TUMORIGENESIS AND NEOPLASTIC PROGRESSION

Recurrent Mutations within the Amino-Terminal Region of β-Catenin Are Probable Key Molecular Driver Events in Sinonasal Hemangiopericytoma

Florian Haller,* Matthias Bieg,† Evgeny A. Moskalev,* Sarah Barthelmeß,* Helene Geddert, Carsten Boltze, Nicolle Diessl, Karin Braumandl,* Benedikt Brors, Heinrich Iro, Arndt Hartmann,* Stefan Wiemann,* and Abbas Agaimy*

From the Institute of Pathology,* and the Department of Otorhinolaryngology, Head and Neck Surgery,** Friedrich Alexander University Erlangen-Nuremberg, Erlangen; the Divisions of Theoretical Bioinformatics,†† Applied Bioinformatics,†† and Molecular Genome Analysis,†† and the Genomics and Proteomics Core Facility,‡ German Cancer Research Center, Heidelberg; the Institute of Pathology,‡ St Vincent’s Hospital, Karlsruhe; the Institute of Pathology,‡ SRH-Klinikum, Gera; the National Center for Tumor Diseases (NCT),** Heidelberg; and the German Consortium for Translational Cancer Research,‡‡ Heidelberg, Germany

Sinonasal hemangiopericytoma (SN-HPC) is an uncommon, site-specific, low-grade mesenchymal neoplasm of probable perivascular myoid cell origin. In contrast to solitary fibrous tumors of soft tissue and sinonasal tract origin, SN-HPCs were recently shown to lack recurrent NAB2-STAT6 fusion variants. Other molecular alterations known to occur in some of soft tissue perivascular myoid cell neoplasms were also absent in SN-HPC; thus, the molecular pathogenesis of SN-HPCs remained unknown. Guided by whole-genome sequencing combined with RNA sequencing of an index case, we analyzed a total of six SN-HPCs for mutations within the amino-terminal region of the gene CTNNB1 (cadherin-associated protein), β 1, 88 kDa, encoding β-catenin. All six cases showed missense mutations, with amino acid substitutions clustering at positions 33 to 45, corresponding to the recognition site of the β-catenin destruction complex. Similar CTNNB1 mutations have been described in a variety of epithelial and mesenchymal neoplasms. These mutations prevent β-catenin phosphorylation and proteasomal degradation but promote its nuclear accumulation and subsequent increased transcription of Wingless-related integration site target genes. Consistent with these molecular findings, β-catenin IHC showed consistent diffuse and strong nuclear staining of the tumor cells in all six SN-HPCs. Our results highlight, for the first time, CTNNB1 mutations as the likely initiating molecular events driving SN-HPC tumorigenesis, which places SN-HPC among the growing family of β-catenin–driven mesenchymal neoplasms. (Am J Pathol 2015, 185: 563–571; http://dx.doi.org/10.1016/j.ajpath.2014.10.019)

Sinonasal hemangiopericytoma (SN-HPC) is a peculiar mesenchymal neoplasm of perivascular myoid cell origin occurring in the nasal cavity and the paranasal sinuses.1,2 It accounts for <0.5% of all sinonasal neoplasms and is characterized by frequent local recurrence, whereas metastases are exceedingly rare.1,3 On the basis of its distinctive morphological features and immunophenotype, SN-HPC has been separated from solitary fibrous tumor (SFT), which can rarely occur in the sinonasal tract, and accounts for <0.1% of all sinonasal neoplasms.1,2 Recently, recurrent translocations involving the genes NGFI-A binding protein 2 (NAB2) and signal transducer and activator of transcription 6 (STAT6) have been identified as the key molecular driver events in pulmonary and soft tissue SFTs,4–6 which are accompanied by aberrant nuclear expression of STAT6.7,8 In a recent study, we demonstrated the absence of NAB2-STAT6 translocations in SN-HPCs in contrast to their presence in SFTs arising in the sinonasal tract.7 The latter harbor the same NAB2-STAT6 translocations as their pulmonary and soft tissue counterparts.8,9 Consistent with this observation, SFTs of the sinonasal tract demonstrated a strong nuclear

