# Galectin-1 Dysregulation Independently Predicts Disease Specific Survival in Bladder Urothelial Carcinoma

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**Abbreviations and Acronyms**

DSS = disease specific survival
FBS = fetal bovine serum
GAL-1 = galectin-1
LCM = laser capture microdissection
PCR = polymerase chain reaction
RT-PCR = reverse transcriptase-PCR
UBUC = bladder urothelial carcinoma
v/v = volume per volume
w/v = weight per volume

Accepted for publication September 26, 2014.
Study received institutional review board approval.
Supported by National Science Council of Taiwan Contracts NSC 101-2632-B-218-001-MY3, NSC 99-2314-B-384-003-MY3 and NSC 99-2313-B-218-001-MY3.

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**Purpose:** Galectin-1 is highly expressed in various tumors and participates in various oncogenic processes. Our previous proteomics investigation demonstrated that galectin-1 is up-regulated in high compared to nonhigh grade lesions. Thus, in the current cohort study we clarified the correlation of galectin-1 over expression with various clinicopathological features and prognosis.

**Materials and Methods:** We selected 185 cases of consecutively treated primary localized bladder urothelial carcinoma for study. Transurethral resection of bladder tumor was performed in all patients followed by radical cystectomy in those with T2 to T4 tumors. Pathological slides were examined to determine cytoplasmic galectin-1 immuno-expression and correlate galectin-1 dysregulation with various clinicopathological factors and disease specific survival.

**Results:** Positive galectin-1 immuno-expression in tumors was significantly linked to pT status ($p = 0.0295$), histological grade ($p = 0.037$), vascular invasion ($p = 0.0287$) and nodal status ($p = 0.0012$). Galectin-1 over expression in tumors significantly predicted disease specific survival at the univariate ($p = 0.0002$) and multivariate levels ($p = 0.03$, HR 2.438, 95% CI 1.090–5.451). In situ hybridization indicated that the *LGALS1* gene was amplified in 43 specimens in an independent cohort of 56 snap frozen tumor specimens. Association analysis showed that an increased *LGALS1* mRNA level was linked to bladder urothelial carcinoma invasiveness ($p = 0.016$) and *LGALS1* gene amplification was significantly associated the amount of GAL-1 protein in tumors ($p < 0.0001$). On the univariate level gene amplification was also closely linked to disease specific survival ($p = 0.0006$).

**Conclusions:** These results reveal that galectin-1 over expression is a possible independent factor for bladder cancer prognosis.

**Key Words:** urinary bladder, carcinoma, galectin 1, gene amplification, prognosis

Urothelial carcinoma is the most common lesion of various heterogeneous tumor types that arise from the urothelial cover of the bladder and ureter. Although UBUC is usually recognized as papillary and noninvasive or superficially invasive lesions, which are often treated with


0022-5347/15/1933-1002/0
THE JOURNAL OF UROLOGY®

http://dx.doi.org/10.1016/j.juro.2014.09.107
Vol. 193, 1002-1008, March 2015
Printed in U.S.A.

curettage, some UBUCs show relentless local recurrence followed by lethal distal spreading.[1]

Today cystoscopy/urine cytology provide high, acceptable specificity to detect high grade UBUCs but lack sensitivity for low grade lesions.[2] Furthermore, bladder cancer recurrence might not be detected in a subset of patients with tumor metastasis, especially those who undergo radical cystectomy. Therefore, identifying prognostic biomarkers by determining UBUC molecular carcinogenesis may offer useful insight to aid in the exploration of better diagnoses/prognoses and novel targeted therapeutic strategies.

