Predictive Value of *N*-Acetylglucosaminyltransferase-V for Superficial Bladder Cancer Recurrence

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Purpose: GnT-V is an enzyme that catalyzes β1–6 branching of *N*-acetylglucosamine on asparagine (*N*)-linked oligosaccharides of cell proteins. GnT-V expression has been closely related to malignant potentials in colon cancer, brain cancer and hepatocellular carcinoma. We determined whether GnT-V expression is predictive of superficial bladder cancer recurrence.
Materials and Methods: The cohort comprised 60 consecutive patients with first time superficial bladder cancer treated with transurethral resection. None of the patients received prophylactic intravesical therapy until recurrence. Paraffin embedded tumor specimens were immunohistochemically examined by the avidin-biotin peroxidase method using monoclonal antibody against GnT-V. Kaplan-Meier survival curves were generated to determine disease-free survival. Univariate and multivariate analyses were done to compare GnT-V expression to other clinical and pathological variables.
Results: GnT-V expression correlated inversely with tumor grade and stage. The positive incidence of GnT-V in G1 to G3 tumors was 7 of 9 (78%), 21 of 43 (49%) and 3 of 8 (38%), respectively. GnT-V was positive in 26 of 44 cases of pTa (60%) and in 5 of 16 of pT1 (31%) disease. The 31 patients with positive GnT-V expression had significantly higher disease-free survival than the 29 with negative GnT-V expression (log rank test $p = 0.0034$). Multivariate analysis revealed that patient age, pT, grade and negative GnT-V expression were independent predictors of recurrence ($p = 0.015$, 0.001, 0.019 and 0.011, respectively).
Conclusions: Immunohistochemical detection of GnT-V is an independent predictor of superficial bladder cancer recurrence.

Key Words: bladder, bladder neoplasms, tumor marker, biological, recurrence, N-acetylglucosaminyltransferases

Patients with superficial bladder cancer are at risk for recurrence and progression. Studies indicate that the recurrence rate is 50% to 80% and the risk of progression is 10% to 25%.[1] Determining which bladder tumors will recur and progress is a problem of major clinical interest and it could be useful for designing clinical followup and treatment strategies.

A number of recent investigations have been performed to determine whether new biological markers can help predict disease recurrence. Among the biological markers of aggressiveness the p53 tumor suppressor gene has been extensively studied.[2] However, the relationship between p53 and superficial bladder cancer recurrence is contradictory.[3,4] Despite extensive studies focused on $p21^{WAF1}$,[5] Ki-67 antigen[6] and vascular endothelial growth factor[7] their clinical evidence as a recurrence predictor is still equivocal.

Aberrant glycosylation occurs in essentially all types of experimental and human cancers.[8] However, glycosylation status in bladder cancer has not been studied extensively except for the roles of ganglioside GM3 and sialyl Lewis X.[9,10]

GnT-V, a key enzyme in the formation of β1-6 branching of asparagine (*N*)-linked oligosaccharides, is the one most strongly linked to tumor metastasis in many glycosyltransferases.[11] Figure 1 shows the structure of β1–6 branching of asparagine (*N*)-linked oligosaccharides and synthetic pathway catalyzed by GnT-V. In colorectal cancer and brain tumors GnT-V expression significantly correlates with distant metastasis.[12,13] In contrast, hepatocellular carcinoma cases with low or no GnT-V expression are more likely to show recurrence than cases with high expression.[14] To our knowledge there have been no reports of the clinical or pathological significance of GnT-V in human bladder cancer. We report that, as defined by its antibody, GnT-V expression is an independent recurrence predictor of superficial bladder cancer, as defined by its antibody.

MATERIALS AND METHODS

Patients. A total of 60 consecutive patients with first time superficial bladder cancer treated with TUR at the department of urology, Sendai Medical Center, Sendai, Japan between 1996 and 2002 comprised the study cohort. Paraffin specimens obtained by biopsy or TUR were used for immunohistochemistry. After TUR patients were followed by routine cystoscopy and urinary cytology at 3-month interval

Submitted for publication March 3, 2005.
Supported by Grant-in-Aid for Scientific Research B-16390459 from the Japan Society for the Promotion of Science and CREST, Japan Science and Technology Agency.
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DOI:10.1016/S0022-5347(05)00013-3

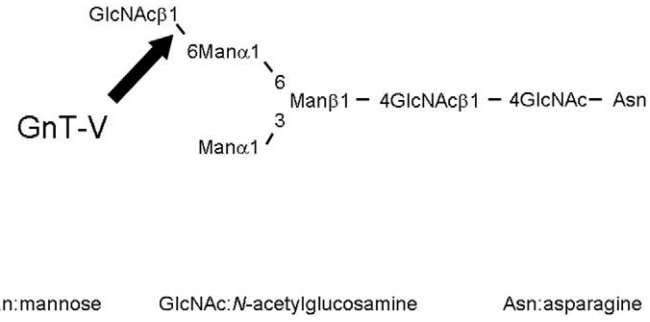

FIG. 1. Structure of β1–6 branching of asparagine (*Asn*) (*N*)-linked oligosaccharides and reaction catalyzed by GnT-V. *N*-acetylglucosamine (*GlcNAc*) is attached to mannose (*Man*) residue of asparagine (*N*)-linked oligosaccharides by GnT-V. Resultant β1–6 branching structure is associated with tumor malignancy. Arrow indicates reaction pathway catalyzed by GnT-V.