Disclosures: None declared.
expression of STAT6, whereas SN-HPCs did not. In the current study, we used whole-genome sequencing combined with RNA sequencing (RNA-seq) in a case of SN-HPC with available fresh-frozen tissue to explore the molecular genetic events in SN-HPCs. We observed a missense mutation at serine 33 (c.98C>T; p.S33F) in the gene CTNNB1 (cadherin-associated protein), β 1, 88 kDa, encoding for β-catenin, a key molecule of the Wingless-related integration site (Wnt) signaling pathway. Missense mutations in the amino-terminal region of β-catenin affecting codons 32 to 45 have been described in a variety of epithelial and mesenchymal neoplasms, including colorectal carcinoma, hepatocellular carcinoma, and desmoid-type fibromatosis, and their function in maintaining aberrant activation of the Wnt signaling pathway has been well demonstrated. By using direct Sanger sequencing of CTNNB1 exon 3 in our recently described cohort of six SN-HPCs, we could demonstrate that missense mutations affecting the amino-terminal region of CTNNB1 are frequent events observed in all six SN-HPCs (100%). Mutations affecting this region have been shown to prevent degradation by the β-catenin destruction complex, thus disrupting an important regulation mechanism resulting in nuclear accumulation of β-catenin. Consistent with this, all six SN-HPCs displayed a diffuse and strong nuclear immunoreactivity for β-catenin. In summary, this study describes, for the first time, recurrent CTNNB1 mutations as the probable key molecular driver events in SN-HPCs, and provides further genetic evidence that SN-HPC is a site-specific distinctive tumor entity that can be clearly separated from other mesenchymal neoplasms of the sinonasal tract, including SFTs.

Materials and Methods

Patient Cohort

The demographic, clinicopathological, and immunohistochemical (IHC) features of the current series of six SN-HPCs (Table 1) have been described recently in detail. All cases have been reclassified by an experienced head and neck soft tissue pathologist (A.A.), according to the current World Health Organization classification of head and neck tumors. The patient’s age ranged from 49 to 82 years (mean, 72 years) with equal sex distribution. The tumors were confined to the sinonasal tract, and none of the patients developed local recurrence or distant metastasis after resection of the primary tumor. The mean tumor size was 4.4 cm (range, 1 to 5.5 cm). All six cases displayed consistent morphological features of SN-HPC (Figure 1, A–D) with ovoid to spindle-shaped tumor cells with blunted or rounded uniform nuclei and pale-eosinophilic cytoplasm. The tumor cells were arranged in short fascicles in some areas, whereas other regions displayed a solid growth with occasional glomoid cellular features. Characteristic hyalinized vascular spaces with staghorn-like configuration and focal prominent peritheliomatous fibrosis were present in all cases. By IHC, all six tumors displayed consistent expression of smooth muscle actin. CD34 immunostaining was limited and patchy in four of the cases, and absent in the other three cases. Four cases were variably positive for CD99, and all six cases were negative for bcl2. None of the tumors harbored a NAB2-STAT6 translocation, and all six tumors were negative for STAT6 immunostaining. From case 1, fresh-frozen tissue was available for whole-genome sequencing and RNA-seq, whereas representative formalin-fixed, paraffin-embedded (FFPE) tissue blocks were used in the other five cases. This study has been approved by the local ethics committee of the University Hospital Erlangen (Erlangen, Germany) (number 217_12 B, 19.09.2012). Signed informed consent was obtained from all participating patients in this study.

Whole-Genome Sequencing

DNA was isolated from the tumor tissue of an SN-HPC (Case 1) with available fresh-frozen tissue using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). Quality of DNA was analyzed with an Agilent 2200 Tapestation (Agilent, Waldbronn, Germany). A total load of 1 μg DNA was fragmented (Covaris E220; Covaris, Woburn, MA) and size was selected to

