis over lipid oxidation (a cancer phenomenon termed the "Warburg effect") give rise to ccRCC progression (Preston et al., 2011; Li et al., 2014). A sialic acid-rich carbohydrate known as Lewis X juts out from cancer cells links the Warburg effect with the metastasize beyond their point of origin (Kannagi, 2004). Aberrant cell surface glycosylation is often due to altered expression or activity of glycosyltransferases and glycosidases. The ST6Gal-I sialyltransferase is an example of a glycosyltransferase commonly upregulated in cancer (Dall'Olio, 2000). This Golgi enzyme adds the negatively charged sugar, sialic acid, in an α 2-6 linkage to the termini of N-glycans (Harduin-Lepers et al., 2001). Accumulating evidences indicate that higher intratumor expression levels of ST6Gal-I are indicator of

¹Key Laboratory of Glycoconjugate Research, MOH, Department of Biochemistry and Molecular Biology, ²Department of Immunology, School of Basic Medical Sciences, ³Department of Urology, Zhongshan Hospital, Shanghai Medical College of Fudan University, Shanghai, China *For correspondence: jjxufdu@fudan.edu.cn

poor prognosis in a variety of human cancers (Bull et al., 2014). Despite this well-known association with cancer aggressiveness, the potential role of ST6Gal-I in the pathogenesis and prognosis of ccRCC remains unknown.

Motivated by this gap in understanding, we used two independent cohorts to analyze and validate the hypothesis that higher tumor protein expression levels of the ST6Gal-I are associated with shorten overall survival and increased risk of recurrence following surgery for ccRCC. Moreover, we explore the specific hypothesis that this association is more pronounced among patients with early stage ccRCC.

Materials and Methods

Patients

A total of 437 patients who underwent radical or partial nephrectomy for ccRCC at Zhongshan Hospital, Fudan University (Shanghai, China) were recruited and divided into two internal cohorts in this study. The study was approved by the research medical ethics committee of Fudan University and informed consent was provided by each patient. The first 296 consecutive ccRCC were recruited between 2003 and 2004 and used as the training cohort. In this study, we updated the clinical status of these patients for an additional five years of follow-up. Thirty one cases were excluded due to loss in follow-up, and the remaining 265 patients were included in the analyses. The second internal cohort included the next 141 consecutive surgical patients with ccRCC during the year of 2008. After excluding tumors with insufficient follow-up (n=15), the remaining 126 ccRCC formed the second internal cohort for the purpose of this validation study. For each patient, clinicopathologic information, including age, gender, ECOG-PS, tumor size, TNM stage, Fuhrman grade and tumor necrosis, was collected from patients' records. Patients were staged using radiographic reports and post reassigned according to 2010 AJCC TNM classification. The University of California Los Angeles Integrated Staging System (UISS) score was used to stratify all patients into three risk groups (low-, intermediate-, high-risk) (Zisman et al., 2002).

Patients with localized RCC were treated with radical or partial nephrectomy. After surgery, patients were evaluated with physical examination, laboratory studies, chest imaging, and abdominal ultrasound or CT scans every six months for the first two years and annually thereafter. Survival status was updated in March 2014. Median follow-up period was 100 months (range, 12-120 months) and 66 months (range, 12-74 months), respectively, in the training and validation cohorts. Overall survival was calculated from the date of surgery to the date of death or to the date of the last follow-up. Recurrence-free survival (RFS) was calculated from the date of curative surgery to the date of recurrence. Patients with tumor metastasis (who has N1 or M1 tumors) at the time of surgery were excluded from the analysis.

Immunohistochemistry

Tissue microarrays were constructed as previously described (Zhu et al., 2008). Primary anti-ST6Gal-I antibody (diluted 1:100; Novus Biologicals, Littleton,

CO) was applied for immunohistochemistry staining. The specificity of this antibody was confirmed by immunohistochemistry and western blotting with peptide competition. A certified pathologist (Z.H.) who was blinded to the clinical data, assess ST6Gal-I immunostaining. A semiquantitative H-score for each sample was calculated multiplying the staining intensities (0: negative, 1: weak staining, 2: moderate staining, 3: strong staining) by the distributions (0: areas that were negative, 1: <25%, 2: 26-50%, 3: 51-75% 4:76-100%), and ranged from 0 to 12 (Wang et al., 2013). To estimate a cut point for dichotomizing ST6Gal-I expression into high versus low expression, we used the training cohort and chose the cut point that maximized the concordance index (Lotan et al., 2013). As a result, tumors with ST6Gal-I expression ≤ 2 IHC score categorized as "low"; those > 2 IHC score were categorized as "high" (Supplementary Figure 1). In the validation cohort, we dichotomized ST6Gal-I expression using the same cut point as for the training cohort.

