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TERT Core Promotor Mutations In Early-Onset Bladder Cancer

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Key words: bladder cancer, early-onset, TERT, mutation, sequencing

Abstract:

Activating mutations in the core promoter of the TERT gene have been described in many different tumor entities. In vitro models showed a two- to fourfold increase in transcriptional activity of the TERT promoter through creation of a consensus binding motif for Ets/TCF transcription factors caused by these mutations. TERT core promoter mutations are the most common mutations in bladder cancer with a frequency between 55,6% and 82,8% described so far, and are independent of stage and grade. Since only few data on molecular alterations of early-onset bladder tumors exist, we assessed the frequency of TERT core promoter mutations in early-onset bladder cancer. Two cohorts of bladder tumors (early-onset patient group; n=144 (age of onset of disease \leq 45 years); unselected, consecutive group; n=125) were examined for TERT core promoter mutations. After microdissection and extraction of DNA the corresponding hot-spot-regions in the TERT core promoter were examined by Sanger-sequencing or a SNaPshot approach. A significantly lower frequency of TERT core promoter mutations was found in tumors from the early-onset cohort compared to the consecutive cohort (57,6% vs. 84,8%, $p < 0,001$). Among the early-onset cohort cases younger than the cohort's median age of 39 years at disease onset showed a significant reduced number of TERT promoter mutations (31/67, 46,3%) than cases aged between 39 and 45 years (52/77, 67,5%; $p = 0,012$). This association was not found in the consecutive cases. Mutation status was independent of tumor stage and grade. We conclude that in tumors from early-onset bladder cancer patients TERT core promoter mutations are not as frequent as in bladder tumors from consecutive cases, but seem to play an important role there as well. In patients below 39 years of age TERT core promoter mutations are a more infrequent event, suggesting different mechanisms of tumorigenesis in these young patients.

Introduction

The TERT gene encodes for the catalytic reverse transcriptase subunit of telomerase, a RNA-dependent polymerase (ribonucleoprotein complex) maintaining telomere length, and preventing replicative senescence and genomic instability. In stem cells telomerase activity is crucial for indefinite replication and the limitless replicative potential associated with increased telomerase activity is one of the six hallmarks of cancer [1].

Recently, activating TERT core promoter mutations were described in familial and sporadic melanoma [2], and could since then be demonstrated in a multitude of sporadic tumors [3]. The TERT core promoter consists of 330 bp upstream the start-codon and of 37 bp of exon 2, and shows mutational hotspots at positions -57, -124 and -146. Mutations in these locations lead to a nucleotide change creating a novel binding motif for E-twenty-six (Ets) transcription factors. In addition, a binding motif for the ternary complex factors (TCFs, which are a subfamily of Ets transcription factors) Elk1 and Elk4 is also generated by these alterations. Elk1 and Elk4 are downstream targets of BRAF and involved in regulating expression of many genes. Creation of these de novo Ets/TCF-binding motifs TERT leads to an increase in TERT promoter activity and gene expression [2].

Several studies shed light on the frequency of TERT core promoter mutations in urothelial carcinoma the bladder. TERT core promoter mutations were found with a frequency up to >80% in bladder cancer [4] and are the most common mutations in bladder cancer known by now. Interestingly, TERT mutations showed neither a correlation to tumor stage or tumor grade, nor to disease outcome, but were suggested as a useful biomarker in the follow-up of bladder cancer patients or for proving the urothelial origin in cancers of unknown primary [5-7].

It is well known that exogenous noxae like exposition to amines, smoking, drugs and radiation therapy are associated with the development of bladder cancer, which is typically a tumor of older people with a mean age of diagnosis being between 65 and 70 years of age. Only a small fraction (approx. 1,5%) of bladder tumors develop before the age of 45 years [8]. These tumors make an interesting cohort, since tumorigenesis in such “early-onset” bladder tumors might be due to a different pathway, or crucial molecular alterations responsible for initiation of urothelial carcinogenesis in general might be discovered by analyzing this cohort. Since only very limited data regarding molecular alterations of early-onset bladder tumors exist, we thought it reasonable to assess the frequency of TERT core promoter mutations in early-onset bladder tumors, maybe even corroborating further incidence for a different tumorigenesis pathway in early-onset bladder tumors.