Table 1  Patient and Tumor Characteristics of Six SN-HPCs with CTNNB1 Mutations

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age/ Sex</th>
<th>Site</th>
<th>Size (cm)</th>
<th>CTNNB1 mutation cDNA</th>
<th>CTNNB1 mutation protein</th>
<th>Nuclear β-catenin expression (IHC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70/M</td>
<td>Nasal cavity and paranasal sinuses right</td>
<td>5.5 × 5.5</td>
<td>c.98C&gt;T</td>
<td>p.S33T</td>
<td>++++</td>
</tr>
<tr>
<td>2</td>
<td>82/F</td>
<td>Nasal cavity and paranasal sinuses (ethmoid) left</td>
<td>1 × 1</td>
<td>c.98C&gt;G</td>
<td>p.S33C</td>
<td>++++</td>
</tr>
<tr>
<td>3</td>
<td>81/M</td>
<td>Ethmoidal sinuses bilateral</td>
<td>5 × 2.5</td>
<td>c.104T&gt;C</td>
<td>p.I35S</td>
<td>++++</td>
</tr>
<tr>
<td>4</td>
<td>74/M</td>
<td>Sphenoidal sinus</td>
<td>3.5 × 1.5</td>
<td>c.98C&gt;G</td>
<td>p.S33C</td>
<td>++++</td>
</tr>
<tr>
<td>5</td>
<td>77/F</td>
<td>Sphenoidal sinus left</td>
<td>5 × 4.5</td>
<td>c.133T&gt;C</td>
<td>p.S45P</td>
<td>++++</td>
</tr>
<tr>
<td>6</td>
<td>49/F</td>
<td>Sphenoidal sinus right</td>
<td>2 × 1</td>
<td>c.101G&gt;A; c.110C&gt;T</td>
<td>p.G34E; p.S37F</td>
<td>++++</td>
</tr>
</tbody>
</table>

Note that the cases have been published previously. *This mutation was homozygous.

F, female; M, male; IHC, immunohistochemistry; ++++, strong and diffuse expression.
SN-HPC, with staghorn-like configurations of sinonasal hemangiopericytoma (SN-HPC). A: SN-HPC covered by intact respiratory epithelium or squamous cell metaplasia. B: The tumor cells are ovoid to spindle shaped with blunt-ended uniform nuclei, sometimes arranged in a whorled pattern around the vessels. C and D: Extravasated erythrocytes and inflammatory infiltrates of mast cells and eosinophilic granulocytes are frequent findings; note the prominent gomoid cellular features. Hylatinized vascular spaces are characteristic for SN-HPC, with staghorn-like configuration (A) and focal prominent perithelomatous fibrosis (D). E: Immunoreactivity for β-catenin is homogeneous diffuse and strong in all six cases. F: Note the strong nuclear staining in the tumor cells, indicating nuclear accumulation of the mutated protein, in contrast to physiological membranous staining in the respiratory epithelium. Original magnifications: ×100 [hematoxylin and eosin (H&E); A]; ×400 (H&E; B–D); ×100 (β-catenin; E); ×400 (β-catenin; F).

Figure 1  Histomorphological features and β-catenin immunohistochemistry of sinonasal hemangiopericytoma (SN-HPC). A: SN-HPC covered by intact respiratory epithelium or squamous cell metaplasia. B: The tumor cells are ovoid to spindle shaped with blunt-ended uniform nuclei, sometimes arranged in a whorled pattern around the vessels. C and D: Extravasated erythrocytes and inflammatory infiltrates of mast cells and eosinophilic granulocytes are frequent findings; note the prominent gomoid cellular features. Hyalinized vascular spaces are characteristic for SN-HPC, with staghorn-like configuration (A) and focal prominent perithelomatous fibrosis (D). E: Immunoreactivity for β-catenin is homogeneous diffuse and strong in all six cases. F: Note the strong nuclear staining in the tumor cells, indicating nuclear accumulation of the mutated protein, in contrast to physiological membranous staining in the respiratory epithelium. Original magnifications: ×100 [hematoxylin and eosin (H&E); A]; ×400 (H&E; B–D); ×100 (β-catenin; E); ×400 (β-catenin; F).

300 bp using an E-Gel system (Life Technologies, Darmstadt, Germany) in the course of library preparation with the NEBNext DNA Library Prep Master Mix Set and the NEBNext Multiplex Oligos (NEB, Frankfurt, Germany) for Illumina (Illumina, Inc., San Diego, CA), using the supplier’s recommendations, and with 10 PCR cycles. Paired-end sequencing (2 × 100 bp) was performed using three lanes of a HiSeq2000 instrument (Illumina, Inc.). In total, 1,301,994,406 reads were collected to obtain a mean coverage of ×33.