Statistical analysis

Statistical analysis was performed by MedCalc Software (version 11.4.2.0; MedCalc, Mariakerke, Belgium) and Stata 12.0 (StataCorp, College Station, TX). Categorical data was analyzed by Fisher exact or χ^2 -test. Survival curves were calculated by Kaplan-Meier method and compared by log-rank test. Univariate and multivariate Cox proportional-hazard models were used to evaluate the hazard ratios of prognostic factors. We analyzed concordance index values to compare the predictive ability of various models with and without the addition of the ST6Gal-I expression variable. All concordance indices were internally validated using a bootstrap methodology proposed by Harrell et al. (Harrell et al., 1996) and therefore represent optimism-corrected estimates of prognostic accuracy. All *p*-values were two-tailed, and differences were considered significant at values of *p*<0.05.

Results

Patient characteristics and associations with ST6Gal-I expression

Clinicopathologic features for the 391 patients under study in the training and validation cohorts are summarized in Table 1. At last follow-up, 103 of 391 patients studied had died.

The mean duration of follow-up was 87.3 months (median, 100 months, range, 12 to 120 months) and 60.2 months (median, 66 months, range, 12 to 74 months), respectively, in the two cohorts. Overall survival rates (the number of patients still at risk) at 3 years and 5 years after nephrectomy were 97.3% (251 patients) and 87.0% (210 patients), respectively, and 87.2% (109 patients) and 77.6% (97 patients), respectively, in the two cohorts. Recurrence-free survival rates at 3 years and 5 years after nephrectomy were 91.2% (231 patients) and 79.4% (185 patients), respectively, and 79.3% (99 patients) and 72.9% (91 patients), respectively, in the two cohorts. ST6Gal-I positive staining was mainly located in the cytoplasm of

tumor cells (Supplementary Figure 2). The median IHC score for ST6Gal-I expression was 2 (range 0-11) in the two cohorts. In our dichotomization of ST6Gal-I, 128 patients (48.3%) and 58 patients (46.0%) had tumors classified as high, respectively, in the two cohorts. In Table 1, we provide a comparison of standard clinicopathologic features by dichotomized ST6Gal-I status (low vs high). Patients with high ST6Gal-I expression were prone to have high-grade tumors ($p<0.001$ and $p=0.016$, respectively) in the two cohorts. A positive correlation between ST6Gal-I expression and the UISS score was also found ($p=0.004$ and $p=0.017$, respectively) in the two cohorts.

High expression of ST6Gal-I is associated with adverse prognosis

Kaplan-Meier analyses indicated that high expression of ST6Gal-I is associated with reduced overall survival ($p<0.001$ and $p<0.001$, respectively) and increased risk of recurrence ($p<0.001$ and $p=0.002$, respectively) in both cohorts (Figure 1A-D). We next evaluated the independent prognostic value of ST6Gal-I levels using multivariate Cox regression analysis, with adjustment for other known pathologic predictors of patient outcome. The multivariate analyses confirmed that ST6Gal-I signature was independently predictive OS and RFS after adjusting for TNM stage, Fuhrman grade, tumor necrosis, ECOG performance status, and after adjusting for the UISS score ($p=0.003$ and $p=0.014$, respectively for OS; $p<0.001$

and $p=0.039$, respectively for RFS; Table 2) in the both cohorts. To quantify the prognostic ability of ST6Gal-I, we provide estimates of the optimism-corrected concordance