Human GAL-1 is a member of the galectin family, proteins with conserved carbohydrate recognition domains that bind galactoside.[3] GAL-1 is highly expressed in various tumors, including prostate and breast cancers, and hepatocellular carcinoma.[4–7] Intriguingly in most lesions GAL-1 expression is associated with aggressiveness and metastasis of these tumors.[8] It participates in various oncogenic processes, including cell transformation,[8] metastasis,[8,9] cell proliferation[9–13] and cell migration.[14] GAL-1 also has an essential role in tumor angiogenesis.[15] Immunohistochemical examination of the brain of immunodeficient mice for GAL-1 expression in human U87 and U373 glioblastoma xenografts demonstrated higher GAL-1 expression in invasive areas of the xenografts than in noninvasive areas.[16] The literature shows that GAL-1 can combine with H-RAS and give rise to H-RAS membrane anchorage. GAL-1 over expression in tumor cells augments membrane linkage of H-RAS and cell transformation.[17,18] Rubinstein et al reported that melanoma cells can secrete GAL-1 protein to escape from T-cell dependent immunity by inducing activated T-cell apoptosis, thus, conferring the immune privilege to tumor cells.[19]

Our previous clinical proteomics examination demonstrated that GAL-1 is up-regulated in high grade UBUC compared to nonhigh grade lesions.[20] However, more specimens were required to verify the clinical significance of GAL-1 in UBUC. In the current study we determined GAL-1 immunohistochemical expression in 185 UBUC specimens to confirm the proteomics data and clarify the relevance of GAL-1 expression to UBUC progression and prognosis.

## MATERIALS AND METHODS

### Case Selection

We retrieved 185 cases of primary localized UBUC consecutively treated between 1997 and 2002 from the archives of Chi-Mei Medical Center under institutional review board guidelines. Tumor histological grade was evaluated according to the recent 2-tiered WHO/ISUP (International Society of Urological Pathology) consensus classification. Clinical stage (pT) of these primary tumors was based on the AJCC (American Joint Committee on Cancer) system, 7th edition system. Transurethral resection of bladder tumor was performed in all cases followed by radical cystectomy for T2 to T4 tumors. Regional lymph node dissection was done only if nodal metastasis was suspected clinically. Excluding patients with poor performance, those with pT3 or pT4 tumors and/or nodal involvement received cisplatin based adjuvant chemotherapy.

In all cases hematoxylin and eosin stained sections were recut and histopathologically reevaluated by 2 pathologists (CFL and HYH). For multiple tumors the sections containing the most invasive area were used for analysis. To determine *LGALS1* mRNA expression and gene dose an independent cohort containing 56 snap frozen tumor specimens was analyzed by real-time RT-PCR and fluorescence in situ hybridization. Of the 56 tumors 29 were classified as noninvasive or superficially invasive (Ta and T1) and 27 were deeply invasive (T2 to T4). Four histologically confirmed benign urothelium samples served as the reference for mRNA expression.

### GAL-1 Immunohistochemistry

Sections were prepared and heated for antigen retrieval as previously described.[21] This was followed by 1-hour incubation with galectin-1 primary antibody (1:200, No. 437400, Invitrogen®) and detection with the ChemMate EnVision™+ Kit (K5001). Two pathologists (CFL and HYH) blinded to clinicopathological data and patient outcome independently assessed UBUC GAL-1 expression. The percent of tumor cells with moderate or strong cytoplasmic immunoreactivity was scored.

Since as shown there was no nuclear staining in our tumor specimen, a labeling index was used, including negative (0)—no tumor cells with moderate or strong cytoplasmic staining, weakly positive (1+)—1% to 24% of tumor cells, moderately positive (2+)—25% to 49%, strongly positive (3+)—50% to 74% and intensely positive (4+)—75% to 100%. Cases with positive GAL-1 cytoplasmic immunostaining in at least 5% of tumor cells (1+) were considered positive.

### Statistical Analysis

The clinical variables used for statistical analysis were stage, grade, nodal status, vascular invasion, perineural invasion, mitotic activity and tumor necrosis. GAL-1 immuno-expression was compared between various parameters with the chi-square test. The end point assessed in the whole cohort was DSS measured from the date of UBUC operation to disease related mortality. In stepwise forward fashion parameters at univariate $p \leq 0.05$ were in principle entered into the Cox regression model to analyze their relative prognostic importance. On all analyses the 2-sided test of significance was used with $p < 0.05$ considered significant. Statistical analysis was done with SPSS® 14.0.

### Cell Lines

Human UBUC T24 cells (high grade and invasive), J82 cells (high grade and invasive), RT4 cells (low grade) and