

ORIGINAL**Serum level of galectin-3 in human bladder cancer**

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Abstract : We examine serum level of galectin-3 in patients with bladder cancer. We used serum samples of 67 patients with urological diseases and classified these patients into bladder cancer group (n=43) and control group (n=24). Galectin-3 concentration was measured by ELISA (Human Galectin-3 Assay Kit, IBL). And we selected the patient with high serum galectin-3 concentration (Urothelial Carcinoma, G3, pT3a pN0M0), we performed immunohistochemical staining with the VECTASTAIN ABC (Avidin Biotinylated enzyme Complex) system. Median value of serum galectin-3 concentration was 1068 pg/ml (range 551-2028) in the cancer group vs 584 pg/ml (range 259-1262) in controls. Serum galectin-3 concentration of the bladder cancer patients was statistically higher than that of controls ($p < 0.0005$). There was no apparent correlation in serum galectin-3 concentration with the clinico-pathological features such as stage and grade. Higher expression of galectin-3 was observed in bladder cancer tissue than in normal bladder tissue. We suggest the measurement of serum galectin-3 is useful for diagnosis of bladder cancer. *J. Med. Invest.* 55 : 127-132, February, 2008

Keywords : *galectin-3, serum, bladder cancer*

INTRODUCTION

Galectins are a family of carbohydrate-binding proteins characterized by a conserved amino acid sequence defined by structural similarities in their carbohydrate-binding domain and affinity for β -galactoside-containing glycoconjugates. It was reported that galectins play a significant role in apoptosis, several of which, such as galectin-1 and galectin-9, were implicated in the promotion of apoptosis of immune and melanoma cells, respectively, whereas galectin-7 induces apoptosis of colon cancer cells. In contrast, galectin-3 was reported to be an antiapoptotic molecule, inhibiting Fas-induced T-cell apop-

toxis as well as epithelial cell apoptosis induced by staurosporine, cisplatin, genistein, and anoikis (1).

Galectin-3 is isolated as a 32 kDa monomer, and consists of three distinct structural domains : the carboxyterminal domain, which appears as the carbohydrate-recognition domain, the N-terminal domain, comprising the 20 NH₂-terminal residues, and the R domain, composed of Pro-Gly-Tyr-rich repeating motif (2). Galectin-3 contains the Asp-Trp-Gly-Arg (NWGR) motif in the C-terminal domain, and it doesn't exist in other galectins. The NWGR motif is critical for the anti-apoptotic function of galectin-3 (3).

Galectin-3 is expressed in normal and neoplastic cells (4). Galectin-3 is localized not only in intracellular space such as the cytoplasm or the nucleus but also in extracellular space such as the cell surface or the extracellular matrix (5).

In certain types of cancers, the expression of galectin-3 is positively correlated to tumor progres-

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sion, such as gastric cancer (6), liver cancer (7), thyroid cancer (8). In other types of cancers, it is inversely correlated to tumor progression, such as head and neck cancer (9), uterine sarcoma (10), breast cancer (11), pancreas cancer (12).

In bladder cancer, galectin-3 mRNA levels were increased in most tumors compared with normal urothelium (13). However, the clinical relevance of this finding is not fully understood. We examined serum level of galectin-3 in patients with bladder cancer.

PATIENTS AND METHODS

Measurement of serum galectin-3 concentration

We studied serum samples of 67 patients who were admitted for urological diseases at our institution between 1996 and 2006. We classified these 67 patients into the cancer group (n=43) and the control group (n=24). Control group consisted of patients with 21 urolithiasis, 1 varicocele and 2 stress incontinence. Clinical data are described in Table 1.

All serum samples were stored at -80°C until this study. All samples were returned to room temperature before use. Serum galectin-3 concentration was measured by ELISA in accordance with the manufacture's instructions (Human Galectin-3 Assay Kit, IBL). Measurable range of this ELISA is from 117.19 to 7500 pg/ml. This ELISA kit has been described and validated elsewhere.