Methods

Tissue samples:

Overall, archival formalin-fixed paraffin-embedded (FFPE) tumor material of 269 cases acquired through transurethral resection of bladder tumors were used for the study (cohort 1: unselected cases: n=125, cohort 2: early-onset-bladder cancer cases (age at time of diagnosis ≤ 45 years): n=144). The tumors were classified and staged according to the WHO classification of bladder tumors [9] and the current AJCC/TNM-classification system [10].

Clinicopathological characteristics of the cases are shown on Table 1. Prior IRB approval (University Hospital Erlangen, Germany) was obtained for the study.

Microdissection and DNA isolation:

Microdissection and isolation of genomic DNA was carried out from FFPE tumor material as described previously [11]. In brief, 5 μm thick serial sections of the tumor tissue were dewaxed and stained with 0.1% methylene blue for 15 seconds. Using an inverted microscope the tumor tissue (identified through matching with a marked HE-stained section reviewed by an experienced surgical pathologist) was scraped off with a sterile needle to obtain a purity of the cells of at least 85%. Isolation of genomic DNA of the microdissected tumor tissue was performed using the High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany) according to the manufacturer's instructions.

TERT promoter mutation analysis:

Mutation analysis of the TERT promoter was performed as described recently. In brief, Sanger-sequencing of the TERT core promoter was done using an ABI Prism

3500 Genetic Analyzer and the Big Dye Terminator v.1.1 Cycle Sequencing Kit (both Applied Biosystems, Foster City, Calif., USA) according to manufacturer's instructions. With DNA isolated from FFPE tissue not always being suitable for Sanger sequencing in cases with non-interpretable results, a SNaPshot assay was used for TERT promoter mutation analysis focusing the three mutations hotspots at -146, -124 and -57. All primers and reaction conditions were described elsewhere in detail [2, 12].

Statistics

A two-sided Fisher exact test was used to evaluate differences in the distribution of TERT mutation status and age of the patients between both cohorts. Statistical analysis was done using SPSS version 13.0 (SPSS, Chicago, IL, USA). P values less than .05 were interpreted as statistically significant.

Results

All investigated cases could be successfully analyzed by Sanger sequencing (n=16) or SNaPshot analysis (n=253) (Figure 1). Overall, there was a significant lower frequency of TERT promoter mutations in the tumors from the early-onset bladder cancer patients (83/144, 57,6%) compared to the unselected, consecutive cohort (106/125, 84,8%; $p < 0,001$; Figure 2). The distribution of the detected TERT promoter mutations in our cohorts was very similar to the results of previously published bladder cancer studies [4, 5] with the majority of the mutated cases (92%) showing the -124C/T or the -146C/T mutations (Table 2). In order to find a possible age dependency of the occurrence of TERT promoter mutations in the tumors from the early-onset bladder cancer cohort we stratified this cohort against the median age (39 years) of the cohort. There was a significant lower frequency of TERT promoter mutations in patients aged <39 years at time of first diagnoses compared to patients ≥ 39 years ($p = 0,012$; Figure 3). No further association was found between TERT mutation and clinico-pathological characteristics of the tumors.

Discussion

In the presented study we analysed TERT promoter mutations in a large cohort of tumors from patients with early-onset disease for the first time, and compared these data to the TERT promoter mutation frequency from an unselected consecutive group of bladder tumors. Data from our consecutive cohort are in line with previously published studies [4-6] and argues for the reliability of the used methods and for a non-biased control group. Interestingly, there was a significant lower frequency of TERT promoter mutations in the tumors from the early-onset patients. This fact points to a possible age-dependent occurrence of TERT promoter mutations in bladder cancer or a different molecular background in early-onset bladder tumors. An age-dependent difference in TERT mutation frequency in bladder cancer was recently observed in two studies, too. Wu and coworkers found a significantly lower mutation frequency in tumors from patients aged younger than 50 years [6]. In this study tumors from patients below 50 years of age showed a mutations frequency of 37,5% vs. 59,6% mutation frequency from the patients ≥ 50 years. Unfortunately, the authors analysed only a small number of tumors from “younger” patients below 50 years of age (n=40) and they also did not comment on the used cut-off of 50 years of age. But despite these limitations data from Wu and colleagues are in line with the results from our study. A study from Wang et al focused on TERT promotor mutation analysis of urothelial tumors from the upper urinary tract [13]. Within a cohort of ureter tumors the mean age of patients with mutated tumors was significantly higher than the mean age of patients with non-mutated tumors (72,4 years vs. 65,1 years). Although both cohorts showed a mean age typical for the regular onset of urothelial tumors the shift towards a lower TERT promoter frequency in an age-dependent manner is remarkable and also strengthens our findings. Interestingly, an age-dependent TERT promoter mutation frequency was also reported from papillary thyroid carcinoma

