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Original article

The expression of Twist has an impact on survival in human bladder cancer and is influenced by the smoking status☆

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Abstract

Objectives: Twist is considered as transcription factor that regulates epithelial mesenchymal transition (EMT) by at least inhibition of E-cadherin expression. EMT is a key event in the tumor invasion process. The purpose of this study is to investigate the expression of Twist but also those of E- and N-cadherin in human primary bladder tumor and to evaluate its prognostic value. As smoking cigarettes is a strong bladder cancer risk factor, we tried to evaluate the impact of the tobacco status on these molecular abnormalities.

Materials and Methods: To delineate on the oncogenic role for Twist in human bladder cancer, we evaluated the E- and N-cadherin but also Twist expression (n = 70) by immunohistochemistry. We evaluated the prognostic value of these expressions. Moreover, we tried to correlate these protein expressions to the smoking status of the patients. Overall survival (OS) and progression-free survival (PFS) were evaluated using the Kaplan-Meier method, and multivariate analysis was performed using the Cox proportional hazard analysis.

Results: Of the 70 bladder tumors, 28 (40%) cases were positive for Twist expression, 16 (23%) cases were negative for E-cadherin expression, and 12 (17%) were positive for N-cadherin expression. When categorized into negative vs. positive expression, Twist was associated with the stage ($P = 0.001$), the grade ($P < 0.001$), the progression ($P = 0.02$), and the E-cadherin expression ($P = 0.01$). Moreover, positive Twist expression clearly predicted poorer PFS ($P = 0.02$). In the multivariate analysis, both positive Twist expression and loss of E-cadherin expression were independent prognostic factors for PFS ($P = 0.046$ and $P = 0.001$, respectively) and only loss of E-cadherin expression for the OS ($P < 0.001$). We also demonstrated that almost 60% (16/28) of patients with Twist-positive expression were current smokers at the time of the diagnosis, corroborating the fact that smoking modulates the expression of EMT markers including Twist.

Conclusion: Positive Twist expression may be a useful prognostic marker for patients with bladder cancer. Its expression seems to be correlated to the tobacco status of the patients.

Keywords: Twist; E-cadherin; Immunohistochemistry; Bladder cancer; Smoking status; Survival

1. Introduction

Bladder cancer is the seventh most common cancer worldwide, accounting for 3.2% of all cancers [1]. In Europe, 36,500 deaths due to bladder cancer occur in males and nearly 13,000 in females. Bladder cancer is one of the malignancies for which the improved understanding of the tumor biology provides an attractive range of molecular markers that could be used as prognostic markers, predictors of response to chemo- or radiotherapy. The aim of the clinicians and scientists is then to characterize the tumors by their molecular profile and to individualize therapies. It is a challenge to investigate the role of these markers as potential clinical targets. It is well known that bladder tumors with the same stage and grade have a heterogeneous clinical

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outcome as they are probably molecularly different. The term of superficial or invasive bladder tumor is confusing as it implies that only one kind of superficial or invasive bladder cancer exists. To understand how these tumors arise, it is going to be very important to define the role of various genes involved in tumorigenesis and tumor progression.

Epithelial-mesenchymal transition (EMT) has emerged as a critical process during cancer progression in which down-regulation or loss of E-cadherin expression (epithelial marker) constitute a molecular hallmark and may be accompanied by N-cadherin neo-expression (mesenchymal marker) [2,3].

The transcriptional repression by repressors such as snail or slug is one mechanism of the E-cadherin expression down-regulation. Recently, it has been postulated that Twist, another promoter repressor of CDH1 (E-cadherin gene), may be involved in tumor progression by silencing E-cadherin expression and EMT induction [4,5].

Twist is considered as a promoter of the EMT, which is a key event in the tumoral invasion step. During this process, the cancer cells lose their interactions with the neighboring cells, change their phenotype (epithelial to mesenchymal), and become able to invade locally and to metastasize.