Table 1. Patient's clinical data

	cancer group	controls
n	43	24
mean age (range)	72 (45-83)	60 (28-85)
sex ratio M/F	34/9	12/12
non muscle invasion	33	-
muscle invasion	10	-
G1	2	-
G2	21	-
G3	20	-

Immunohistochemical staining

After radical cystectomy, both bladder cancer tissue and normal bladder tissue were embedded in OCT compound and stored at -80°C . We selected the patient with high serum galectin-3 concentration, and cut these tissue in thickness of 5 microme-

ters at -18°C and put section on the slide. We performed hematoxylin eosin staining, and chose the section by which a core is stained clearly. The good bladder cancer tissue and normal bladder tissue could be obtained. The histopathological diagnosis of selected bladder cancer tissue was urothelial carcinoma (UC), G3, pT3a pN0M0.

Immunohistochemical staining was performed with the VECTASTAIN ABC (Avidin Biotinylated enzyme Complex) system. The brief procedure was as follows. Sections were air dried, and fixed with acetone. We rinsed sections in tap water and washed in buffer. After incubating sections for 30 minutes with diluted normal blocking serum, the primary antibody (rat monoclonal anti-Gal-3 antibody, TIB 166) was incubated for 60 minutes at room temperature. Following incubation for 30 minutes with diluted biotinylated secondary antibody solution, we added VECTASTAIN ABC Reagent and waited for 30 minutes at room temperature. We incubated sections in peroxidase substrate solution until desired stain intensity develops. Cancer tissue and normal tissue were stained for the same hour at the same time. The expression of galectin-3 was evaluated using light microscopy at $\times 400$ magnification and the results interpreted according to the intensity of immunoreactive product in the bladder cancer cells. The immunohistochemical results were evaluated as : weak, less than 20% positive cells with weak intensity ; moderate, 20-50% positive cells with moderate intensity ; and strong, more than 50% positive cells with strong intensity.

Statistical analysis

Statistical analysis was performed using SPSS-II[®]. To compare results of measurement of serum galectin-3 concentration in two groups, we used Mann-Whitney test. Differences were considered significant at $p < 0.05$.

RESULTS

Serum level of galectin-3

Serum galectin-3 concentration was increased in the cancer group. Median value of serum galectin-3 concentration was 1068 pg/ml (range 551-2028) in the cancer group vs. 584 pg/ml (range 259-1262) in controls ($p < 0.0005$, Fig. 1A). We divided 43 bladder cancer patients into 33 non muscle invasive group and 10 muscle invasive group, and examined these samples. There was no significant difference

in serum galectin-3 concentration between the two groups (Fig. 1B). We also divided these 43 patients into 20 patients with G3 and 23 patients with G1 or G2. There was no apparent correlation in serum level of galectin-3 between the two groups (Fig. 1C).

