c-*kit* expression in small cell carcinoma of the urinary bladder: prognostic and therapeutic implications

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The prognosis for small cell carcinoma of the urinary bladder is poor, and strategies for improved therapy are needed. Targeted therapy against the c-*kit* proto-oncogene has been successful in the management of gastrointestinal stromal tumor. We investigated the expression of c-*kit* in 52 cases of small cell carcinoma of the urinary bladder. Specimens with more than 10% of cells demonstrating strong membrane staining were considered to have positive immunostaining for c-*kit*. c-*kit* expression was detected in 21 of 52 specimens from these patients. Among the 21 specimens, seven had less than 10% staining, and were considered to be negative. Nine had 11–50% staining, and five had more than 50% staining. Overall, 14 of 52 (27%) small cell carcinomas of the urinary bladder were positive for c-*kit* expression. During a median follow-up of 11 months, 60% of the patients died of bladder cancer. No association was found between c-*kit* expression and survival or other clinicopathologic parameters. Five-year cancer-specific survivals for c-*kit*-positive and c-*kit*-negative tumors were 9 and 15%, respectively ($P = 0.36$). A significant proportion (27%) of small cell carcinomas of the urinary bladder expressed c-*kit*, suggesting that it may prove useful as a therapeutic target in small cell carcinoma of the urinary bladder.

Small cell carcinoma of the urinary bladder is a distinct histological and biologic disease entity with an aggressive clinical course, poor prognosis, and an average life expectancy of only a few months.[1–3] Consequently, strategies for improved therapy of this highly lethal cancer are needed. Therapy directed at tumor-specific targets has been successful in improving survival in some cancers. The proto-oncogene c-*kit* encodes a transmembrane tyrosine kinase receptor c-*kit* (also called CD117), which has been shown to be involved in hematopoi-esis and carcinogenesis.[4,5] Overexpression of c-*kit* has been detected in several malignancies, including gastrointestinal stromal tumor, carcinoma of breast, and small cell carcinoma of the lung.[6–8] Gastrointestinal stromal tumor has been successfully treated with therapy targeted against c-*kit*.[9] We studied the expression of c-*kit* in a large series of small cell carcinomas of the urinary bladder and correlated the findings with other clinicopathologic parameters.

Materials and methods

Patients

In total, 52 patients with small cell carcinoma of the urinary bladder were identified from Indiana University (Indianapolis, IN, USA), Case Western

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Reserve University (Cleveland, OH, USA), North-western University (Chicago, IL, USA), University of Chicago (Chicago, IL, USA), and Cordoba University (Cordoba, Spain). The medical records were reviewed. Formalin-fixed paraffin-embedded tissue specimens were available for all cases. Sections stained with hematoxylin and eosin were examined. All tumors fulfilled the criteria established for small cell carcinoma by the WHO classification system.[10] The diagnosis was further supported by immuno-histochemical stains and, in some cases, electron microscopic examination. Pathologic staging was performed according to the 2002 TNM (tumor, lymph nodes, and metastasis) classification system.[11] This research was approved by the Institutional Review Boards in accordance with the Institutional Committee for the Protection of Human Subjects.

Immunohistochemistry

Immunohistochemical staining was performed with the avidin–biotin complex method of Hsu *et al*.[12] In brief, serial 5 μm-thick sections of formalin-fixed, paraffin-embedded tissue samples were used for the studies. Slides were deparaffinized twice in xylene for 5 min and rehydrated through graded ethanol solutions to distilled water. Antigen retrieval was carried out by heating sections in citrate buffer (DAKO Target Retrieval Solution S1699, DAKO Corporation, Carpintera, CA, USA) for 15 min. Endogenous peroxidase activity was inactivated by incubation in 3% H_2O_2 for 15 min. Nonspecific binding sites were blocked using Protein Block (DAKO) for 20 min. Tissue sections were then incubated with the purified rabbit polyclonal anti-body against c-*kit* (IgG, 1:50 dilution; Oncogene, Boston, MA, USA) for 30 min at room temperature, followed by biotinylated secondary antibody (DAKO) and peroxidase-labeled streptavidin. 3,3-Diaminobenzidine was used as the chromogen in the presence of hydrogen peroxide. Sections from gastrointestinal stromal tumors were used as positive controls. Negative controls were performed using blocking serum in place of primary antibody. Positive and negative controls were run in parallel with each batch, and appropriate results were obtained.

