Human Pathology (2010) 41, 1172–1177

ELSEVIER

Human
PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Loss of PTEN/MMAC1 activity is a rare and late event in the pathogenesis of nephroblastomas ☆

Christine Grill MSc [a,1], Christian Guelly PhD [b], Birgit Ebner PhD [b], Ivo Leuschner MD [c], Cornelia Hauser-Kronberger PhD, MD [a], Gerald Hoefler MD [d], Barbara Guertl MD [d,*]

[a] *Department of Pathology General Hospital and Paracelsus Medical University Salzburg Muellner Hauptstrasse 48, A-5020 Salzburg, Austria*
[b] *Centre of Medical Research, Medical University of Graz, Stiftingtalstrasse 24, A-8010 Graz*
[c] *Kiel Pediatric Tumor Registry, Institute of Pathology, Christian Albrechts University Kiel, Michaelisstr. 11, D-24105 Kiel*
[d] *Institute of Pathology, Medical University of Graz, Auenbruggerplatz 25, A-8036 Graz*

Received 1 September 2009; accepted 8 October 2009

Keywords:
PTEN;
Nephroblastoma;
Nephrogenic rest

Summary Recent genetic investigations of nephroblastomas point to an activation of the Wnt pathway. Data indicate however that activation might be partly due to cross talk of different signaling pathways including the tumor suppressor gene PTEN (phosphatase and tensin homolog on chromosome 10). Therefore, we examined expression and chromosomal aberrations of PTEN in nephroblastomas of different subtypes and the corresponding nephrogenic rests. Loss of heterozygosity was analyzed by high-resolution melting analysis of 4 different single nucleotide polymorphisms. Results were confirmed by sequence analysis of the polymerase chain reaction products. In addition, an intragenic insertion-deletion polymorphism of the PTEN gene was investigated. Protein expression was assessed by immunohistochemistry. Twenty-two nephroblastomas and their corresponding nephrogenic rests were included in the study. In the high-resolution melting analysis, 15 samples were homozygous, 6 were heterozygous, and for 1 sample results could not be obtained for technical reasons. None of the samples showed loss of heterozygosity. Nineteen of the tumors and corresponding nephrogenic rests were also examined immunohistochemically. All tumors showed cytoplasmic positivity, with the exception of 1 tumor that showed complete loss of staining. In 1 tumor, the epithelial component showed distinct cytoplasmic staining, whereas the immature muscle and hyaline cartilage were negative. All nephrogenic rests exhibited positive cytoplasmic staining of all components. Our results establish that inactivation of PTEN is a rare and late event in the pathogenesis of nephroblastomas.

1. Introduction

Nephroblastomas are morphologically and genetically complex embryonal renal tumors of childhood. Histologically, nephroblastomas present with 3 cell types differentiating toward blastema, epithelium, and stroma. Each component can be present within a tumor to a varying degree. The diverse cell types may also express a varying degree of differentiation [1].

☆ This project was partly funded by the Thyssen Stiftung (Koeln, Germany), project number AZ.20.06.0.046, and the Salzburger Krebshilfe (Salzburg, Austria) (nonprofit organizations).

* Corresponding author.
[1] Present address: Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Iceland, Reykjavik, Iceland.

0046-8177/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.humpath.2009.10.024

Nephroblastomas are thought to develop from nephrogenic rests (NRs), their precursor lesions, as subclonal proliferation. NRs are abnormally persistent metanephric remnants of embryonal kidney development. They can be identified in about 1% of children without renal tumors at autopsy. In 30% to 44% of kidneys removed for nephroblastoma, incidental NRs can be found [2]. Classification of NRs is based on their topographic distribution within the renal lobe: perilobar rests can be found subcapsular between the renal pyramids; and intralobar rests, in between the pyramids [3,4]. A more recently developed subclassification takes not only the topographic site into consideration, but also the tumorigenic potential. NRs are therefore classified as obsolete, sclerosing, dormant, hyperplastic, and neoplastic (adenomatous) NRs [1,2]. It is very important to identify incidental NRs, as they harbor an association with bilateral and metachronous contralateral nephroblastomas [5,6].