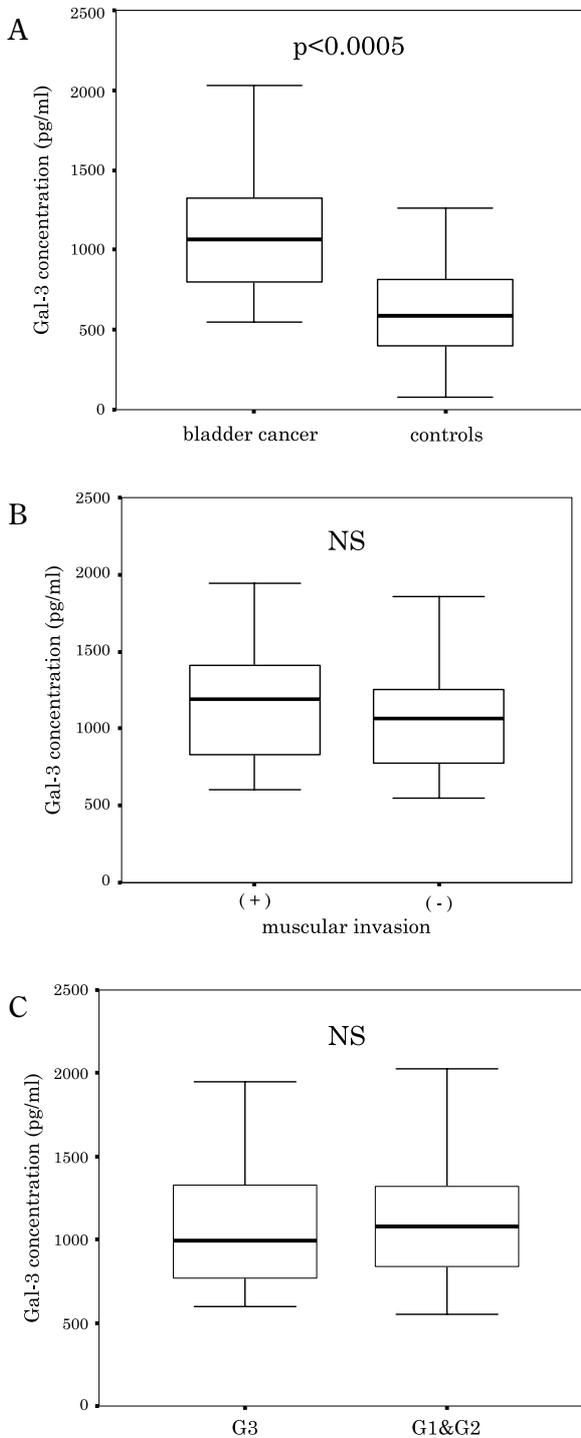


Fig. 1. Analysis of serum galectin-3 concentration ; Serum galectin-3 concentration of the cancer group was statistically higher than that of controls (A). There was no apparent correlation in galectin-3 concentration between non muscle invasive group and muscle invasive group (B). No significant difference was seen in galectin-3 concentration between G3 and G1 or G2 by Mann-Whitney test (C).

Based on data from the patients with bladder cancer, receiver operating characteristic (ROC) curve was obtained. The ideal cutoff value of serum galectin-3 concentration was recommended to be 586 pg/ml. Using this cutoff value, the sensitivity and specificity were 97.7% and 54.2%, respectively.

Immunohistochemical staining of galectin-3

We selected one patient with high serum galectin-3 concentration, and studied immunohistochemical staining of galectin-3 in bladder cancer tissue and normal bladder tissue. The histopathological diagnosis of selected bladder cancer tissue was UC, G3, pT3a pN0M0. Higher expression of galectin-3 was observed in bladder cancer tissue than in normal bladder tissue. The cytoplasm was stained strongly in bladder cancer tissue, but almost no cytoplasm was stained in normal tissue (Fig. 2). We performed immunostaining of galectin-3 to other three samples (UC, G3, pT2b or pT3a pN0M0). We evaluated all bladder cancer tissue as strong, and all normal tissue as weak according to our criteria. There was no correlation between the level of serum galectin-3 and degree of staining. The pattern of staining was same as the above.

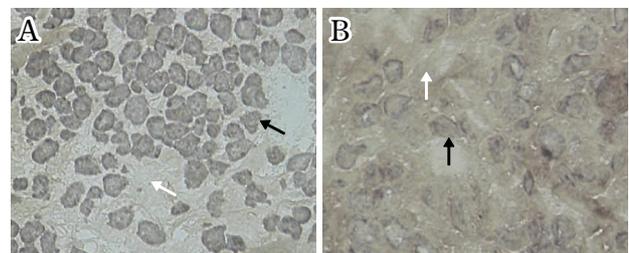


Fig. 2. Immunohistochemical staining of galectin-3 ; Black arrow indicates nucleus and white arrow indicates cytoplasm. Higher expression of galectin-3 was observed in bladder cancer tissue (B) than in normal bladder tissue (A). The cytoplasm was stained strongly in bladder cancer tissue, but almost no cytoplasm was stained in normal tissue ($\times 400$). We evaluated bladder cancer tissue as strong, and normal tissue as weak according to our criteria.

DISCUSSION

Galectin-3 is a member of the galectin gene family that is expressed at elevated levels in a variety of neoplastic cell types and has been associated with cell growth, cellular adhesion process, cell proliferation, transformation, metastasis, and apoptosis (14-20). Galectin-3 has both anti-apoptotic and proapoptotic function depending on cell's differentiation status and tissue type (21). It was reported that

the Asp-Trp-Gly-Arg (NWGR) motif (3) and phosphorylation (22) are critical for the anti-apoptotic function of galectin-3. Fukumori, *et al.* mentioned that secreted extracellular galectin-3 was related to pro-apoptotic function of galectin-3, but its mechanisms are quite complicated and there are many unanswerable questions (23).

The expression of galectin-3 is up-regulated in various types of cancer. Several reports have indicated its involvement in carcinogenesis (24, 25). One possible reason for this is the anti-apoptotic activity of galectin-3. Some reports have proposed mechanisms by which galectin-3 protects cells from apoptosis. Takenaka, *et al.* have shown that nuclear export of galectin-3 is important for its anti-apoptotic activity (26).

