Mutational Activation of the β-Catenin Proto-Oncogene Is a Common Event in the Development of Wilms’ Tumors 1

Robert Koesters,2 Ruediger Ridder, Annette Kopp-Schneider, David Betts, Volker Adams, Felix Niggli, Jakob Briner, and Magnus von Knebel Doeberitz

Department of Surgery, University of Heidelberg, 69120 Heidelberg, Germany [R. K., R. R., M. v. K. D.]; Central Division of Biostatistics, Deutsches Krebsforschungszentrum, 69120 Heidelberg, Germany [A. K.-S.]; Children’s Hospital of Zurich, 8032 Zurich, Switzerland [D. B., F. N.]; Heart Center, University of Leipzig, 04289 Leipzig, Germany [V. A.]; and Institute of Histological and Cytological Diagnostics, 5001 Aarau, Switzerland [J. B.]

Abstract

Activation of β-catenin-mediated transcription is the nuclear end point of organ-specific Wnt signaling. In the developing kidney, Wnt-4, a secreted glycoprotein, acts as an autoinducer of the mesenchymal to epithelial transition that underlies normal nephron development. Dysregulation of this epithelial transformation process may lead to Wilms’ tumors (WTs). In this study, we investigated the potential role of the β-catenin proto-oncogene, a candidate downstream target molecule of Wnt-4 signaling, in the development of WTs. In 6 of 40 tumors (15%), mutation analysis revealed heterozygous missense mutations or small deletions that result in the loss of important regulatory phosphorylation sites within the β-catenin protein. These findings indicate that activating β-catenin mutations may play a significant role in the development of WTs and establish a direct link between Wilms’ tumorigenesis and the Wnt signal transduction pathway governing normal kidney development.

Introduction

WTs or nephroblastoma, an embryonic kidney-derived tumor, is one of the most common solid pediatric malignancies affecting about 1 in 10,000 children. Most WTs are sporadic and unilateral, whereas familial and bilateral tumors occur in only 1% and 7% of the cases, respectively. WTs have been proposed to fit a “two-hit” model of carcinogenesis (1). According to this model, WTs are caused by two independent mutational hits. In sporadic cases, both hits occur as somatic mutations, whereas in inherited cases one mutation is already present in the germ line and only the second mutation is acquired somatically. This intriguingly simple two-hit model, however, has been complicated by the fact that the two hits required may affect the two alleles of several distinct WT suppressor genes. These have been mapped to chromosomes 11p13, 11p15, 16q, and 1p (2–6). Thus far, only the WT1 gene, residing at 11p13, has been molecularly characterized (7, 8). This gene encodes a transcription factor that plays a pivotal role in the development of the kidney and also the gonads. Constitutional mutations of this gene lead to Denys-Drash syndrome, a triad of pseudohermaphroditism, glomerulopathy, and WT (9). The WT1 gene has been found to be mutationally inactivated in a subset of WTs (10).

β-Catenin is involved in the developmental Wnt/wingless signal transduction pathway (11, 12). Oncogenic activation of this protein has been found to occur in several human cancers and is caused by mutations affecting a specific degradation targeting box near the NH2 terminus of the β-catenin protein (13–17). In the developing kidney, Wnt-4 is required for the transition of metanephric blastema to renal epithelial cells (18), and signaling is likely to occur via β-catenin. Because WTs are the result of molecular alterations that lead to disturbance of this mesenchymal-to-epithelial transition, we considered the β-catenin proto-oncogene a candidate gene to be involved in Wilms’ tumorigenesis.

Materials and Methods

Patient Samples. Surgical resection specimens were obtained from 34 patients undergoing surgery for WT at the Kinderspital Zurich, Inselspital Bern, Kantonsspital Chur, and University Hospital of Geneva. After resection of the tumor, part of the material was immediately snap-frozen in liquid nitrogen and stored at -80°C. Formalin-fixed and paraffin-embedded samples of all tumors were examined by a pathologist at the University Hospital of Zurich.

DNA Preparation and PCR Amplification. DNA was isolated from snap-frozen tissues, as described (19). Exon 3 of β-catenin was amplified by PCR using primers bcat-fwd1 (5'-GCCGTATGGAGGTAATGGA-3') and bcat-rev1 (5'-GCTACTGTTGAGTGGAA-3'). Reactions contained 2 mM MgCl2 and 1 unit of TaKaRa Ex-Taq polymerase. Cycling conditions were: initial denaturation for 5 min at 94°C, 35 cycles of denaturation for 30 s at 94°C, 30-s annealing at 55°C, and 30-s elongation at 72°C, followed by a final elongation step for 7 min at 72°C.

