Frequency of TERT promoter mutations in prostate cancer

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Abstract

Objective: Recently, recurrent mutations within the core promoter of the human telomerase reverse transcriptase (TERT) gene generating consensus binding sites for ETS transcription factor family members were described in melanomas and other malignancies (e.g. bladder cancer, hepatocellular carcinoma). These mutations were discussed as early drivers for malignant transformation.

In prostate cancer (PrCa) TERT expression was associated with a poor prognosis and a higher risk for biochemical recurrence. The underlying mechanisms for high TERT expression in PrCa are still not clarified in detail. To date, data on TERT promoter mutation analysis in PrCa are sparse. Therefore, we performed sequence analysis of the core promoter region of the TERT gene in an unselected cohort of prostate tumors.

Methods: Sections from 167 formalin-fixed, paraffin-embedded and cryopreserved prostate tumors were used for DNA isolation. After precise microdissection the mutation hotspot region within the TERT core promoter (-260 to +60) was analysed by direct Sanger sequencing or SNaPshot analysis.

Results: All cases could be analysed successfully. Mutations within the core promoter of the TERT gene could not be detected in any of the cases. All tumors showed wildtype sequence.

Conclusion: TERT core promoter mutations reported from several other malignancies could not be detected in our unselected cohort of prostate cancers. These data indicate that alterations within the core promoter of the TERT gene play no important role in prostate carcinogenesis.

Introduction

The search for recurrent mutations in single genes within tumor genomes is driven by the hope of uncovering important driver alterations that lead to a better understanding of carcinogenesis. The availability of new and sensitive high-throughput sequencing technologies sped up this search and led to the identification of new mutation hotspots in a variety of cancer entities. Although a new definition of a recurrent gene mutation was suggested in terms of combining recurrent alterations in the scale of a pathway rather than in the scale of only one single gene, discovering new recurrent gene mutations is still a worthwhile approach in cancer research [1].

Recently, mutations within the promoter of the telomerase reverse transcriptase (TERT) gene that encodes the catalytic subunit of the telomerase were identified in familial and sporadic melanoma with a high frequency [2]. The immortality of cells is still a classical hallmark of tumors and reactivation of telomerase leading to telomere maintenance remains a fundamental process in carcinogenesis. Alterations within the coding region of the TERT gene are a rare event in cancers. Therefore the identification of recurrent mutations within the core promoter of the TERT gene leading to new binding motifs for transcriptions factors of the ETS family attracted great interest among the cancer research field [3]. The consequences of these mutations are still not completely understood but they lead to a 2-4 fold increased transcriptional activity in vitro [4]. Subsequently, these mutations were found in several other malignancies, e.g. bladder carcinoma, thyroid cancer or cancers of the nervous system, and were discussed as early drivers for malignant transformation [3].

Expression and reactivation of the telomerase were also described as important features of prostate cancer (PrCa). Telomerase activity was found in up to 100% of analysed PrCa cases [5]. Interestingly, high expression of telomerase components not always results in mandatory telomerase activity [6]. In addition, significant associations between TERT expression and aggressive behavior of prostate tumors have been reported [7]. Recently promising in vitro data were published showing both telomerase as an important target of an anti-androgen therapy for PrCa, and the usefulness of boron derivatives as a telomerase inhibitor in PrCa cells [8, 9]. These data suggest telomerase inhibition as a reasonable approach for a PrCa therapy. The molecular and cellular pathways involved in telomerase reactivation in PrCa are still not clear. Expression of TERT and the activity of telomerase were shown to be regulated by androgen receptor (AR) signaling whereas exogenous expression of AR surprisingly led to inhibition of TERT transcription in PrCa cells [10, 11]. The genomic region of the TERT gene (chromosome 5p15.33) was not described as a region containing copy number alterations in prostate tumors making gene amplification as a reason for TERT expression in PrCa unlikely [12]. Less is known about TERT promoter mutations in PrCa. To date only three studies with a combined number of 49 prostate tumors reported a sequence analysis of the TERT promoter and found no evidence for involvement of TERT promoter mutations in PrCa [13-15]. These data already indicate that the cellular mechanisms of telomerase reactivation in PrCa are only poorly understood and further clarification is needed. As TERT promoter mutations are a potential mechanism for a possible telomerase reactivation we wanted to further the discussion of this topic for PrCa. We therefore analysed the core promoter region of the

TERT gene containing the reported mutation hotspots in the largest series of PrCa reported so far.