recently. Liu and coworkers found a significant higher mutation frequency in patients aged >45 years compared to patients aged ≤45 years [14]. In addition, a study on medullablastomas reported TERT mutations to be significantly more frequent in an older cohort of pediatric patients (median age: 16 years) compared to a very young patient cohort (median age: 6 years) [15]. All these data gave evidence for a minor role of TERT promoter mutation in tumors from patients with early-onset disease.

It is difficult to explain this age-dependency. Focusing on bladder cancer it could be shown recently that TERT promoter mutations lead to increased levels of TERT mRNA, TERT protein, telomerase enzymatic activity and telomere length in vitro [16]. Moreover, increased levels of TERT mRNA were shown to be associated with a reduced disease-specific survival suggesting high levels of TERT mRNA as a marker for biological aggressiveness in bladder cancer [16]. Our findings of a low TERT promoter mutation frequency in bladder tumors from young patients fits well to this observation as bladder tumors in young patients showed a low biological aggressiveness, and patients with early-onset disease displayed a favorable course of disease in most cases investigated [17, 18].

TERT promoter mutations were described as being frequently concomitant with FGFR3 mutations in bladder tumors especially in tumors with low grade and low stage [19]. Based on the limited molecular data available on early-onset bladder tumors FGFR3 mutations are a rare event bladder tumors from young adults [20, 21]. As FGFR3 and TERT promoter mutation being the most frequent alterations in bladder cancer the low frequency of both mutations in tumors from patients with early-onset disease argue for a specific and discrete molecular background of tumorigenesis in these patients. Own studies on epigenetic changes in bladder tumors underlined a discrete molecular background in these cases as patients below

19 years of age showed a low rate of epigenetic alterations compared to elderly patients [22].

Taken together, this first report on TERT promoter analysis in bladder cancer from patients with early-onset disease showed a lower mutation frequency as reported from patients with regular disease-onset. These data further strengthen the suggestion of a distinct molecular tumorigenesis in young bladder cancer patients.

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Competing Interests

The authors have declared that no competing interest exists.

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Figure legend

Figure 1: Representative examples for TERT promoter mutation analysis. **A:** Sanger sequencing showing a -124 “C => T” mutation. **B:** SNaPshot-analysis with wildtype-sequence for -124 “C” and -146 “C” and typical mutation at -124 and -146 (arrows).

Figure 2: Distribution of TERT promoter mutations between early-onset bladder cancer cohort and the consecutive cohort.

Figure 3: Distribution of TERT promoter mutation within the early-onset bladder cancer cohort stratified according to cohort’s mean age.

Table 1: Clinico-pathological characteristics of the study cohorts

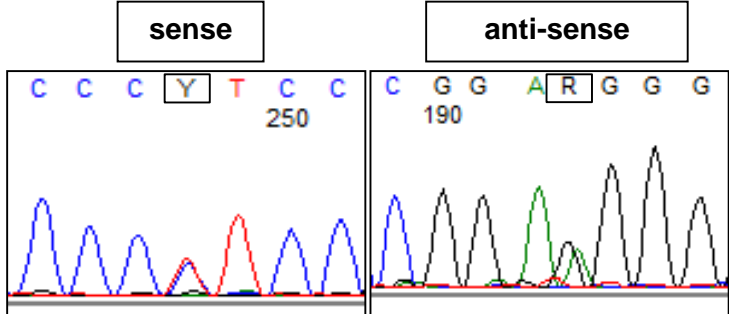
	Consecutive Cohort		Early-Onset Tumor Cohort	
Number	n=125		n=144	
Age (years)	Median: 71 Mean: 70,7	Range: 29-94	Median: 39 Mean: 36,6	Range: 11-45
Stage	Papilloma Cis Ta T1 ≥T2 unknown	n=2 n=1 n=78 n=35 n=8 n=2		n=5 n=0 n=83 n=14 n=27 n=16
Grading	G1 G2 G3 unknown	n=33 n=56 n=17 n=19		n=43 n=48 n=30 n=24
Gender	Male Female unknown	n=95 n=30 n=0		n=100 n=24 n=21

Table 2: Distribution of the TERT core promoter mutations in the two study cohorts.

