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Original contribution

Significance of TWIST expression and its association with E-cadherin in bladder cancer

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Summary Recently, TWIST, a basic helix-loop-helix transcription factor, has been reported to play a key role in the metastatic progression of several types of human cancer. The aim of this study was to investigate the significance of TWIST expression in bladder cancer using tissue microassays generated from 226 bladder tissue specimens. Using immunohistochemical staining, we studied TWIST expression levels in nonmalignant bladder tissues (n = 37), primary bladder cancer tissues (n = 164), and 25 cases of matched lymph node metastatic lesions. The association between TWIST expression levels and tumor staging and grading, as well as metastatic potential, was analyzed by statistical analysis. Our results showed that TWIST protein expression was significantly higher in bladder cancer specimens compared with nonmalignant tissues ($P < .001$), indicating its positive role in the development of bladder cancer. In addition, increased TWIST expression levels were associated with advanced-stage and high-grade tumors, suggesting its involvement in the progression of this cancer. Furthermore, TWIST expression was much higher in the metastatic lesion compared with its primary site ($P < .05$). More importantly, the increased TWIST expression in bladder cancer specimens was correlated with decreased membranous expression of E-cadherin, a cell adhesion molecule that plays a key role in the metastatic progression of human cancer. Our results demonstrate TWIST as a novel positive factor in the development and progression of bladder cancer and suggest a marker for advanced bladder cancer.

1. Introduction

Bladder cancer is the fourth most common cancer in men, the ninth most common cancer in women, and the second leading cause of death among genitourinary malignancies in the United States [1]. Transitional cell carcinoma (TCC) accounts for over 95% of bladder cancer. TCC of the bladder is a heterogeneous disease, and 70% to 80% of newly diagnosed bladder cancers are superficial tumors at diagnosis that are confined to the urothelium or the lamina propria (stages Ta/T1). Although these tumors can be managed with endoscopic resection and intravesical chemotherapy, the recurrence rate is high at 30% to 70%, and up to 30% will progress to muscle-invasive diseases (T2 or

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higher). Although radical cystectomy may be able to achieve 5-year survival for 50% of the patients with invasive cancer, nearly half of the remaining patients will develop distant metastasis within 2 years, and the 5-year survival rate is as low as 6% [2,3]. Early detection of molecules that enable cancer cells to invade or metastasize may help to identify high-risk superficial tumors with invasive tendency or advanced tumors with metastatic potential to facilitate clinical treatment.

Recently, up-regulation of TWIST, a highly conserved basic helix-loop-helix transcription factor, has been reported in several types of cancer, and its expression levels are found to be correlated with advanced tumor stage and metastatic potential [4-9]. For example, a recent study on 570 patients with melanoma found that patients with high TWIST expression in the primary tumors had a significant shorter survival time than those with lower TWIST expression (P = .0028) [4]. Increased TWIST was also prominent in invasive cancer specimens as well as animal xenografts compared with the primary sites [5,6,10,11]. In osteosarcoma, up-regulation of TWIST is reported to be a predictor in patients with poor response to chemotherapy [12]. These results suggest that TWIST may be a potential marker for advanced cancer. One of the mechanisms responsible for the positive effect of TWIST on metastasis is reported to be its ability to promote epithelial-to-mesenchymal transition (EMT) [5,7,10], which is a key step during embryonic morphogenesis and was recently implicated as a key step for the progression of primary tumor to metastatic stage [13]. One of the hallmarks of EMT is the loss of E-cadherin, a central component of cell-cell adhesion junctions, and the loss of which has been frequently found in metastatic tumors [14]. Several in vitro studies have reported that the TWIST-induced metastasis is associated with down-regulation of E-cadherin [5,7], indicating its involvement in the TWIST-mediated metastasis. However, the association between TWIST and E-cadherin has not been established in clinical studies. The aim of the current study was to investigate the prognostic significance of TWIST in bladder cancer and its association with E-cadherin expression. Using immunohistochemical staining, we studied the expression of TWIST and E-cadherin in a total of 226 bladder tissue samples and correlated it with tumor staging, grading, and metastatic potential. Our results showed that increased TWIST was inversely correlated with E-cadherin expression, which was associated with advanced tumor stage and metastatic potential. Our results suggest that TWIST may be a novel marker for early identification of potentially invasive bladder cancer.

2. Materials and methods

2.1. Bladder cancer tissue microarray construction

A total of 260 bladder tissues, consisting of 186 primary bladder cancers, 45 adjacent normal tissues, and 30 matched regional lymph node metastases, were obtained from archives of formalin-fixed, paraffin-embedded surgical (transurethral resection or cystectomy) specimens at the Institute of Urology, Peking University, Beijing, China. All the original slides of the specimens were reviewed for histopathological staging (1997 UICC TNM classification) and grading (World Health Organization classification) by 2 independent pathologists. The representative areas of tumor and nontumor tissues were marked for tissue microarray (TMA) construction. Briefly, tissue cylinders were taken from the selected regions of the donor block and then punched precisely into a recipient paraffin block using a tissue-arraying instrument (Beecher Instruments, Silver Spring, MD). A total of 2 to 4 representative tissue samples (a "core set," 0.6 mm in diameter) from each area were selected, and total of 805 tissue cores were generated from 260 tissue blocks. Multiple sections (5 μm thick) were cut and mounted onto microscope slides.

Hematoxylin-eosin–stained TMA sections were evaluated histopathologically by 2 pathologists in a blinded fashion to validate the diagnostic morphology of each array spot. Of the 260 donor tissues, 226 were informative for data analysis, including 164 primary tumors, 37 normal tissues, and 25 matched lymph node metastases. The clinicopathological characteristics of the 164 informative bladder cases are shown in Table 1. The noninformative samples included lost of samples, unrepresentative samples, and samples with too few tumor cells (<50 cells).

2.2. Immunohistochemistry staining

Immunohistochemistry (IHC) studies were performed using 2-step Envison+ kit (DAKO, Glostrup, Denmark) for detection of both TWIST and E-cadherin expression. TMA sections were deparafinized and rehydrated. Endogenous

Table 1 Summary of pathological and clinical data

Characteristics	Informative cases in TMAs (%)
Median age (range), y	65 (42-83)
Sex	
Male	130
Female	34
Stage	
Ta-1	76
T2-4	88
Grade	
G1	41
G2	44
G3	79
Lymph node metastasis	
N0	30
N+	25
Surgical procedure	
TUR	84
Cystectomy	80

Abbreviations: N0, tumor without lymph node metastasis; N+, tumor with lymph node metastasis; TUR, transurethral resection.