Materials and methods

Patients and Tissue Samples

Overall, 167 unselected, archival prostate tumors (formalin-fixed and paraffin-embedded tissue samples: n=119; snap-frozen tissue samples: n=48) were investigated. All patients were Caucasians. The tumors were diagnosed according to the WHO classification of prostate tumors and staged according the TNM system [16, 17]. Characteristics of the study participants are shown in Table 1. Prior institutional review board (University Hospital Erlangen) approval was obtained for molecular analysis on archival material.

Tissue microdissection and DNA isolation

DNA was extracted from prostate tumors after precise manual microdissection (purity of tumor cells >85%) of serial sections (5µm) using the High Pure PCR Template Preparation Kit (Roche GmbH, Mannheim, Germany) according to manufacturer's instructions. DNA quality and quantity was determined using the Synergy2 Multi-Detection Reader (BioTek, Bad Friedrichshall, Germany) according to the manufacturer's instructions.

TERT promoter analysis using Sanger sequencing

One part of the core promoter (-260 to +60) of the TERT gene containing the described mutation hotspots was amplified by PCR using primers (sense: 5'- att cgc ggg cac aga cgc -3'; antisense: 5'- tcg cgg tag tgg ctg cgc -3') obtained from Metabion (Martinsried, Germany) in a total volume of 25 µl containing approx. 150 ng DNA, 0.2 mM dNTP (Promega), 0.18 µM primers, 5% DMSO and 0.0025 U/µl GoTaq (Promega, Mannheim, Germany). The thermal cycling conditions were as follows: initial denaturation for 3 min at 95℃, 45 cycles of denaturation at 94℃ for 1 min, annealing at 69.3℃ for 1 min, elongation at 72℃ for 1 min and final primer extension at 72℃ for 10 min. Gradient PCR was used for optimization of cycling conditions. After amplification, PCR-products (size: 335 bp) were purified using the Qiagen Dye Ex 2.0 TM Spin Kit according to the manufacturer's conditions. Sequence analysis was performed with PCR primers using Applied Biosystems Big Dye Terminator v1.1 Cycle Sequencing Kit and an Applied Biosystems ABI 3500 Genetic Analyzer.

TERT promoter analysis using SNaPshot analysis

A previously reported SNaPshot assay (Life Technologies Corp., Carlsbad, CA, USA) was used for detection of hotspot mutation at positions -57, -124 and -146. Capillary electrophoresis and detection of fluorescence-labeled products were performed using an Applied Biosystems ABI 3500 Genetic Analyzer. A detailed description of the method can be found elsewhere [18].

Results

The analysis of the most frequent mutation hotspots within the core promoter of the TERT gene was successful in all available cases. Mutation analysis using Sanger sequencing could be performed in 108/167 cases. (Figure 1) In 59/167 cases the core promoter region could not be amplified in one part due to insufficient DNA quality (e.g. DNA degradation, low DNA concentration). In these cases SNaPshot analysis of mutation sites at -57, -124 and -146 could be performed (Figure 1). In none of the analysed cases a promoter mutation could be found, all cases showed a wildtype sequence.

Discussion

In the presented study we performed sequence analysis of the core promoter region of the TERT gene in a comprehensive and representative cohort of PrCa samples. The results of our study suggest that TERT promoter mutations are not involved in the development of PrCa as no mutation was detected in any of the investigated cases. These data are in line with previously published studies on only very small cohorts and corroborate the minor importance of TERT promoter alterations in PrCa [13-15]. Meanwhile a study investigating the whole genomes of 57 PrCa cases was published [19]. Apart from already known data from exome analyses this study displayed the spectrum of whole-genome alterations in prostate tumors. Here only one TERT missense mutation was detected (p.R819C) but no promoter mutations were reported. This study also strengthens our findings and should together with our data finalize the actual discussion on TERT mutations in PrCa.