Whole-genome sequencing reads were aligned against the 1-KG phase 2 reference genome (hs37d5; 1000 Genomes project, http://www.1000genomes.org/data, last accessed October 13, 2014) using an in-house pipeline, as described previously.13 The workflow uses bwa version 0.6.2-r126-tpx14 and allows for soft-clipped reads requiring a PHRED-based base quality score of at least 20. Each sequencing lane was aligned separately and subsequently merged using samtools version 0.1.17.15 PCR duplicates were marked using PICARD tools version 1.95 (http://picard.sourceforge.net, last accessed October 13, 2014).

For single-nucleotide variation calling, we performed the method, as described recently, combining samtools mpileup version 0.1.17 and beftools.13,15 Gene annotation and functional impact assignment within coding portions of genes was done by ANNOVAR16 using the GENCODE gene model version 17.17 Single-nucleotide variations were compared against dbSNP version 135 (http://www.ncbi.nlm.nih.gov/SNP, last accessed October 13, 2014), Online Mendelian Inheritance of Man (http://www.omim.org, last accessed October 13, 2014), and COSMIC (Catalogue of Somatic Mutations in Cancer) version 66 (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic, last accessed October 13, 2014). In addition, breakpoints of translocations were identified using the CREST (clipping reveals structure) algorithm, which makes use of soft-clipped reads to discover genomic locations with a high proportion of partially mapped reads.19

RNA-Seq

RNA was isolated from the same tumor (Case 1) using the RNeasy Plus Mini Kit (Qiagen). Quality of RNA was assessed using an Agilent Bioanalyzer 2100 (Agilent). For RNA-seq, 1 μg of total RNA was fragmented to a median length of 150 bp (Covaris), and library preparation was done with an Illumina TruSeq RNA Sample Preparation Kit version 2, following the supplier’s instructions (Illumina, Inc.). Paired-end sequencing (2 × 100 bp) was performed, and 171,763,248 reads were collected using a HiSeq2000 instrument (Illumina, Inc.).

Paired-end RNA-seq reads of single lanes were aligned against the 1-KG phase 2 reference genome (hs37d5; 1000 Genomes project, http://www.1000genomes.org/data, last accessed July 21, 2014) separately using STAR version 2.3.0e.20 For improving the accuracy of the alignment, we supplied a splice junction database that is based on the GENCODE gene model version 17.17 After alignment, single lanes were merged using samtools version 0.1.1715 and marked for PCR duplicates using PICARD tools version 1.95 (http://picard.sourceforge.net, last accessed July 20, 2014). Reads per kilobase of transcript per million mapped reads values per gene were calculated using custom perl scripts on the basis of the following formula:

\[
\text{rpkm} = \left( \frac{\text{reads(exonSum)}}{\text{readsTotal}} \right) \times \frac{1000}{\text{(reads/10}^6)}
\]

Here, \(\text{reads}\) is the number of mapped non–duplicate-marked reads in the gene, \(\text{exonSum}\) is the total length of all exons among the gene, and \(\text{readsTotal}\) is the total number of mapped non–duplicate-marked reads among all exons. For defining exonic and intragenic regions, we used the gene model of The Reference Sequence Database.21

Gene-fusion detection on RNA-seq data was performed using DEFUSE version 0.6.1.22 Because DEFUSE aligns the reads independently, we supplied the hg19 reference genome and the ENSEMBL version 69 gene and transcript model.23
Mutation Analysis of CTNNB1 Exon 3 by Sanger Sequencing

DNA was isolated from FFPE tumor samples using the QIAamp DNA FFPE Tissue Kit (Qiagen). PCR amplification of CTNNB1 exon 3 was performed in 50-μL reactions that contained approximately 200 ng DNA, 1.5 mmol/L MgCl₂, 200 mmol/L dNTP, 500 mmol/L primers, and 1.25 U Tag DNA polymerase (Genaxxon Bioscience GmbH, Ulm, Germany). Amplification was started by an initial denaturation step at 94°C for 3 minutes. The first amplification cycle was denaturation at 94°C for 30 seconds, annealing at 65°C for 30 seconds, and elongation at 72°C for 40 seconds. This procedure was continued for 10 cycles, reducing the annealing temperature by 1°C each cycle, followed by 45 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 1 minute, and elongation at 72°C for 40 seconds. The sequences of the PCR primers were as follows: 5′-TTTGATGGAGTTGGACA-TGG-3′ (forward) and 5′-CCAGCTACTTGTTCTTGAGT-GAA-3′ (reverse). Approximately 5 μL of each reaction was examined on 2% agarose gels. Amplicons were sequenced with Sanger chemistry by using the following sequencing primers: 5′-GCCATGGAACCAGACAGAAA-3′ (forward) and 5′-AAAAATCCCTGTACCACACTCA-3′ (reverse).