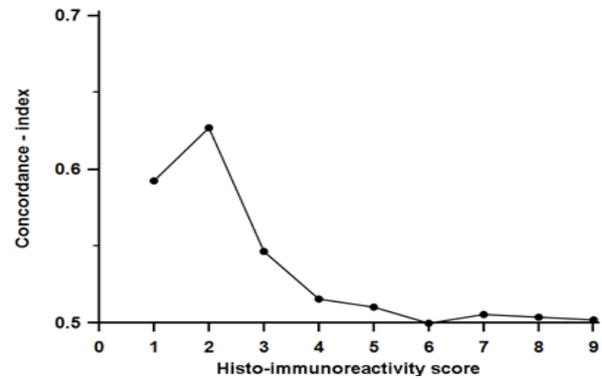


Figure 1. The Optimal Cutoff Value of ST6Gal-I was Obtained by Concordance-index Analysis. The concordance index (c-index) at different cutoff values of overall survival time was calculated to determine the optimal cutoff value of ST6Gal-I histo-immunoreactivity score (H-score) in tumors of training cohort. The optimal value of cutoff point was two due to its best predictive value (c-index, 0.627). Under these conditions, specimens with H-score 1-2 and 3-9 were classified as low and high. ST6Gal-I β -galactosidase a 2, 6 sialyltransferase-1, IRS immunoreactivity score, c-index concordance index

Table 1. Patient Characteristics and Associations with ST6Gal-1 Expression

Variable	Training cohort (n = 265)				p	Validation cohort (n = 126)				p
	Patients		ST6Gal-1 expression			Patients		ST6Gal-1 expression		
	No.	%	Low (n=137)	High (n=128)		No.	%	Low (n=68)	High (n=58)	
Age, years										
Mean \pm SD	56.9 \pm 12.4		57.0 \pm 12.1	56.7 \pm 12.7	0.873	54.0 \pm 10.6		54.8 \pm 9.9	54.0 \pm 11.4	0.691
Gender					0.818					0.079
Male	185	69.8	97	88		95	75.4	56	39	
Female	80	30.2	40	40		31	24.6	12	19	
Tumor size, cm					0.262					0.416
Mean \pm SD	4.6 \pm 2.6		4.5 \pm 2.4	4.8 \pm 2.8		4.6 \pm 2.6		4.5 \pm 2.5	4.8 \pm 2.8	
TNM stage					0.171					0.331
I	169	63.8	93	76		93	73.8	50	43	
II	33	12.4	18	15		15	11.9	10	5	
III	61	23	26	35		16	12.7	8	8	
IV	2	0.8	0	2		2	1.6	0	2	
Fuhrman grade					<0.001					0.016
1	47	17.7	32	15		32	25.4	22	10	
2	121	45.7	71	50		60	47.6	35	25	
3	68	25.7	25	43		19	15.1	5	14	
4	29	10.9	9	20		15	11.9	6	9	
Tumor necrosis					0.182					0.361
Absent	207	76.5	112	95		97	77	55	42	
Present	58	23.5	25	33		29	23	13	16	
ECOG-PS					0.918					0.379
0	224	84.5	115	109		111	88.1	62	49	
≥ 1	41	15.5	22	19		15	11.9	6	9	
UISS					0.004					0.017
LR	101	38.1	65	36		73	57.9	46	27	
IR	150	56.6	67	83		46	36.5	21	25	
HR	14	5.3	5	9		7	5.6	1	6	

*Bold values are statistically significant ($p<0.05$); ST6Gal-1 β -galactosidase alpha-2-6 sialyl transferase-1, ECOG-PS Eastern Cooperative Oncology Group performance status, UISS the University of California Los-Angeles Integrated Staging System

Table 2. Multivariable Associations of ST6Gal-1 Expression with Overall Survival and Recurrence-free Survival in the Training and Validation Cohorts