The expression of c-*kit* was graded as previously described.[7] The entire section was scanned at low magnification, and hot spots were preferentially scored. At least 1000 cells were analyzed. The cutoff value of 10% for positive staining is based on the criteria used in the literature for c-*kit* staining,[7] and other tumor markers, such as Her-2/neu in breast cancers.[13,14] The authors recognize that there is a need for standardization of c-*kit* immunostaining evaluation in bladder tumors.

Statistical Analysis

All statistical tests were two-sided, with a *P*-value of 0.05 or less considered to be statistically significant.

SAS version 8.2 was used for the statistical analysis. SPLUS was used for Kaplan–Meier survival curves. Fisher's exact test for differences in frequencies was used for analyzing the c-*kit* expression and survival and other clinicopathologic parameters.

Results

Totally, 52 patients were analyzed. The male to female ratio was 4:1. The patients' ages ranged from 36 to 85 years (mean, 67 years). All patients except one had advanced disease (T2 or above) at presentation. Pathologic stage was T1 (1 patient, 2%), T2 (26 patients, 50%), T3 (22 patients, 42%), and T4 (three patients, 6%), respectively. Among patients with known clinical history, 57% (21/37) of patients had lymph node metastases at the time of radical cystectomy; four had distant metastases at presentation. Four patients underwent transurethral resection of bladder tumor. Overall, 55% received chemotherapy, while only 27% received radiation.

Expression of c-*kit* was evaluated by immunohis-tochemistry in specimens from 52 patients. The staining was predominantly located on the cell membrane (Figure 1), in keeping with the fact that c-*kit* is a membrane-bound tyrosine kinase receptor. The normal urothelium and stromal cells were negative for c-*kit* immunostaining. The expression of c-*kit* was graded as previously described.[7] Speci-mens were considered to be positive when more than 10% of tumor cells showed positive immuno-staining. Of the 52 specimens, 38 samples (73%) were negative (31 samples with no staining, seven with less than 10% staining). In all, 14 samples (27%) were positive for c-*kit* expression (nine between 11 and 50%, five with more than 50% staining).

There was no correlation between c-*kit* expression and survival ($P = 0.36$) (Figure 2). For c-*kit* positive tumors, 2- and 5-year cancer-specific survi-vals were 34 and 9%, respectively. For c-*kit* negative tumors, 2- and 5-year cancer-specific survivals were 41 and 15%, respectively. There was no correlation of c-*kit* expression with other clinicopathological characteristics including age ($P = 0.79$), gender ($P = 0.41$), history of smoking ($P = 0.58$), clinical stage ($P = 0.20$), pathologic T stage ($P = 0.51$), lymph node metastasis ($P = 0.41$), and distant metastasis ($P = 0.99$).

Discussion

Overexpression of c-*kit* has been detected in 28–78% of cases of small cell carcinoma of the lung.[7,8,15] Mutation of c-*kit* is uncommon, as detected by polymerase chain reaction-single-strand conformational polymorphism.[16] There have been conflicting reports on the prognostic significance of c-*kit* expression. Potti *et al*[7] observed a significantly worse prognosis in small cell lung carcinoma
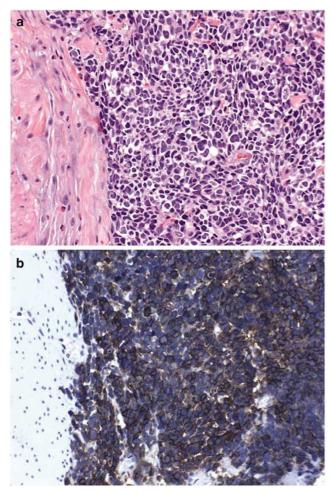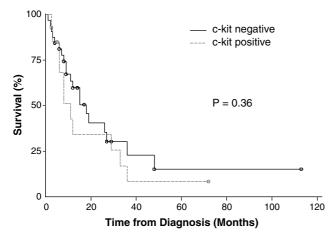
Figure 1 Small cell cancer of the urinary bladder. (**a**) The tumor invades into muscularis propria wall (hematoxylin staining, original magnification ×200). (**b**) Positive c-*kit* immunoreactivity (hematoxylin counterstain, original magnification ×200).