Distance from ATG start site (bp)	Base change	Consecutive Cohort (n)	Early-Onset Tumor Group (n)
-57	A > C	6	0
-124	C > T	75	52
-124	C > A	2	1
-146	C > T	22	24
-57 and -124	A > C and C > T	1	0
-124 and -146	C > T and C > T	0	5
-124 and -146	C > A and C > T	0	1

Figure 1:

A



B

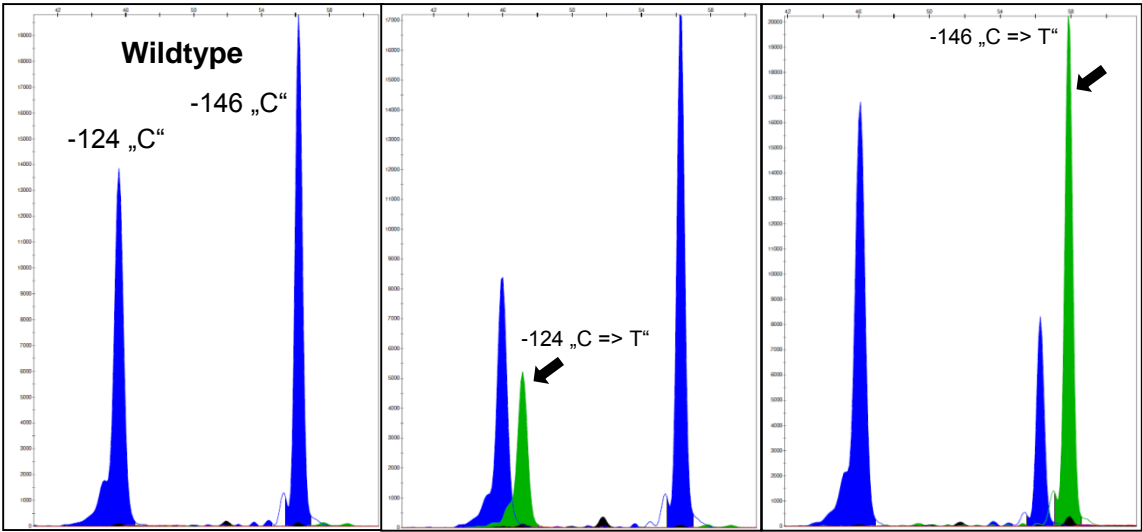


Figure 2:

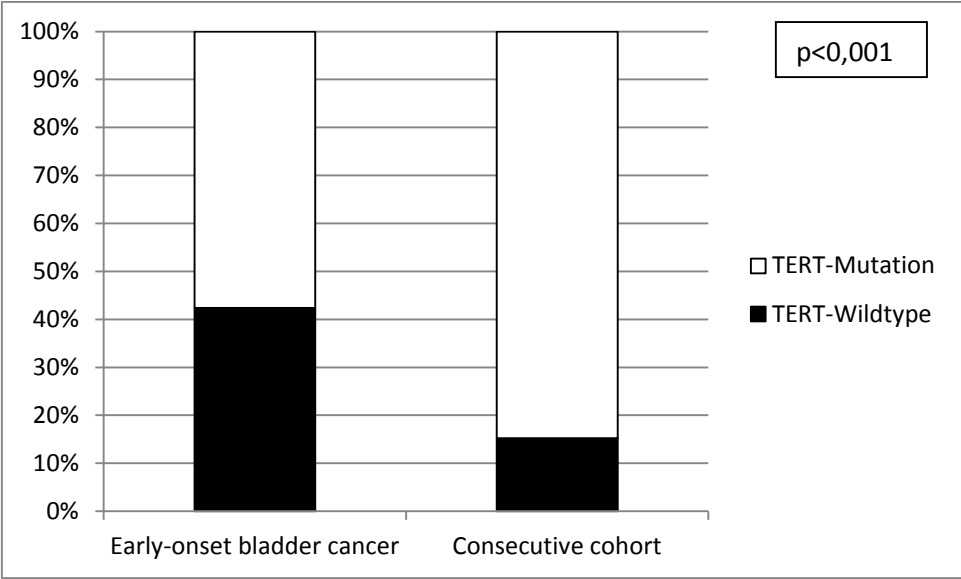


Figure 3