ST6Gal-1 (high vs low)	Training Cohort		Validation Cohort		
	HR (95%CI)	p value	HR (95%CI)	p value	
OS	Adjusted for age	2.629 (1.581-4.371)	<0.001	4.841 (2.167-10.812)	<0.001
	Adjusted for age+gender	2.637 (1.586-4.387)	<0.001	5.108 (2.287-11.413)	<0.001
	Adjusted for age+tumor size	2.600 (1.562-4.329)	<0.001	4.945 (2.217-11.027)	<0.001
	Adjusted for age+TNM stage	2.439 (1.463-4.067)	<0.001	4.396 (1.956-9.882)	<0.001
	Adjusted for age+Fuhrman grade	2.114 (1.252-3.569)	0.005	3.402 (1.500-7.717)	0.003
	Adjusted for age+tumor necrosis	2.549 (1.529-4.249)	<0.001	4.823 (2.137-10.887)	<0.001
	Adjusted for age+ECOG	2.604 (1.566-4.331)	<0.001	4.642 (2.080-10.360)	<0.001
	Adjusted for age+UISS score	2.175 (1.302-3.635)	0.003	2.875 (1.246-6.633)	0.014
RFS	Adjusted for age	4.525 (2.688-7.615)	<0.001	3.471 (1.685-7.151)	<0.001
	Adjusted for age+gender	4.512 (2.680-7.597)	<0.001	3.471 (1.685-7.151)	<0.001
	Adjusted for age+tumor size	4.503 (2.673-7.588)	<0.001	3.592 (1.740-7.414)	<0.001
	Adjusted for age+TNM stage	4.198 (2.489-7.080)	<0.001	3.198 (1.544-6.625)	0.002
	Adjusted for age+Fuhrman grade	3.598 (2.111-6.132)	<0.001	2.527 (1.207-5.294)	0.015
	Adjusted for age+tumor necrosis	4.387 (2.603-7.394)	<0.001	3.533 (1.697-7.355)	<0.001
	Adjusted for age+ECOG	4.543 (2.697-7.653)	<0.001	3.284 (1.597-6.753)	0.001
	Adjusted for age+UISS score	3.662 (2.166-6.193)	<0.001	2.208 (1.047-4.657)	0.039

*Bold values are statistically significant (p<0.05); ST6Gal-1 β-galactoside α-2-6 sialyl transferase 1, ECOG-PS Eastern Cooperative Oncology Group performance status, UISS the University of California Los-Angeles Integrated Staging System

Table 3. Optimism-corrected Concordance Indices in the Training and Validation Cohorts

	Training Cohort		Validation Cohort		
	Without ST6Gal-1	Including ST6Gal-1	Without ST6Gal-1	Including ST6Gal-1	
OS	Age	0.606 (<0.001)	0.664 (<0.001)	0.572 (<0.001)	0.711 (<0.001)
	Age+gender	0.606 (<0.001)	0.664 (<0.001)	0.576 (<0.001)	0.727 (<0.001)
	Age+tumor size	0.647 (<0.001)	0.699 (<0.001)	0.691 (<0.001)	0.765 (<0.001)
	Age+TNM stage	0.697 (<0.001)	0.727 (<0.001)	0.683 (<0.001)	0.787 (<0.001)
	Age+Fuhrman grade	0.688 (<0.001)	0.719 (<0.001)	0.741 (<0.001)	0.784 (<0.001)
	Age+tumor necrosis	0.611 (<0.001)	0.664 (<0.001)	0.634 (<0.001)	0.743 (<0.001)
	Age+ECOG	0.613 (<0.001)	0.684 (<0.001)	0.627 (<0.001)	0.747 (<0.001)
	Age+UISS score	0.700 (<0.001)	0.721 (<0.001)	0.803 (<0.001)	0.844 (<0.001)
RFS	Age	0.522 (<0.001)	0.679 (<0.001)	0.615 (<0.001)	0.689 (<0.001)
	Age+gender	0.546 (<0.001)	0.695 (<0.001)	0.616 (<0.001)	0.692 (<0.001)
	Age+tumor size	0.614 (<0.001)	0.727 (<0.001)	0.692 (<0.001)	0.734 (<0.001)
	Age+TNM stage	0.665 (<0.001)	0.750 (<0.001)	0.695 (<0.001)	0.760 (<0.001)
	Age+Fuhrman grade	0.700 (<0.001)	0.757 (<0.001)	0.760 (<0.001)	0.772 (<0.001)
	Age+tumor necrosis	0.563 (<0.001)	0.695 (<0.001)	0.649 (<0.001)	0.697 (<0.001)
	Age+ECOG	0.574 (<0.001)	0.712 (<0.001)	0.633 (<0.001)	0.691 (<0.001)
	Age+UISS score	0.671 (<0.001)	0.748 (<0.001)	0.795 (<0.001)	0.814 (<0.001)

*The optimism, calculated from 150 bootstrap samples, is in parentheses and is an estimate of overfitting; ST6Gal-1 β-galactoside alpha-2-6 sialyl transferase 1, ECOG-PS Eastern Cooperative Oncology Group performance status, UISS the University of California Los-Angeles Integrated Staging System

Table 4. Age-adjusted Association (Hazard Ratio and 95% Confidence Interval) of High ST6Gal-1 Expression with OS and RFS Stratified by UISS Score (Training Set Only)