Galectin-3, which has been reported to be expressed in the nucleus, in the cytoplasm and on the cell surface, can be secreted into the stroma (5). It has been shown to be up-regulated in some cancers such as thyroid carcinoma, hepatocellular carcinoma and lymphoma, and down-regulated in others including breast, uterine and pancreas cancer (27).

Expression pattern of galectin-3 is altered in many types of cancers. Therefore, several attempts to use galectin-3 expression as a diagnostic indicator are under development. Inohara, *et al.* demonstrated that expression of galectin-3 in fine needle aspirates could be a diagnostic marker for thyroid cancer (28). Aron, *et al.* mentioned that galectin-3 was strongly expressed in smears of papillary thyroid carcinoma. However, since it is also expressed in a variety of benign lesions, its role as a pre-surgical marker for differentiating benign from malignant thyroid nodules is limited (29). Nakamura, *et al.* reported that strong expression of galectin-3 in colorectal cancer correlated with cancer progression, liver metastasis, and poor prognosis for patients (30). Shimamura, *et al.* studied decreased expression of galectin-3 was associated with advanced stage, tumor de-differentiation, and metastasis in ductal adenocarcinoma of the pancreas (12).

The expression of galectin-3 is down-regulated in some of urological cancers, suggesting that galectin-3 has a tumor suppressive role in urological organs. The mechanism by which galectin-3 promotes cancer progression has been reported. However, little is known about tumor suppressive functions of galectin-3 (31).

In prostate cancer, Van den Brule, *et al.* have reported galectin-3 was usually not expressed or decreased compared with the normal glands (32). In

renal cell carcinoma, it has been shown galectin-3 expression was significantly higher in low-grade conventional (clear cell) RCCs, indolent chromophobe RCCs (33, 34).

On the contrary, Cindolo, *et al.* have reported that increased galectin-3 mRNA expression compared to basal levels of normal bladder samples was observed in many bladder cancer samples with no apparent correlation with the clinico-pathological features such as stage and grade (13). We thought if the role of galectin-3 could be solved clinically, it was useful for diagnosis, prognostic prediction and selection of anticancer therapies. Thus we tried to measure the serum level of galectin-3 in bladder cancer patients.

We used ELISA kit to measure serum galectin-3 concentration in patients with bladder cancer. To our knowledge, this is the first report using ELISA kit to measure serum galectin-3 concentration in patients with bladder cancer. We demonstrated that serum galectin-3 concentration in bladder cancer patients was statistically higher than normal control patients ($p < 0.0005$).

However, there was no statistical difference in either histological grade or pathological stage with bladder cancer patients. Those results are consistent with these as Cindolo, *et al.* has reported *in vitro*.

In immunohistochemical staining of galectin-3, the expression of galectin-3 was apparently higher in bladder cancer tissue than in normal bladder tissue.

A standard method for diagnosis of bladder cancer is urine cytology and cystoscopy. However, cystoscopy is invasive and expensive. Several urinary markers for bladder cancer have been investigated. Nuclear matrix protein-22 (NMP22), bladder tumor antigen (BTA) and urine cytology are major urinary markers for diagnosis of bladder cancer. Sensitivity and specificity were previously reported with 85% and 91.3% for NMP22, 67% and 80.8% for BTA, 44% and 100% for urine cytology (35). However, Poulakis, *et al.* have demonstrated the specificity of urinary markers obviously became low with pyuria in cystitis and urolithiasis (36). Contrary to urinary markers, serum level of galectin-3 is not affected by pyuria. For that reason we consider that the measurement of serum galectin-3 will be helpful to diagnosis of bladder cancer, if bladder cancer is clinically suspected.

As the results of this study, the possibility that galectin-3 will be useful in diagnosis of bladder cancer was suggested. However, there was no signifi-

cant difference in the stage and grade in our study. Further studies are needed to characterize the role of galectin-3 in bladder cancer. We will examine the level of urine galectin-3, and evaluate the utility through a combination of serum galectin-3 and urine markers including urine galectin-3. The combined use of these markers might be able to improve accuracy of diagnosis of bladder cancer.

In conclusion, the serum galectin-3 concentration of the bladder cancer patients was statistically higher than that of controls. This result suggests that the measurement of serum galectin-3 concentration is helpful to diagnose bladder cancer.

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