DNA Sequencing. PCR fragments were purified using High Pure PCR Purification kit (Roche Diagnostics), essentially as recommended by the manufacturer. For cloning, purified PCR fragments were ligated into the vector pCR 2.1 using the TA cloning system (Invitrogen). Plasmid DNA was purified by using High Pure Plasmid Isolation kit (Roche Diagnostics). Sequencing reactions were set up using either 30 ng of purified PCR fragment or 250 ng of plasmid DNA as template and 10 pmol of sequencing primer in a total reaction volume of 10 µl following a dye terminator protocol (Big Dye; Perkin-Elmer). The sequencing reactions were run on an ABI Prism 310 DNA Sequencer (Perkin-Elmer). Sequencing primers of β-catenin exon 3 were bcat-fwd2 (5’-GGAGTGGACATGCGCTT-3’) and bcat-rev2 (5’-CCTGTTCCCCACCATACAGG-3’). All mutations found were verified by sequencing both of the DNA strands and by analyzing a second, independently generated PCR amplicon.

Western Blot Analysis. Snap-frozen tissue samples cut into 20-µm sections were used for total cellular protein extraction in lysis buffer (50 mM Tris-HCl (pH 7.5), 150 mM sodium chloride, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 0.02% sodium azide, and 1 mM phenylmethylsulfonyl fluoride). Aliquots of 100 µg of soluble protein were electrophoresed on 8% SDS-polyacrylamide gels, transferred to polyvinylidene difluoride membranes (Millipore), and probed with monoclonal antibodies specific for β-catenin (1:1000 dilution; Transduction Laboratories) or β-actin (1:1000; ICN). Horseradish peroxidase-conjugated rabbit anti-mouse IgG (Dianova) was used as secondary antibody. The membranes were developed using an enhanced chemiluminescence system (Amersham-Pharmacia).
Results

To examine whether WTs bear mutations in β-catenin, the conserved GSK-3β phosphorylation consensus region encoding the regulatory degradation targeting box of β-catenin was amplified by PCR, and the resulting PCR fragments were subjected to direct sequencing. By screening 41 WT specimens derived from 40 patients, we detected seven samples that displayed sequence ambiguities in their primary PCR products, indicating the presence of nonidentical alleles. On cloning, we identified in-frame deletions, as well as single nucleotide changes, that have occurred in these tumors (Table 1). Tumors NT4, NT8, NT38, and S.M. were found to harbor a common deletion of codon 45 that causes loss of a functionally important serine phosphorylation site. It is of importance to note that NT4 and NT8 were local and metastatic recurrences of the same tumor (NT3), respectively, and, therefore, provided independent evidence for the distinct β-catenin mutation found in this patient. Unfortunately, no material was available from the primary tumor in this case. Missense mutations within codon 45 were observed in NT21 and M.M. tumors and result in substitution of serine by cysteine or proline, respectively. One mutation was found in codon 41 (NT9) and replaces a threonine phosphorylation site by alanine. Representative examples of the mutational analysis are shown in Fig. 1. Taking into account that tumors NT4 and NT8 both were recurrent tumors of the same patient (NT3), 6 independent tumors among a total number of 40 (15%) have been identified that contained mutations of β-catenin (Fig. 2). All mutations were found in a heterozygous state in the tumor, and no mutations were found in the normal kidney when available (NT21, NT38, S.M.). All of the affected tumors were of early stage and with no histopathological evidence for anaplastic progression (Table 1). In two cases, a sporadic tumor (S.M.) and a Denys-Drash associated-tumor (NT21), β-catenin mutations were found in tumors also known to harbor mutations in the WT1 gene (20, 21).

Mutations in the degradation targeting box should lead to accumulation of β-catenin protein. In accordance with this, Western blot analysis demonstrated increased levels of β-catenin protein in tumor tissues harboring a mutated β-catenin allele as compared with normal kidney tissue (Fig. 3).

Discussion

In this study, we have analyzed the status of the β-catenin proto-oncogene in a panel of WTs. β-Catenin is a dual-function protein that acts as a key regulator of the cadherin-mediated cell-cell adhesion system, as well as a downstream effector of the Wnt-signaling pathway (22). Activation by Wnt/wingless leads to inhibition of the GSK-3β kinase that normally causes phosphorylation of the NH2 terminus of β-catenin and targets its cytoplasmic pool for ubiquitin-mediated degradation (11, 12). On accumulation, β-catenin interacts with members of the Lef-1/TCF family to generate a functional transcription factor complex that causes constitutive activation of target genes (e.g., c-myc and cyclin D1; Refs. 23 and 24). Stabilization of β-catenin may result from mutations within the β-catenin gene.