		UISS-LR		UISS-IR		UISS-HR	
		HR (95%CI)	p value	HR (95%CI)	p value	HR (95%CI)	p value
OS	ST6Gal-1 low	1.0 (reference)	-	1.0 (reference)	-	1.0 (reference)	-
	ST6Gal-1 high	5.084 (1.356-19.058)	0.016	1.934 (1.04-3.599)	0.038	1.25 (0.362-4.322)	0.725
RFS	ST6Gal-1 low	1.0 (reference)	-	1.0 (reference)	-	1.0 (reference)	-
	ST6Gal-1 high	19.859 (2.564-153.852)	0.004	3.396 (1.839-6.271)	<0.001	0.993 (0.288-3.421)	0.992

*Bold values are statistically significant (p<0.05); HR hazard ratio, CI confidence interval, ST6Gal-1 β-galactoside alpha-2-6 sialyl transferase-1, UISS the University of California Los-Angeles Integrated Staging System

indices for models without and with adjustment for ST6Gal-I in Table 3. For OS, the C-indices of UISS were 0.7 and 0.803, respectively, and improved to 0.721 and 0.844, when incorporated with ST6Gal-1 in both cohorts. For RFS, the C-indices of UISS were 0.671 and 0.795, respectively, and improved to 0.748 and 0.814 when incorporated with ST6Gal-I in both cohorts.

High ST6Gal-I expression is a predictor of adverse prognosis in patients with low-risk disease defined by the UISS score

To illustrate the potential prognostic value of ST6Gal-I, we evaluated the ability of dichotomized ST6Gal-I to further stratify patients following initial classification by UISS score. The OS of patients with different UISS

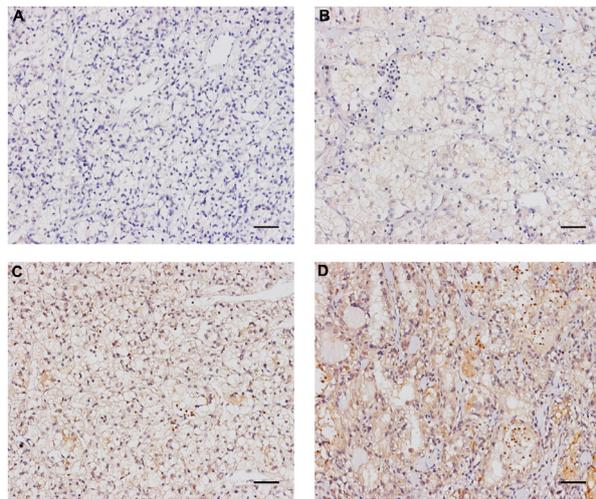


Figure 2. H-score Examples. (A) Case A, H-score = $(0*4) + (1*0) + (2*0) + (3*0) = 0$; (B) Case B, H-score = $(0*2) + (1*2) + (2*0) + (3*0) = 2$; (C) Case C, H-score = $(0*1) + (1*2) + (2*1) + (3*0) = 4$; (D) Case D, H-score = $(0*0) + (1*1) + (2*2) + (3*1) = 8$

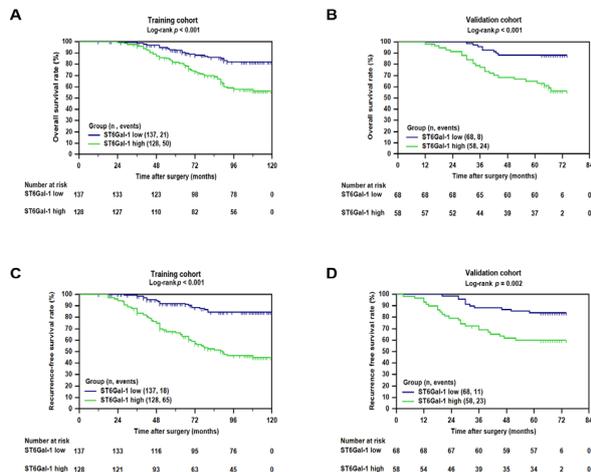


Figure 3. Estimated Overall Survival and Recurrence Free Survival Following Surgery by Dichotomized ST6Gal-I Expression for Patients with Clear Cell Renal Cell Carcinoma. (A), OS for training cohort; (B), RFS for training cohort; (C), OS for validation cohort; (D), RFS for validation cohort