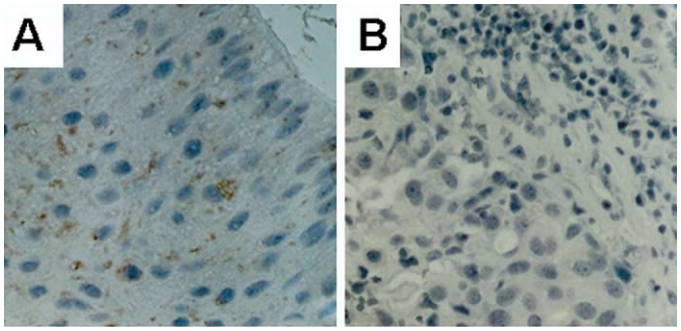

FIG. 2. Immunohistochemistry of bladder cancer using anti-GnT-V monoclonal antibody. GnT-V was positive in low grade bladder cancer (*A*) but negative in high grade invasive cancer (*B*). Since intracellular localization of GnT-V was done Golgi apparatus, GnT-V was clearly detected in Golgi pattern around nuclei. Reduced from ×200.

until recurrence. None of the patients received prophylactic intravesical therapy until recurrence. Table 1 lists patient clinical and pathological characteristics. Mean followup was 25.5 months (range 3 to 78). Written consent was obtained from all patients. The tumor staging system was based on the American Joint Commission on Cancer staging system.[15]

Immunohistochemistry. Paraffin embedded tumor specimens were immunohistochemically examined by the avidin-biotin peroxidase method using monoclonal antibody against GnT-V.[12] Paraffin embedded samples were cut at 3 μm and subjected to hematoxylin and eosin staining, and immunohistochemistry. Immunohistochemical staining was performed as previously described using monoclonal antibody against human GnT-V.[12–14] Briefly, deparaffinized specimens were reacted with anti-GnT-V monoclonal antibody as the primary antibody. Anti-mouse Ig antibody conjugated with horseradish peroxidase was used as the secondary antibody and peroxidase activity was visualized with aminoethylcarbazol solution (Nichirei, Tokyo, Japan). A control experiment was done by omitting the primary antibody from the staining procedure and no specific staining was found. Immunostaining results were evaluated while blinded to all clinical data. Based on the staining status of Golgi apparatus specimens with 10% or more positive cancer cells were considered GnT-V positive, as described previously.[12–14]

Statistical analysis. The chi-square test was used to assess the association of the GnT-V status with clinical and pathological parameters. Recurrence-free survival was evaluated by Kaplan-Meier curves. Differences between the groups were evaluated by the log rank test. We used Cox proportional hazards regression analysis to test the association of GnT-V expression with other clinical and pathological variables, including patient sex, age, pathological stage, tumor diameter, single or multiple lesions and tumor grade for the prediction of recurrence. We used the statistical program SPSS 12.0 (SPSS, Chicago, Illinois).

TABLE 1. *Patient clinical and pathological characteristics*

Mean age ± SD	67.7 ± 10.7
No. men (%)/women	47 (78)/13 (22)
No. tumor grade (%):	
G1	9 (15)
G2	43 (72)
G3	8 (13)
No. pathological stage (%):	
pTa	44 (73)
pT1	16 (27)

RESULTS

Figure 2 shows representative microphotographs of immunohistochemistry with anti-GnT-V monoclonal antibody. Since the intracellular localization of GnT-V is the Golgi apparatus, GnT-V was clearly detected in the Golgi pattern by immunohistochemistry. Table 2 lists GnT-V status and pathological parameters. GnT-V expression inversely correlated with tumor grade and stage. The incidence of positive GnT-V expression in bladder cancer was significantly higher in low grade/less invasive cancer than in higher grade/invasive cancer. The positive incidence of GnT-V in G1 to G3 tumors was 7 of 9 (78%), 21 of 43 (49%) and 3 of 8 (38%), respectively. GnT-V was positive in 26 of 44 cases of pTa (60%) and 5 of 16 of pT1 (31%) disease.

The 31 patients with positive GnT-V expression had significantly higher disease-free survival than the 29 with negative GnT-V expression (log rank test p = 0.0034, fig. 3). Univariate analysis showed that male sex, tumor size less than 1.5 cm, grade, pT and negative GnT-V expression were significant predictors of recurrence (p = 0.009, 0.012, 0.016 and 0.005, respectively, table 3). Multivariate analysis revealed that patient age, pT, grade and negative GnT-V expression were independent predictors of recurrence (p = 0.015, 0.001, 0.019 and 0.011, respectively, table 3).

DISCUSSION

After TUR mandatory cystoscopy and urine cytology are required for the routine followup of superficial bladder can-

TABLE 2. *GnT-V status and pathological parameters*

	No. GnT-V Pos/Total No. (%)
Tumor grade:	
G1	7/9 (78)
G2	21/43 (49)
G3	3/8 (38)
p Value	0.021
Pathological stage:	
pTa	26/44 (60)
pT1	5/16 (31)
p Value	0.019