In this study, we investigated 70 primary bladder tumors for the expression of the E- and N-cadherin and Twist, and tried to find out a correlation between these protein expressions but also to evaluate their prognostic significance by immunohistochemical analysis. Considering our results, we showed that Twist overexpression was inversely correlated to the E-cadherin level but we failed to show any correlation with the N-cadherin neo-expression. Both Twist and E-cadherin were associated with several clinicopathological parameters and may be considered as prognostic factors for survival. We also noticed a correlation between the smoking status of the patients and Twist expression.

2. Materials and methods

2.1. Patients

The study included 70 patients with a primary bladder tumor. The tissues were obtained from patients who had undergone a transurethral resection or a partial/total cystectomy between 1999 and 2001 at the Urology Department, Besancon University Hospital, France. None of the patients had received preoperative treatment. All patients were classified according to the 1997 UICC TNM classification for the stage and OMS 2004 for the grade (LMP: low malignant potential; LG: low grade; HG: high grade). Immunostaining was evaluated by 2 independent pathologists (B.K. and M.E.F.) to validate the diagnosis. Each sample was used after written consent was obtained from the patients. The clinicopathological data of the tumors are shown in Table 1. Tumor recurrence/progression was defined based on clinical, radiological, or histological diagnoses.

Table 1
Clinicopathological data of the bladder tumors

Characteristics	Data (percentage)
Median age (range), y	69 (49–84) [female: 68; male: 72]
Sex	
Male	52 (74%)
Female	18 (26%)
Ratio M/F	2.8
Stage	
Ta	35 (50%)
T1	18 (27%) [4pT1a; 14pT1b]
≥T2	17 (23%)
Grade	
OMS 2004	
LMP	12 (17%)
LG	24 (34%)
HG	34 (49%)
Carcinoma in situ	14 (40%) [7pT1; 7 ≥ pT2]
Lymph nodes around the bladder	4/10
Surgical procedure	
TUR	57
Cystectomy	13 (3 partial, 10 total)
Recurrence	19 (27%)
pTa	13 (68%) [4 LMP; 8 LG; 1 HG]
pT1	5 (36%) [1 pT1aHG; 1 pT1bLG; 3 pT1bHG]
≥pT2	1 (6%) [HG]
Progression	11 (16%)
pTa	2 (18%) [LG]
pT1	5 (45%) [HG]
≥pT2	4 (37%) [1 LG; 3 HG]
Death	33 (47%)
pTa	11 (33%) [2 LMP; 5 LG; 4 HG]
pT1	11 (33%) [pT1aLG; pT1bLG; 7pT1bHG]
≥pT2	11 (33%) [2 LG; 9 HG]

TUR; transurethral resection; LMP: low malignant potential; LG: low grade; HG: high grade.

2.2. Immunohistochemistry

Immunohistochemistry was performed to evaluate the altered expression of proteins involved in the bladder cancer invasiveness, namely E- and N-cadherin, and Twist. We chose representative paraffin sections of primary bladder tumor tissues from 70 patients, previously fixed in Formol 4%. We used tissue sections of 4 μm on gelatinized slides for the common hematoxylin-eosin staining and the anti E- and N-cadherin antibodies application and silanized slides for the anti Twist antibody application. All steps of the immunohistochemistry were done using the Benchmark Automate (Ventana, Tucson, AZ) according to the manufacturer's instructions. The slides were incubated in the automate with an antibody against N-cadherin (monoclonal, clone 3B9; Zymed Laboratories Inc., Montrouge, France, dilution 1/200), E-cadherin (monoclonal, clone 4A2C7, Zymed, dilution 1:40) and Twist (polyclonal, clone H81; Santa Cruz Biotechnology Inc., San Diego, CA, dilution 1:50). Then, the slides were manually dehydrated and mounted. Positive controls included normal urothelium from patients with benign urological disease for the E-cadherin staining, a high