There are several lines of evidence that genomic variations but not mutation might influence TERT expression and disease risk in PrCa. In a large case-control study an intronic single nucleotide polymorphism in the TERT gene (rs2242652, C ⇔ T) was found that was strongly associated with increased PrCa risk. Because of this strong correlation it was suggested that the SNP might have a functional relevance. Indeed, further evaluation showed an increased TERT expression in bengin prostate tissue from patients who underwent radical prostatectomy depending on the SNP variants [20, 21]. This constant evaluated expression of TERT might provide a possible predisposition for PrCa. Another influence on TERT expression might be length polymorphisms in variable number tandem repeats (VNTR). Recently, it could be shown that the TERT gene

contains five VNTRs that are located within introns 2 and 6. A large case-control study found a significant higher prostate cancer risk for individuals carrying rare VNTR2-2nd alleles than for individuals with common alleles. These VNTRs were also discussed having an enhancer function for gene transcription. In vitro studies on PrCa cell lines analyzing the activity of the TERT promoter in combination with different VNTR variants clearly showed an increased luciferase activity for the VNTR2-2nd variants [22, 23]. These effects might also be expected for TERT expression and could also increase the individual risk for PrCa.

Beside these genomic influences TERT expression is also regulated by several cellular processes in PrCa. Matsamura and colleagues analysed the impact the phosphorylation status of Fas-associated death domain-containing protein (FADD) on TERT expression in PrCra [24]. FADD has a crucial role in the formation of the death-inducing signaling complex and is also involved in cell cycle regulation. The phosphorylated form of FADD was highly expressed in PrCa with lower Gleason score and was inversely associated with a shorter recurrence-free survival after prostatectomy. In parallel cases with high levels of phosphorylated FADD also showed only low TERT expression suggesting a direct influence of FADD-phosphorylation on TERT expression. Shimada and colleagues found significant differences between FADD phosphorylation levels and clinicopathological outcomes for Gleason score 3 + 4 and 4 + 3 [25]. These data indicate that Gleason 4 + 3 tumors should be considered as high-risk tumors and transition from non-phosphorylated to phosphorylated FADD stimulating agents (e.g. paclitaxel) might be added to different therapy options. As high TERT expression correlates with aggressive PrCa levels of TERT and non-phosphorylated FADD might

be potent biomarkers for the biological behavior of PrCa. Furthermore, different factors can regulate TERT expression positively or negatively. Several transcription factors (e.g. SP1), hormones (e.g. androgen) and the P13K/Akt and MAP kinase pathways can up- regulate TERT transcription (reviewed in [26]). In addition recently the down-regulation of TERT by six microRNAs (let-7g*, miR-133a, miR-138-5p, miR-342-5p, miR-491-5p, and miR-541-3p) has been reported [27].

Summing up high expression of TERT is unlikely caused by promoter mutations or other genomic alterations in PrCa. TERT expression is more likely caused by the influence of diverse cellular pathways and might display cell cycle activity and increased proliferation.

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Table 1: Characteristics of study patients

	PrCa-Cases	
Number:	n =167	
Age (years):	Median: 66	Range: 46-87
	Mean: 64,9 (± 6,7)	
Stage:	Organ-confined disease	n = 76
	Non-organ-confined disease	n = 89
	No data available	n = 2
Gleason Score:	Median: 7	Range: 3-10
Gleason Sum:	<7	n = 52
	=7	n = 54
	>7	n = 54
	No data available	n = 7

Figure legends

Figure 1: Representative examples for Sanger sequencing and SNaPshot analysis of the promoter mutation hotspots at -57 and -124. Upper lane: Sanger sequencing of DNA from prostate tumor showing wildtype sequence for TERT promoter position -57 (A) and position -124 (G). DNA from the melanoma cell line SK-MEL-28 showing “A => C” mutation at position -57. DNA from the bladder cancer cell line RT112 showing “G => A” mutation at position -124. Lower lane: corresponding SNaPshot analyses. Arrows indicate mutations in the promoter sequence.

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