Pathol. Oncol. Res. (2010) 16:189–191
DOI 10.1007/s12253-009-9205-6

Is Predisposition for Nephroblastoma Linked to Polymorphisms of the WTX Gene?

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Received: 3 February 2009 / Accepted: 31 August 2009 / Published online: 13 September 2009

Abstract Inactivation of Wilms′ tumor X (WTX) gene has been linked to the pathogenesis of a varying percentage of nephroblastomas. In contrast, germline mutations of WTX were identified to cause bone dysplasia, but not to induce the development of nephroblastomas. In our study we investigated whether tumor promotion of nephroblastoma by inactivation of WTX gene is linked to certain single nucleotide polymorphisms (SNPs). Therefore 8 SNPs—distributed over the whole length of the WTX gene—were investigated by high resolution melting curve analysis (HRMA) and sequencing of genomic DNA from nephroblastoma patients (NB) and controls. No difference was detected in the 8 SNPs investigated, which were distributed over the whole length of the gene. Additionally, sequence analysis of the coding part of the WTX gene of the tumor samples revealed no chromosomal aberration. Our study indicates, that inactivation of WTX appears to be a late event in tumorigenesis of nephroblastoma in a subgroup of nephroblastomas.

Keywords WTX · Nephroblastoma · SNP · HRMA · Polymorphism

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Introduction

Nephroblastomas (Wilms' tumors) are embryonal neoplasms of the kidney, affecting 1 in 8000 to 10000 children [1]. The molecular pathogenesis of nephroblastomas appears to be heterogeneous. Recently, WTX, a novel tumor suppressor gene was identified on the X-chromosome. This gene is inactivated by a monoallelic "single-hit" event. In the first investigation the frequency of this inactivation in nephroblastomas was estimated to be 30% [2]. In further examinations, however, only 7 % of 102 nephroblastomas showed a functional somatic nullizygosity of the WTX gene [3]. We hypothesized that this difference might be due to a linkage between inactivation of WTX gene in nephroblastomas and a specific genetic background indicated by a certain SNP pattern of the WTX gene. Similar observations have been documented in larynx carcinomas with a specific SNP of the Galphas gene linked to a significantly higher risk for lethal outcome [4].

Material and Methods

DNA Extraction

Patients' samples Selected paraffin sections of kidneys removed from nephroblastomas were deparaffinated

and stained with Papanicolau´s stain followed by manual microdissection of normal kidney tissue and tumors [5]. Isolation of genomic DNA was performed by QIAamp®DNA mini kit (QIAGEN, Germany) according to the manufacturer's instructions.

Control samples DNA isolation from peripheral blood samples was performed by the MagNA Pure Compact Nucleic Acid Isolation Kit I on the MagNA Pure Compact Instrument (Roche, Austria).

High Resolution Melting Analysis (HRMA)

Primers were designed using Primer3 software (http://frodo.wi.mit.edu/) (primers and characterisation of SNPs see Table 1). HRMA was performed using the LC480 instrument (Roche Diagnostics GmbH, Germany) and the LightCycler 480 High Resolution Melting Master kit according to the manufacturer's instructions. To detect sequence variations the GeneScanning Software v1.3 (Roche Diagnostics GmbH, Germany) was used.

Sequencing

To confirm HRMA results, PCR products were column purified after HRMA using SigmaSpin Post-Reaction Purification columns (Sigma-Aldrich, Austria). The purified PCR products were used as templates in DNA sequencing reactions using BigDye Terminator v1.1 chemistry (Applied Biosystems, US). After BigDye Terminator removal, using Sigma Spin Post Reaction Clean-up Plates, the samples were run on an ABI Prism AB3730 capillary sequencer and SeqScape v2.5 Software (Applied Biosystems, US) was used for data analysis.

To detect sequence aberrations in the coding region of the WTX gene 17 overlapping PCR products were amplified from the corresponding tumor samples and sequenced as described above. The appropriate sequence information was obtained from Genebank sequence NM_152424.

