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Analysis of genetic events in 17p13 and 9p21 regions supports predominant monoclonal origin of multifocal and recurrent bladder cancer

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Summary

Clonality was tested in 86 tumours from 25 patients with recurrent and multifocal superficial bladder transitional cell carcinomas (TCCs) using the analysis of TP53 mutations and of LOH in the 17p13 and 9p21 regions. Tumours from the majority of individuals showed either absence or presence of the same TP53 mutation and/or an identical LOH pattern, with the same allele lost in all tumours. Only two pairs of tumours from two patients had discordant findings, which were incompatible with monoclonality. Therefore, our results rather support the monoclonal model of development of highly recurrent superficial bladder TCCs.

Keywords: Transitional cell carcinoma (TCC); Bladder cancer; Clonality; Recurrent cancer; LOH; TP53 mutations

1. Introduction

Bladder cancer is the second most common malignancy of the genitourinary tract with a male to female ratio of 3:1 [1]. About 90% of all bladder cancers are transitional cell carcinomas (TCCs) derived from the urothelium, and more than 80% of them are superficial, non-muscle-invasive tumours (classified as Ta, T1 or Tis). Approximately 70% of all patients with superficial bladder TCCs develop recurrences after transurethral resection (TUR), and in 10–20% of patients the progression to muscle invasion (T2–T4) occurs [2]. Nearly 30% of all patients present at diagnosis with multifocal disease—simultaneous occurrence of several spatially distinct tumours at different sites of the bladder wall [3].

Simultaneous or metachronous development of multiple superficial bladder TCCs evokes the question of possible monoclonal nature of these tumours. During the last years, two hypotheses have been proposed: the monoclonal hypothesis, and the field cancerization hypothesis. According to the monoclonal model, the progeny of a single malignant cell proliferates and spreads throughout the urothelium either via intraluminal seeding (when the transformed cell is fully released from the primary tumour), or via intraepithelial migration of the malignant cell. On the contrary, the field cancerization model proposes independent transformation of

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numerous urothelial cells at multiple sites as a result of accumulation of carcinogenic events, leading to the growth of multiple unrelated tumours [4].

Various molecular genetic methods or their combinations can be used to determine the clonality of multiple synchronous or recurrent tumours in one patient including X-chromosome inactivation analysis, molecular cytogenetic techniques (FISH, CGH), loss of heterozygosity (LOH) analysis, and single-gene mutation analysis [5–11]. Several studies published up to now presented evidence both for and against the monoclonal model of bladder cancer, but the majority of molecular studies favoured the monoclonal origin of multiple tumours in one patient. Most of these studies concentrated on advanced-stage invasive carcinomas [5–8]. On the other hand, several studies, which found evidence for the existence of more than one tumour clone, particularly in the early stages of bladder carcinoma, supported the field cancerization hypothesis [9–11].

We attempted to address this question by the analysis of genetic events in the 17p13 and 9p21 regions of the human genome, harbouring the TP53 and CDKN2A genes known to play a role in bladder tumorigenesis [12], in a large series of well characterized multifocal and recurrent superficial bladder TCCs. TP53 gene mutations and LOH at several TP53 and CDKN2A intragenic and extragenic polymorphic DNA markers were analysed in a total of 86 tumours from 25 patients sampled during a period of 5 years. The patients were selected for highly recurrent disease, and the study was particularly focused on the analysis of the genetic profile of the early stages of the disease. Our results support the monoclonal model of development of multifocal and recurrent superficial bladder TCCs.

2. Materials and methods

2.1. Patients and samples

The study comprised 25 patients with Ta or T1 bladder TCCs (22 males and three females, mean age 69.8 years) treated with TUR in the period from April 2000 to May 2002 in whom one or more tumour recurrences during the follow-up period (till December 2004) appeared (Table 1). DNA testing was performed after obtaining of informed consent from each patient. TUR was performed using standard techniques with the removal of all visually identifiable tumour tissue. After TUR, the patients were followed and treated according to the current convention and depending on the general health status and age of each individual patient (in G3 and multiple T1G2 tumours intravesical bacillus Calmette-Guerin immunotherapy and in TaG2 or multiple G1 tumours intravesical chemotherapy were administered). Tumour progression was defined as the development of muscle invasive disease, and disease generalization as the appearance of distant metastases.

In total, tissue samples from 86 different tumours were obtained. Blood samples were available from all patients for the analysis of marker informativity. Tumours were classified according to the TNM system [13]: 50 superficial urothelial tumours were classified as Ta, 32 tumours as T1, and four recurrent tumours were muscle invasive (three T2 and one T3). Tumour grading was performed according to the criteria defined by the WHO International Classification of Tumours [14]: 21 tumours were G1, 58 tumours were G2, and seven were G3 (Table 1). The tumour samples were frozen in liquid nitrogen and stored at −70 °C until DNA extraction.

2.2. Analysis of TP53 mutations and of LOH in the 17p13 and 9p21 regions

DNA isolation from the peripheral blood lymphocytes and from the frozen tissue samples was performed using the QIAamp DNA Blood Midi Kit and QIAamp DNA Mini Kit (Qiagene). TP53 mutations were screened in exons 5–9 using direct sequencing of PCR products as described previously [15]. LOH analysis was performed for at least one of seven polymorphic DNA marker loci in the TP53 gene region of 17p13, and for at least one of three markers in the CDKN2A gene region of 9p21 (Table 2). Genotypes for TP53 codons 72 and 213 were analysed by restriction digestion [16,17]. The other markers were microsatellites (STRs), and their typing was performed using capillary electrophoresis with laser-induced fluorescence detection. FAM-labelled primers were used for typing of the 9p21 markers. Amplification of the 17p13 markers was performed with non-labelled primers, and the resulting unlabelled PCR products were detected on a multicapillary sequencer using counter-current migration approach in which intercalation takes place upon contact of the DNA fragments and opposite-migrating SYBR Green II dye molecules [18]. The STR polymorphism in polyA tail of one of the Alu sequences in intron 1 of the TP53 gene [19] was amplified by a semi-nested PCR. LOH was determined by the comparison of the genotypes from blood lymphocyte and tumour DNAs in individual patients.

2.3. Statistical analysis

The correlation between the presence of genetic alterations on 17p13 or 9p21 and disease progression or disease generalization was analysed by the Fisher's factorial test.

3. Results

3.1. Clinical course of the disease

During the course of the study, five patients out of the total of 25 (20%) showed tumour progression and