PTEN is a tumor suppressor gene located on chromosome 10q23.3 influencing cell proliferation, apoptosis, and cellular migration. PTEN is involved in a variety of signal transduction pathways, but also acts via the Akt/PI3K pathway [7]. Inactivation of PTEN has been implicated in the development of a wide variety of malignancies. In several studies, a role of PTEN in the pathogenesis of different types of renal cell carcinomas has been established. Compared with normal renal tissue and oncocytomas, expression of PTEN is significantly reduced or not detectable in renal cell carcinomas [8], also paralleled by increased p-Akt activation [9]. There is strong evidence that the mechanism of inactivation of PTEN in renal cell carcinomas is due to an allelic loss on chromosome 10q23.3 rather than mutations of this gene [10]. Investigations for genomic imbalances with comparative genomic hybridization in nephroblastomas demonstrated loss of chromosomal material at chromosome 10q in some of the cases [11].

The aim of this study was to establish whether inactivation of PTEN gene plays a role during the pathogenesis of nephroblastomas. In addition, we investigated whether changes of PTEN are early or late events during this process by including corresponding NRs of these tumors into our study.

2. Methods

2.1. Samples

All samples were obtained from the children's tumor registry at the Institute of Pediatric Pathology, University of Kiel. Tissue samples had been fixed in 10% buffered formaldehyde solution and embedded in paraffin wax. Sections from paraffin blocks were obtained.

2.2. DNA extraction

Areas of normal tissue, NRs, and nephroblastomas were selected for microdissection after deparaffination and stained with Papanicolaou stain. Areas of interest from tumors and NRs were microdissected manually under microscopic control from 3 to 5 sections, depending on the size of the selected area, as described elsewhere [12]. The microdissected tissue was directly transferred into Eppendorf tubes containing ATL buffer. Genomic DNA was extracted with the QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

2.3. Immunohistochemistry

Sections of 3 μm were deparaffinated followed by pretreatment for 40 minutes in a water bath at 98°C in target retrieval solution at pH 9 (DAKO, Glostrup, Denmark) and a cooling phase for 20 minutes at room temperature. Endogenous peroxidase was blocked with 3% H_2O_2 (DAKO) for 10 minutes at room temperature. PTEN antibodies (Abcam, Cambridge, United Kingdom) were diluted 1:100 in antibody diluent (Dako). Samples were incubated for 30 minutes at room temperature. Dako EnVision Detection System (Dako) was used as detection system incubating slides for 30 minutes at room temperature followed by 10-minute treatment with 3,3′-diaminobenzidine + substrate. Specimens were counterstained with hematoxylin.

2.4. Loss of heterozygosity analysis

2.4.1. High-resolution melting analysis

Primers for high-resolution melting analysis (HRMA) were designed using Primer3 software (http://frodo.wi.mit.edu/) (primers and characterization of single nucleotide polymorphisms in Table 1). Samples were amplified in duplicate 20-μL reactions using the LightCycler 480 High-Resolution Melting Master Kit (Roche Diagnostics GmbH, Vienna, Austria) and analyzed on a LightCycler480 instrument (Roche Diagnostics GmbH). Amplification products were denaturated at 95°C for 1 minute, for reassociation cooled down to 40°C for 1 minute, and then melted from 65°C to 95°C with 25 signal acquisitions per degree. Sequence variations were detected using the Gene Scanning Software v1.5 (Roche Diagnostics GmbH).

2.5. Sequencing reaction

Polymerase chain reaction (PCR) products of the HRMA were purified using either SigmaSpin Post-Reaction Purification Columns (Sigma-Aldrich, Vienna, Austria) according to the manufacturer's instructions or Macherey-Nagel (Marchery-Nagel GmbH & Co., Düren, Germany) microtiter plates for amplicons with a length of more than 150 base pairs (bp). Purified PCR products were applied as templates in DNA sequencing reactions using BigDye Terminator v1.1 chemistry (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. After BigDye Terminator removal with SigmaSpin Post-Reaction Clean-