score were substratified by the dichotomized ST6Gal-I expression (Figure 2A-C). We noted that this was primarily evident among low-risk patients (log-rank $p=0.007$, Figure 2A) and, to a slightly lesser extent, intermediate-risk patients (log-rank $p=0.021$, Figure 2B). In contrast, ST6Gal-I expression had a limited ability to stratify high-risk patients on UISS score (log-rank $p=0.621$, Figure 2C). Again, the RFS of patients with different UISS score were substratified by the dichotomized ST6Gal-I expression (Figure 2D-F). We noted evidence that the potential prognostic value of ST6Gal-I was evident among low-risk and intermediate-risk patients classified by UISS score (log-rank $p<0.001$ and $p<0.001$, respectively, Figure 2D and E). In contrast, ST6Gal-I expression had a limited ability to stratify patients with high UISS score (log-rank $P=0.677$, Figure 2F). In table 4, we provide the age-adjusted

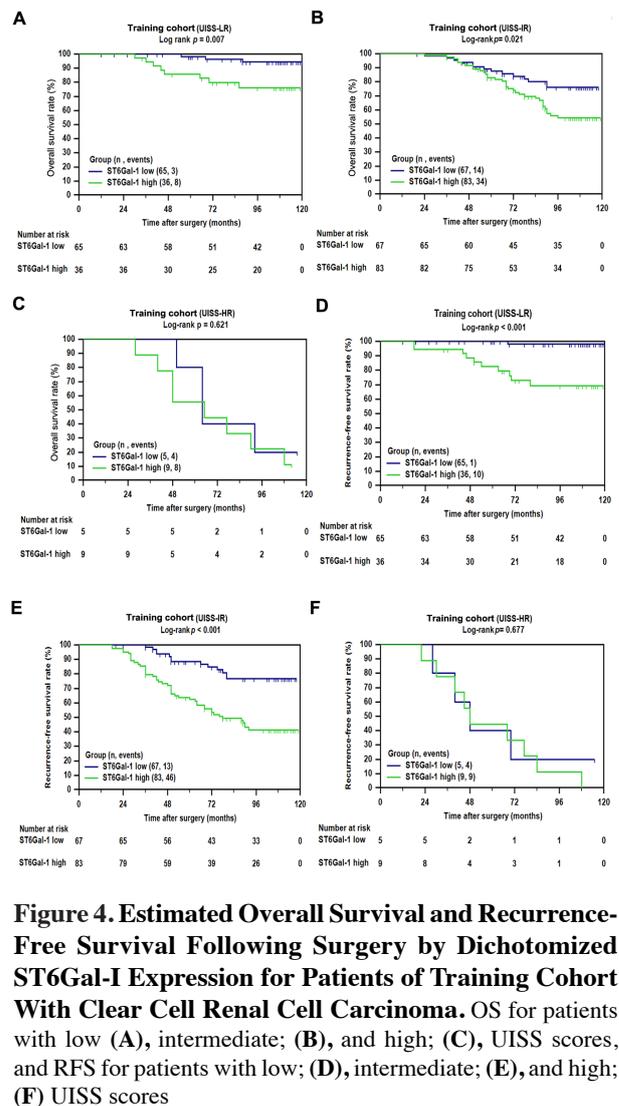


Figure 4. Estimated Overall Survival and Recurrence-Free Survival Following Surgery by Dichotomized ST6Gal-I Expression for Patients of Training Cohort With Clear Cell Renal Cell Carcinoma. OS for patients with low (A), intermediate; (B), and high; (C), UISS scores, and RFS for patients with low; (D), intermediate; (E), and high; (F) UISS scores

HR and 95% CIs that correspond with our stratified Kaplan-Meier curves. Given that in our validation cohort only seven patients who were classified as high risk using the UISS score experienced death and recurrence, we did not attempt stratified analyses in this cohort.