Results

HRMA Results

Four amplicons containing 8 different SNPs were investigated in samples of genomic DNA from normal renal tissues of 8 female nephroblastoma patients (NB) and from 20 female controls. The 201 base pair (bp) amplicon located at the 5′ end of the gene showed the same melting characteristics in all samples. This result was confirmed by the sequencing of amplicons derived from two NB and one control sample which all demonstrated C on both alleles. A 138 bp PCR product amplified from an intronic region also revealed identical melting curves in all samples. Sequence data of one control DNA showed a T on both alleles. The third amplicon spanning 319 bp was located in the middle of the gene and contained four SNPs. Because of technical reasons one NB sample could not be amplified. The remaining NB samples showed the same melting curve as the controls. Sequencing of genomic DNA of 2 NB samples and 1 control sample demonstrated C for the first two SNPs, A for the third and G for the fourth SNP. The fourth amplicon spanning 341 bp was located at the 3´end of the gene and contained two SNPs. All samples revealed identical melting curves and sequence data showed G for both alleles in both SNPs.

Sequence Analysis

The whole coding region of the WTX gene was examined by sequence analysis for mutational aberrations in the tumors of the 8 female NB. No sequence alteration was detected.

Table 1 Primer pairs for HRMA analysis and characterisation of SNPs

Amplicon	Size	Sequences	SNP	Chromos. Position	Type
1	201 bp	fw: 5′- GATCCGCAGGGCCTTGTCTCTACG-3′	rs35150885	X: 63342798-63342798	upstream
		rev: 5′-ATCGGTTTCGGTTTTCACGCACTC-3′			
2	138 bp	fw: 5′-TGGCACCTGTCTGATCTCGCTCTA-3′	rs5918862	X: 63336767-63336767	intronic
		rev: 5′- CCACTTACTTGCCACTCCCATCTT-3′			
3			rs12838095	X: 63330807-63330807	intronic
	319 bp	fw: 5′- GTTGGTATTTGGGCATTTGGAGGATT-3′	rs12838085	X: 63330806-63330806	intronic
		rev: 5′- GAGGTGATATGAGGGTGGGTCTAAG-3′	rs12846431	X: 63330772-63330772	intronic
			rs12839713	X: 63330771-63330771	intronic
4	341 bp	fw: 5′- AATCACTTGCCACTTTCTGCTACC-3′	rs1010866	X: 63320998-63320998	downstream
		rev: 5′- TTTGCTTTTAATTGGCTTTGCTTGAT-3′	rs6624669	X: 63320841-63320841	downstream

Discussion

Nephroblastoma is associated with more than 50 clinical conditions and chromosomal abnormalities with the majority resulting in clinically defined syndromes. However, the majority of identified causative genes do not show a functional interaction in the development of nephroblastoma [6]. In a very recent examination, germline mutations of the WTX gene have been linked to a pedigree of syndromic patients with sclerosing skeletal dysplasia. None of the surviving patients, however, had a history of nephroblastoma [7]. In contrast to these findings inactivation of WTX has been described in the pathogenesis of an extremely divergent number of nephroblastomas ranging from 7% to 30% [1, 2]. In our study we investigated the possibility that pathogenesis of nephroblastomas is linked to a subset of SNPs within the WTX gene. However, we were not able to identify a subset of SNPs linked to the development of nephroblastoma. Additionally no mutation in the coding region of the WTX gene was found in the tumors. Our results indicate, together with the lack of association of germline mutations of WTX with development of nephroblastoma, that inactivation of WTX appears to be a late event in tumorigenesis of nephroblastoma in a subgroup of nephroblastomas acting in cooperation with other genetic changes.

Acknowledgements This project was partly funded by the Thyssen Stiftung (non-profit organisation), project number AZ.20.06.0.046.

We thank Angelika Hofmann, Christine Grill and Sandra Sunitsch for extraction of the genomic DNA.

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