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Research Article

Urokinase gene 3'-UTR T/C polymorphism is not associated with bladder cancer

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Abstract

Urokinase degrades basement proteins and is hypothesized to play a role in cancer progression. We investigated the hypothesis of C/T polymorphism in the 3'-untranslated region (3'-UTR) of the urokinase gene being associated with the development of bladder cancer. Such an association seems unlikely, since the genotype distributions in 114 bladder cancer patients did not differ from those of 105 controls.

Key words: urokinase, bladder cancer, single nucleotide polymorphism.

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Introduction

Gene polymorphisms have been found to be associated with the susceptibility to bladder cancer or its severity, such as in the p53 and p21 genes (Chen *et al*., 2000; Chen *et al*., 2002). Disclosing other genes associated with bladder cancer could provide further testing for the identification of individuals at risk, improving preventive medicine and, ultimately, appropriate treatment for subtypes of the disease.

Urokinase, a plasminogen activator, cleaves plasminogen into plasmin, hence stimulating fibrinolysis and degrading major basement membrane glycoproteins, such as fibronectin and laminin (Stump *et al*., 1986; Liotta *et al*., 1981; Salo *et al*., 1982). Therefore, urokinase appears to play a role in tumor invasion and progression. Indeed, the expression of urokinase plasminogen activator and its receptor has been reported to be associated with the clinical course of bladder cancer (Seddighzadeh *et al*., 2002), and elevated levels of urokinase have been described in bladder tumors and other malignancies (Testa and Quigly, 1990; Sappino *et al*., 1987; Hasui *et al*., 1994; Schlechte *et al*., 1989). Urokinase is therefore hypothesized to be a useful marker for predicting tumor progression and recurrence.

The urokinase gene is located at chromosome 10q24. Polymorphisms include a C/T transversion at the 3' UTR (+4065 nucleotide) described by Tripputi *et al*. (1985), and a C/T substitution in exon 6 and a T/C substitution in intron 7 (Conne *et al*., 1997). Herein, we investigated if the 3' UTR T/C substitution is associated with bladder cancer, one of the most common urological malignancies in Taiwan.

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Patients and Methods

A total of 110 patients with bladder cancer (78 males and 32 females, age range 42-80 years; mean 65.8 ± 12.2 years) from the central area of Taiwan and attending the China Medical College Hospital were enrolled in this study. All patients had transitional cell carcinoma (TCC) and were classified into invasive and non-invasive cancer groups, according to pathological grading and clinical course. There were 61 patients with non-invasive tumors (Ta and T1 according to the American Joint of Cancer Committee, AJCC) and 49 patients with invasive tumors (T2a and T2b following AJCC staging). The control group consisted of 105 healthy volunteers from the same city area (65 males and 40 females; age range 40-73 years; mean 54.7 ± 10.4 years) who had no family history of stone disease or cancer. They were submitted to renal ultrasonography and routine tests for urinary microscopic hematuria, in order to exclude renal calcification. Informed consent was obtained from all participants. The genomic DNA was obtained from peripheral blood, using the Genomaker DNA Extractor kit (Bloosm, Taiwan). Polymerase chain reaction (PCR) was carried out in a total volume of 50 μL, containing 50 ng genomic DNA, 2-6 *p*mole of each primer,

1X Taq polymerase buffer (1.5 mM $MgCl_2$), and 0.25 units of AmpliTaq DNA polymerase (Perkin Elmer, Foster City, U.S.A.). The primers were designed according to STS Accession No. G27040: 5'-CCGCAGTCACACCAAGGA AGAG-3' and 5'-GCCTGAGGGTAAAGCTATTGTC GTGCAC-3'. The cycling conditions were as follows: one cycle at 94 °C for 5 min, 35 cycles at 94 °C for 30 s, 58 °C for 30 s, 72 °C for 40 s, and one final extension cycle at 72 °C for 7 min. The 210-bp PCR product was digested with 2 units of *ApaL* I (New England Biolabs, Beverly, U.S.A.), yielding two fragments of 185-bp and 25-bp for allele T, resolved after agarose gel electrophoresis. Considering that the 25bp fragment could be lost, the results were confirmed by direct sequencing of the purified PCR products (QIAEX II kit, Qiagen, Germany), using a dRhodamine DyeDeoxy Terminator Sequencing kit (PE Applied Biosystems, Foster City, CA) in an ABI Prism 377 DNA Sequencer (PE Applied Biosystems). Genotype frequencies were compared in contingency tables, using either the chi-square or Fisher's exact test.

Results and Discussion

The frequencies of the genotypes in bladder cancer patients and controls are shown in Table 1. No significant difference was detected between the two groups ($\chi^2 = 1.222$; df = 1; p = 0.27). When patients were compared according to tumor grading (Table 2), there was a statistically significant difference between groups I and II (p = 0.029, Fisher's exact test). However, when group I was compared to (II + III), a nonsignificant p figure was obtained (p = 0.088; Fisher's exact test). Therefore, our data do not support the hypothesis that CT heterozygosis is a risk factor for poorer tumor grading. In addition, no difference was found between patients with invasive and with non-invasive tumors ($\chi^2 = 0.173$; df = 1; p = 0.677) (Table 2).

Taken together, these findings are not indicative of an association of the urokinase gene 3'-UTR C/T polymorphism with the occurrence of bladder cancer, although the possibility of an increased risk for tumors in heterozygotes to develop a poorer differentiation deserves to be further investigated.

Table 1 - Genotype frequencies of urokinase gene 3'-UTR T/C polymorphism in patients with bladder cancer and in normal individuals.

Individuals	Genotypes			Total
	CC	CT	TT	
Cancer patients	104	8	0	110
Controls	101	4	0	105

Table 2 - Genotype frequencies of 3'-UTR T/C polymorphism of the urokinase gene in patients with bladder cancer, according to pathological grading and clinical staging.

	Genotypes		
	CC	CT	Total
Tumor grade (AJCC)			
I	22	4	26
II	58	1	59
III	22	3	25
Clinical Staging			
Non-invasive	56	5	61
Invasive	46	3	49

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