Discussion

The functional contribution of ST6Gal-I to a malignant phenotype has yet to be elucidated; however, escape from apoptosis, metastasis formation, and resistance to therapy may play a prominent role (Liu et al., 2011; Swindall and Bellis, 2011; Swindall et al., 2013; Bull et al., 2014). Elucidation of the exact mechanisms by which these occur has not been as straightforward. Currently, it is believed that ST6Gal-I functions as an inhibitor of cellular apoptosis primarily through $\alpha 2, 6$ -sialylation of FasR by preventing the initiation of death-inducing signaling complex (DISC) and hindering the binding of the Fas-associated adaptor molecule FADD to the FasR death domain (Swindall and Bellis, 2011). Next to Fas-mediated apoptosis, ST6Gal-I has been reported to mediate resistance to anoikis through $\alpha 2, 6$ -sialylation of $\alpha 5\beta 1$ -integrin by preventing its binding to galectin-1 and the subsequent induction of anoikis (Zhuo and

Bellis, 2011). In addition to its antiapoptotic activity, it has been shown that ST6Gal-I plays a critical role in less differentiation and therapy resistance in cancer (Hedlund et al., 2008; Swindall et al., 2013).

Data from other investigators on sialylation and RCC aggressiveness are limited. Borzym-Kluczyk and Radziejewska (Borzym-Kluczyk and Radziejewska, 2013) investigated the expression of $\alpha 2$, 6-linked sialic acids on renal cancer tissues using *Sambucus nigra* agglutinin (SNA) lectins histochemistry and found that secreted $\alpha 2$, 6-linked sialic acids were significantly increased by cancerous cells when compared to normal and intermediate renal tissue. However, conclusions from that investigation were limited by the SNA lectin histochemistry, giving that the expression of $\alpha 2$, 6-sialylated oligosaccharides is controlled at multiple levels. To the best of our knowledge, the current investigation is the first study to demonstrate an independent association between higher ST6Gal-I protein expression and an increased risk of death and recurrence following surgery for clinical localized ccRCC. Although the mechanism underlying ST6Gal-I overexpression in ccRCC remains to be established, it has been demonstrated that ST6Gal-I is upregulated in response to the activation of RAS, and increased substrate availability for sialic acid biosynthesis (Seales et al., 2003; Almaraz et al., 2014).

Better definition of signal message pathways playing a role in the development of RCC has caused an increase in preclinical and clinical studies into directed treatment of RCC (Tanriverdi, 2013). An evolving appreciation of the therapeutic limits of the current agents (including antiangiogenic, molecularly targeted, and immunotherapeutic) has heightened the need for continued exploration of RCC biology and investigation of novel approaches to RCC management (Philips and Atkins, 2014). Sialic acid-linked glycans are aberrantly expressed in many epithelial cancers, which could affect differentiation, adhesiveness and invasion of tumor cells. Our immunohistochemical examination in RCC displayed a subcellular cytoplasmic pattern for ST6Gal-I, apparently localized to the Golgi apparatus. This implies its potentially crucial role in acquired renal carcinogenesis through aberrant sialic acid-linked glycosylation. ST6Gal-I could be a specific diagnostic tumor marker for ccRCC. As ST6Gal-I appear in external secretions and could be secreted into body fluids as a quantitative soluble marker (Lee et al., 2012). Moreover, current data suggest that ST6Gal-I is an ideal therapeutic target. P-3Fax-Neu5Ac is a fluorinated sialic acid analogue that globally inhibits sialyltransferases and strongly hinders tumor cell adhesion to ECM and migration *in vitro* and tumor engraftment in a mouse model *in vivo* (Bull et al., 2013). Obviously, however, the utility of P-3Fax-Neu5Ac as therapeutic modalities requires much further study.

There are limitations to the current investigation that should be mentioned. Our choice of a designation for high ST6Gal-I expression was based on an analysis of concordance index. Although this approach is reasonable for exploratory investigations, prospective analyses are warranted now for validation of our results. Other limitations include small population size of both cohorts and recruitment from the same institution. The large

sample size is an improvement of ccRCC prognosis that is limited by low statistical power. Additional strengths of the current study include the adjustment for known predictors of ccRCC outcome.

In conclusion, our findings show that ST6Gal-I expression in ccRCC is significantly related to histopathological phenotypes with poor prognoses, for example, those with high Fuhrman's grade or high UISS score. Moreover, outcomes of patients whose RCCs had high ST6Gal-I were markedly worse compared with those with low ST6Gal-I ccRCCs. These analyses indicate, for the first time, that ST6Gal-I is a novel and useful independent predictor of poor prognosis in patients with low and intermediate-risk ccRCCs determined by UISS score.

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