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The prognostic significance of nuclear CSE1L in urinary bladder urothelial carcinomas☆

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Abstract Prognosis of urinary bladder urothelial carcinomas may be challenging; many tumors with similar histopathologic features show significantly different clinical outcomes. CSE1L, the chromosome segregation 1-like protein, is both a cytoplasmic and nuclear protein. We investigated the cytoplasmic/nuclear expression pattern of CSE1L to determine its potential prognostic significance. In immunohistochemical analysis, nonneoplastic urothelium showed faint CSE1L staining, whereas all tumors in the bladder cancer specimens had significant staining for CSE1L (100%, or 38/38). CSE1L cytoplasmic/nuclear staining was defined based on relative staining intensity. A total of 20 (52.6%) of 38 cancer specimens had strong nuclear CSE1L staining, and 44.7.3% (17/38) of the samples had strong cytoplasmic CSE1L staining. Bladder urothelial carcinomas with high CSE1L nuclear staining had a significantly lower overall survival rate (log-rank test, $P = .011$). CSE1L expression was not correlated with tumor stage, likely reflecting the faultiness of current urothelial carcinoma evaluation methods. Our results suggest that nuclear CSE1L may play an oncogenic role in bladder tumor progression and that immunohistochemical staining of nuclear CSE1L may be useful for the prognosis of bladder urothelial carcinomas.

Keywords: Urinary bladder cancer; CSE1L; Prognosis; Tumor markers; Urothelial carcinomas

1. Introduction

Urothelial carcinoma (transitional cell carcinoma) is the most common tumor in human urinary bladder. Patients with advanced stage or grade of bladder urothelial carcinomas usually have poor prognosis [1]. The 5-year survival rates are 95%, 75%, 60%, 35%, and 10% of patients for the Ta, T1, T2, T3, and T4 tumors, respectively [2,3]. However, although most bladder urothelial carcinomas are low-grade diseases, the tumors have high recurrent rates: about 50% to 70% of patients with non–muscle-invasive tumors will develop tumor recurrence within 5 years, although the tumors are completely resected [2,3]. Patients with a history of bladder carcinoma in situ are more likely to develop aggressive upper tract urothelial carcinoma [4]. Moreover, the recurrent bladder cancer has higher death rates [5]. The optimal treatment regimen depends upon patient's specific clinical characteristics with regard to renal function including medical comorbidities; tumor location, grade, and stage;

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and the status molecular markers [6]. Urothelial carcinomas often reveal diverse morphological and immunologic features that frequently lead to diagnostic challenges [7]. It is needed to find molecular markers that are associated with the clinical-pathologic correlation and behavior of bladder urothelial carcinomas so as to achieve more accurate prognosis to aid clinicians in the management of the disease [8].

The chromosome segregation 1-like protein (CSE1L) is the human homologue of the yeast chromosome segregation protein (CSE1) [9]. Pathologic studies have shown that CSE1L is highly expressed in most cancers and that its expression correlates with advanced cancer grade and stage [10-19]. Recent studies have shown that CSE1L is a secretory protein and that it is implicated in the invasion and metastasis of cancer [20-23]. The prognostic evaluation of bladder urothelial carcinomas may be challenging [24-26]. The expression status and prognostic significance of CSE1L in bladder urothelial neoplasms have not been studied. CSE1L is located in both the cytoplasm and nucleus of cell. Cytoplasmic CSE1L is associated with microtubules, whereas nuclear CSE1L has been shown to regulate the transcriptional activity of p53 protein, a major tumor suppressor protein that is associated with the aggressive clinical outcome and poor prognosis of bladder tumors [9,27-29]. We reported here that high CSE1L nuclear staining is associated with poor prognosis of patients with bladder urothelial carcinoma. The results suggest that nuclear CSE1L plays a role in the development of urothelial carcinoma and that immunohistochemical analysis of CSE1L distribution in a tumor is a useful ancillary tool for the prognosis of bladder urothelial carcinomas.

2. Materials and methods

2.1. Patients and samples

The retrospective observation study used anonymous unlinked and "as excess" samples approved by the ethics committees of the Taipei Medical University Hospital, Taipei, Taiwan. In this study, we enrolled 38 patients with urothelial carcinoma of urinary bladder who had undergone surgical resection, following the institutional review board–approved guidelines. The clinical stages and grades for each patient were classified according to the TNM classification system and the World Health Organization classification system [30,31].

2.2. Analyses of CSE1L expression by immunohistochemistry

The paraffin-embedded bladder cancer specimens and paired nontumor tissue sections (4 μm) were deparaffinized in xylene and rehydrated in graded alcohol. Antigen retrieval was performed by treatment with boiling citrate buffer (10 mmol/L, pH 6.0) for 20 minutes. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in water, and nonspecific staining was blocked by incubation with 5% bovine serum albumin for 1 hour at room temperature. After incubation with a 200-fold dilution of anti-CSE1L antibody (clone 3D8; Abnova, Taipei, Taiwan) for 20 minutes at room temperature and thorough washing 3 times with phosphate-buffered saline, the slides were incubated with a horseradish peroxidase/Fab polymer conjugate for another 30 minutes. The sites of peroxidase activity were visualized by using diaminobenzidine (3,3'-diaminobenzidine tetrahydrochloride) as the substrate and counterstained with Mayer's hematoxylin. Paraffin-embedded sections of normal colonic epithelium with homogeneous CSE1L staining were included as the positive controls. In the negative control, the primary antibody was omitted and replaced by phosphate-buffered saline.

2.3. Immunohistochemical score system

Immunohistochemical evaluation incorporated both intensity and distribution of staining, yielding a subjective score and histologic score (H-score). We adopted the semiquantitative scoring system, incorporating the staining intensity and distribution of staining. Each tumor was given a score according to the intensity of the nuclear or cytoplasmic staining (no staining, 0; weak staining, 1+; moderate staining, 2+; and strong staining, 3+) and confirmed by 2 expert pathologists. The immunoreactive H-score was determined by multiplying the staining intensity and percentage of stained cells with the minimum score of 0 and a maximum score of 300 [32]. We defined a score of 200 or higher as highly positive.

2.4. Statistical analysis

CSE1L expression was assessed based on the intensity of the immunohistochemical staining. The primary outcome was overall survival, which was defined as the time from the initiation of surgery to death because of disease-related death or to the date of the last follow-up. Significant differences in the clinicopathologic variables between each group were tested using the Fisher exact test. The distribution of overall survival was estimated using the Kaplan-Meier analysis and log-rank test. The prognostic significance of the variables was evaluated. The variables in the model included nuclear and cytoplasmic expression of CSE1L, tumor grade, clinical stage, T status, and lymph node metastasis. The analyses were performed using the SPSS version 15.0 (SPSS Inc, Chicago, Ill), and $P < .05$ (2-tailed test) was considered statistically significant.

3. Result

3.1. Patient characteristics and CSE1L immunohistochemical expression

Baseline characteristics of the patients are shown in Table 1. In total, 38 patients, including 28 men and 10 women,