β-Catenin IHC

The IHC was performed on freshly cut sections (3 μm thick) of representative FFPE tumor blocks on a fully automated slide preparation and staining system (Benchmark XT System; Ventana Medical Systems Inc., Tuscon, AZ). After pretreatment with buffer CC1 for 95°C for 64 minutes, a mouse monoclonal antibody directed against amino acids 571 to 781 of β-catenin (clone 14/β-catenin, catalog number 610154; BD Biosciences, Heidelberg, Germany) was used in a 1:50 dilution with incubation at 37°C for 32 minutes. Tissue from desmoid-type fibromatoses and scar tissue were used as external positive and negative controls, respectively. In the SN-HPCs, only nuclear staining was considered, whereas physiological strong membranous staining of respiratory epithelium was available as internal positive control in all cases (Figure 1). In addition, IHC staining with antibodies directed against CD34, α smooth muscle actin, bcl2, CD99, and STAT6 has been performed on all cases, as reported previously.9

Results

Deciphering Key Molecular Driver Events in a Case of SN-HPC by Whole-Genome Sequencing and RNA-Seq

The integrative analysis of whole-genome sequencing and RNA-seq data from the index case (Case 1) revealed the absence of a fusion gene that was represented on both genomic DNA and RNA levels. Alignment of the genomic sequence of the tumor DNA to the reference genome and application of filters, as described in Materials and Methods, revealed 12 nonsynonymous somatic mutations within 11 protein-coding genes, including a missense mutation in exon 3 of the CTNNB1 gene (c.98C>T; p.S33F) (Table 2). The CTNNB1 mutation was present on both the genomic DNA and the RNA levels, and this specific missense mutation has been reported in different types of epithelial and mesenchymal neoplasms before (mutation ID COSM5669). The integrative analysis of genes with nonsynonymous somatic mutations in comparison to the RNA-seq expression data revealed a high RNA abundance only for CTNNB1, whereas the other genes were only rarely represented on the RNA level (Table 2).

According to the major role of β-catenin as a central effector molecule of the Wnt pathway and its important function as transcriptional coactivator in the T-cell factor (TCF)/β-catenin transcriptional complex, we compared the RNA abundance of selected genes with known function in Wnt signaling and TCF/β-catenin target gene expression (Table 3). In detail, we evaluated 51 Wnt signaling components with either agonistic or antagonistic effect on the pathway, the 5 components of the β-catenin destruction complex, 11 components and cofactors of the TCF/β-catenin transcriptional complex, and 5 well-known TCF/β-catenin target genes. Interestingly, the highest RNA abundance with reads per kilobase of transcript per million mapped reads values >150 was observed for CTNNB1 itself, for three TCF/β-catenin target genes (the transcription factors FOS, JUN, and TWIST1) and for four components of the Wnt pathway with antagonistic function (APCDD1, NKD1, NKD2, and WIF1). In contrast, most Wnt agonists displayed low RNA abundance. Collectively, these RNA expression data further support the functional effect of the β-catenin mutation in the index case, demonstrating up-regulation of TCF/β-catenin transcriptional target genes in the absence of agonistic Wnt signaling components upstream of the mutated β-catenin.