Figure 2 Kaplan–Meier survival curves for patients with small cell cancer of the urinary bladder stratified according to c-*kit* expression.

patients with c-*kit* overexpression, whereas other investigators have noted a trend toward better prognosis in such patients.[17] In this study, we found

that a significant proportion (27%) of small cell carcinomas of the urinary bladder had positive c-*kit* expression, raising the question of whether therapy targeted against c-*kit* may be beneficial for some patients with small cell carcinoma of the urinary bladder.

The c-*kit* gene encodes a tyrosine kinase receptor related to platelet-derived growth factor (PDGF)/colony-stimulating factor 1 (CSF-1).[18] The c-*kit* pathway has been shown to be involved in a number of physiological and pathological processes, including hematopoiesis, spermatogenesis, melanogenesis, and oncogenesis.[4,15] Two mechanisms of c-*kit* activation exist: autocrine and/or paracrine activation by its ligands; and ligand-independent activation by mutation. Ligand-dependent activation of c-*kit* depends on the engagement of its ligand, the stem cell factor. The binding of c-*kit* by stem cell factor induces phosphorylation and activation of the c-*kit* signal transduction pathway. This type of activation occurs during some normal physiological processes, and during oncogenesis of some cancers.[8,19] It has been shown that some tumor cells express both c-*kit* and stem cell factor which appears to be capable of protecting the cells against apoptosis.[19] Ligand-independent constitutive activation of c-*kit* involves mutations, mainly in exon 11, the region between the transmembrane and tyrosine kinase domains. c-*kit* protein derived from mutation is constitutively activated without engagement by its ligand, stem cell factor. Stable transfection of mutant c-*kit* complementary DNA has been shown to induce malignant transformation, suggesting that the mutations contribute to tumor development.[20] Mutation of c-*kit* is common in gastrointestinal stromal tumor,[20] whereas mutation is uncommon in small cell carcinoma of the lung and colon.[16,21] It has been suggested that c-*kit* expression does not accurately identify responders/nonresponders to STI-571 therapy for gastrointestinal stromal tumors. The presence or absence of activating mutations of c-*kit* is most predictive of clinical response.[22] c-*kit* expression does not always correlate with the presence of activating mutation.[16]

STI-571 (Gleevec, Novartis, Basel, Switzerland), inhibiting c-*kit* phosphorylation and its subsequent activation, has been utilized in the successful treatment of gastrointestinal stromal tumor.[9] The role of STI-571 in the treatment of other cancers is not well established. Whether the encouraging results of STI-571 treatment on gastrointestinal stromal tumor can be translated to small cell carcinoma of the urinary bladder is uncertain. STI-571 inhibits the growth of Ewing's sarcoma cell lines; the degree of inhibition correlates with the levels of c-*kit* expression. Nevertheless, the inhibition is less pronounced than in gastrointestinal stromal tumor, and high doses of STI-571 are required to accomplish inhibition.[23] At the present time, there are no *in vitro* or *in vivo* models available to test the effectiveness of target therapy using

STI-571 in small cell carcinoma of the urinary bladder. In small cell lung carcinoma patients treated with first-line chemotherapy, the expression of c-*kit* is lost in about half of the patients whose cancers expressed c-*kit* prior to chemotherapy.[24]

There is currently no randomized clinical trial for the management of small cell carcinoma of the urinary bladder. Current experience in the treatment of small cell carcinoma of the urinary bladder is based on anecdotal reports, some published more than a decade ago. Considering the generally poor prognosis of small cell carcinoma of the urinary bladder, it may be reasonable to consider the therapeutic use of STI-571 in patients with c-*kit*-positive tumors.

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