Table 1  β-Catenin mutations found in 41 WT specimens

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/sex/laterality</th>
<th>Histology</th>
<th>Stage</th>
<th>WT1 status</th>
<th>Mutation</th>
<th>Amino acid change</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT3</td>
<td>3 yr/F/left</td>
<td>Favorable</td>
<td>II</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>NT4</td>
<td>3½ yr</td>
<td>Favorable</td>
<td>1st relapse</td>
<td>wt</td>
<td>Δ 3bp</td>
<td>Δ Ser45</td>
</tr>
<tr>
<td>NT8</td>
<td>4 yr</td>
<td>Favorable</td>
<td>2nd relapse</td>
<td>wt</td>
<td>Δ 3bp</td>
<td>Δ Ser45</td>
</tr>
<tr>
<td>NT9</td>
<td>1 yr/F/left</td>
<td>Favorable</td>
<td>I</td>
<td>wt</td>
<td>ACC→GCC</td>
<td>Thr41Ala</td>
</tr>
<tr>
<td>NT21</td>
<td>½ yr/M/left/bilateral</td>
<td>Favorable</td>
<td>V</td>
<td>Arg394Trp</td>
<td>TCT→TGT</td>
<td>Ser45Cys</td>
</tr>
<tr>
<td>NT38</td>
<td>4 yr/F/right</td>
<td>Favorable</td>
<td>II</td>
<td>wt</td>
<td>Δ 3bp</td>
<td>Δ Ser45</td>
</tr>
<tr>
<td>M.M.</td>
<td>½ yr/M/right</td>
<td>Favorable</td>
<td>I</td>
<td>wt</td>
<td>TCT→CCT</td>
<td>Ser45Pro</td>
</tr>
<tr>
<td>S.M.</td>
<td>½ yr/M/left</td>
<td>Favorable</td>
<td>II</td>
<td>Arg301Stop</td>
<td>Δ 3bp</td>
<td>Δ Ser45</td>
</tr>
</tbody>
</table>

* Zinc-finger region analyzed.
* n.a., data not available; wt, wild type.
  * First relapse of NT3 (regional).
  * Second relapse of NT3 (distant metastasis).
  * Denys-Drash-associated WT.
  * Additional cleft palate.

Fig. 1. Nucleotide sequences of β-catenin mutations in four WT specimens. Electropherograms for the regions comprising codon 41 (NT9) and codon 45 (M.M., NT21, and S.M.) showing wild-type (wt) sequences derived from control samples (top), sequences derived from direct sequencing of tumor (tu)-derived PCR fragments (middle), and sequences of isolated mutant alleles as obtained after subcloning (bottom). The affected codons are boxed, and the affected nucleotides are indicated by an arrow.
itself and constitutes an important oncogenic step in several human cancers (13–17).

Our findings indicate that β-catenin mutations represent a common event in the development of WTs (15% in our patient group) and seem to occur more frequently than mutations of WT1 (10%) or p53 (5%; Refs. 10 and 25). The latter have been found only in the rare anaplastic variants of WTs. The β-catenin mutations found in WTs in each case eliminated a functionally important GSK-3β phosphorylation site at the NH₂ terminus of the β-catenin protein. Such mutations have been shown to lead to stabilization of the β-catenin protein and exert a dominant effect at the level of β-catenin/TCF-mediated transcription (14). Furthermore, the mutations found in WTs are identical to mutations that have been previously shown to occur in other human cancers (13–17). Together, these data imply that β-catenin mutations are causatively involved in the development of WTs.

Accumulating evidence suggests an important physiological role of the Wnt-signaling pathway during normal organ development. In the embryonic kidney, Wnt-4 is required for the mesenchymal-to-epithelial transition (18). It is a fatal malfunctioning of the latter process that normally should differentiate into the epithelial components of the nephron. Because β-catenin is likely to be a downstream mediator of Wnt-4 signaling, the finding of activating mutations in the β-catenin gene in WTs underlines the importance of the Wnt signal transduction pathway in the developing mammalian kidney.

Collecting more data on β-catenin mutations and uncovering the identity of the remaining WT genes is required to clear up the complex picture of WT genetics. We are confident that the finding of β-catenin mutations in WTs opens up new perspectives in research on WTs, as well as on normal kidney development.

Acknowledgments

We thank R. Moos, D. Koppenhoefer, and A. Siermann for technical assistance. The help provided by Dr. S. I. Peter is also gratefully acknowledged.

References


Fig. 2. Schematic representation of the location of β-catenin mutations in WTs. Boldface type, residues representing potential GSK-3β phosphorylation sites at codons 33, 37, 41, and 45. Amino acid substitutions are indicated above. Δ, deleted codon 45.

Fig. 3. Western blot analysis of tumor (tu) and normal kidney (kid)-derived tissue lysates from patients NT21 and S.M. using monoclonal antibodies specific for β-catenin and β-actin. Both tumors bear a mutated β-catenin gene.
Mutational Activation of the \( \beta \)-Catenin Proto-Oncogene Is a Common Event in the Development of Wilms' Tumors

Robert Koesters, Ruediger Ridder, Annette Kopp-Schneider, et al.


Updated version Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/59/16/3880

Cited articles This article cites 24 articles, 13 of which you can access for free at:
http://cancerres.aacrjournals.org/content/59/16/3880.full#ref-list-1

Citing articles This article has been cited by 27 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/59/16/3880.full#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/59/16/3880. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.