<table>
<thead>
<tr>
<th>Gene</th>
<th>cDNA</th>
<th>Protein</th>
<th>RPKM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTNNB1</td>
<td>c.C98T</td>
<td>p.S33F</td>
<td>541.1</td>
</tr>
<tr>
<td>NPEP5</td>
<td>c.T1058G</td>
<td>p.F353C</td>
<td>31.9</td>
</tr>
<tr>
<td>DENND1A</td>
<td>c.G2407C</td>
<td>p.A803P</td>
<td>11.68</td>
</tr>
<tr>
<td>POUSF2</td>
<td>c.G1329T</td>
<td>p.L443F</td>
<td>0.83</td>
</tr>
<tr>
<td>CHIT1</td>
<td>c.C1049G</td>
<td>p.A350G</td>
<td>0.01</td>
</tr>
<tr>
<td>CHIT1</td>
<td>c.G1046A</td>
<td>p.W349X</td>
<td>0.01</td>
</tr>
<tr>
<td>DSPP</td>
<td>c.G3103A</td>
<td>p.D1035N</td>
<td>0</td>
</tr>
<tr>
<td>RHAG</td>
<td>c.C527T</td>
<td>p.A176V</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2 List of Nonsynonymous Somatic Mutations in Protein-Coding Genes Determined by Whole-Genome Sequencing Compared to RNA Abundance Determined by RNA-Seq in a SN-HPC (Case 1)

The RPKM value is proportional to the RNA abundance of a gene. RPKM, reads per kilobase of transcript per million mapped reads.
Frequent CTNNB1 Mutations in a Cohort of SN-HPCs

Collectively, the findings in the index case encouraged us to analyze five additional SN-HPCs for mutations in CTNNB1 by Sanger sequencing. The missense mutation affecting serine 33 (p.S33F) found in the index case (Case 1) by whole-genome sequencing was confirmed (Figure 2A). Strikingly, all additional five SN-HPCs harbored similar missense mutations in the amino-terminal region of CTNNB1 (Figure 2B–F, and Table 1). Two cases also had amino acid substitutions at serine 33 (p.S33C), one case had a substitution at isoleucine 35 (p.I35S), one case had a substitution at serine 45 (p.S45P), and one case had two mutations with substitution of glycine 34 (p.G34E) combined with a homozygous substitution of serine 37 (p.S37F).

According to the well-known mechanism of nuclear accumulation of β-catenin with mutations in the amino-terminal region, the tumors were analyzed for β-catenin expression by IHC. Consistent with the molecular findings, all six SN-HPCs demonstrated a homogeneous diffuse and strong nuclear staining for β-catenin (Figure 1, E and F).

**Discussion**

Identification of Recurrent CTNNB1 Mutations in SN-HPC

Until now, the molecular pathogenesis of SN-HPCs remained obscure. Namely, the recurrent NAB2-STAT6 fusions described...
recently in most soft tissue tumors included in the family of SFT/HPC could not be detected in any of six SN-HPCs examined in a recent study.9 Another recent study failed to demonstrate any rearrangements of NOTCH1-3 genes in five cases of SN-HPCs analyzed.24 Accordingly, absence of similar alterations in SN-HPCs suggested a different, albeit yet un-
known, molecular pathway involved in these morphologically distinct tumors confined to the sinonasal tract.

Herein, we present, for the first time, strong evidence that recurring missense mutations in exon 3 of the CTNNB1 gene, leading to substitutions of amino acids in the amino-terminal region of β-catenin, are the probable key molecular driver event in SN-HPCs. These mutations were detected in all six SN-HPCs examined in the current study and were accompanied by consistent nuclear accumulation of β-catenin, thus providing strong evidence that the reported CTNNB1 mutations are correlated to an oncogenic functional status of β-catenin in a manner reminiscent of desmoid-type fibromatosis and other β-catenin—driven neoplasms.11,25,26

Regulation and Function of β-Catenin in the Wnt Signaling Pathway

β-Catenin is a key regulatory molecule in the Wnt pathway, an important signaling network controlling embryonic development and adult tissue homeostasis with effects on cell proliferation and differentiation.10 In the absence of activating Wnt signals, cytosolic β-catenin is constantly degraded and its levels are low. After Wnt activation, β-catenin is stabilized through phosphorylation and translocates to the nucleus, where it forms a transcriptionally active complex with members of the TCF/LEF family of transcription factors and coactivates the expression of pro-survival and proliferative genes.10,27 According to its fundamental role in connecting the transcriptional response to on and off states of Wnt signaling pathway, activation of β-catenin is tightly regulated, but make it susceptible to oncogenic mutations observed in a variety of epithelial and mesenchymal neoplasms.11 Specifically, mutations affecting the amino-terminal region at positions 32 to 45 abrogate the phosphorylation-dependent degradation through a multi-protein assembly called the β-catenin destruction complex.11,12 In a first step, serine 45 is phosphorylated by casein kinase 1. Phosphorylated serine 45 then primes further phosphorylation of threonine 41 and subsequently of serines 37 and 33 by glycogen synthase kinase 3 (GSK3).

Additional components of the destruction complex are the adenomatosis polyposis coli (APC) protein, the scaffolding protein Axin, and the E3-ubiquitin ligase β-TrCP.11,12 Collectively, these proteins form the β-catenin destruction complex, which directs phosphorylated β-catenin to proteasomal degradation.

CTNNB1 Mutation in SN-HPC Disrupts the Phosphorylation Motive Necessary for β-Catenin Degradation

The mutations found in five of six SN-HPCs in the current study directly affected the phosphorylation sites, serine 33 (three cases), serine 37 (one case), and serine 45 (one case), whereas the sixth case harbored a mutation at position 35 with substitution of isoleucine to serine. The substitutions of serines in the first five cases directly foreclose the phosphorylation of β-catenin by GSK3 at serines 33 and 37 and by casein kinase 1 at serine 45, respectively.11,12 In the sixth case, the installation of serine at position 35 disrupts the S/T-X-X-X-pS/T sequence motive necessary for phosphorylation of serine 33 by
In summary, all six SN-HPCs harbored mutations abrogating the phosphorylation and subsequent proteosomal degradation of β-catenin by the β-catenin disruption complex, which is consistent with a nuclear accumulation of β-catenin demonstrated by diffuse and strong nuclear immunoreactivity in all six cases.

Expression Analysis of Wnt Signaling Components and β-Catenin Transcriptional Targets Supports Functional Relevance of CTNNB1 Mutation in SN-HPC

The RNA-seq data available for the index case enabled us to compare the RNA abundance of 73 components of Wnt signaling and TCF/β-catenin transcriptional response. In detail, 51 Wnt signaling components with agonistic or antagonistic effect on the Wnt pathway, the 6 components of the β-catenin destruction complex, 11 components and cofactors of the TCF/β-catenin transcriptional complex, and 5 TCF/β-catenin target genes were evaluated for their RNA abundance in relation to their main functional effect on Wnt signaling and TCF/β-catenin target gene expression. We observed a high RNA abundance for the TCF/β-catenin target genes FOS, JUN, and TWIST1, which constitute three important transcription factors involved in cell proliferation, differentiation, and transformation. Notably, the four components of the Wnt signaling pathway with highest RNA abundance (APCDD1, NDK2, WIFI, and NKD1) have an antagonistic effect on the pathway, and constitute a physiological negative feedback loop with intent to dampen the activated Wnt signaling. In contrast, Wnt signaling components with agonistic effect had a rather low RNA abundance. Collectively, these RNA expression data indicate transcriptional activation of TCF/β-catenin target genes located downstream of the mutated β-catenin in the absence of agonistic Wnt signaling components upstream of β-catenin, thus further supporting the functional relevance of the observed β-catenin mutation and nuclear β-catenin accumulation.

CTNNB1 Mutations in Other Mesenchymal Neoplasms

Desmoid-type fibromatosis is the prototype soft tissue neoplasm driven by alterations affecting the Wnt signaling network. Although desmoid-type fibromatosis in patients with familial adenomatous polyposis coli (Gardner syndrome) occurs in the context of APC germ-line mutations, sporadic cases of desmoid-type fibromatosis frequently harbor mutations affecting serine 45 and threonine 41 of CTNNB1. These amino acid substitutions abrogate β-catenin phosphorylation through casein kinase 1 and GSK3 and confer to oncogenic stabilization and nuclear accumulation of β-catenin, which can be visualized by nuclear immunoreactivity. Moreover, recurrent mutations in the amino-terminal region of CTNNB1 have also been reported in 75% of nasopharyngeal (juvenile) angiofibromas, and recently, in 88% of palisaded myofibroblastoma of lymph node (synonym: intranodal hemorrhagic spindle cell tumor with amianthoid fibers). In both entities, CTNNB1 mutations were also accompanied by strong nuclear β-catenin immunoreactivity. Nasopharyngeal (juvenile) angiofibroma is a rare lesion with striking predominant for young males. It occurs in the posterolateral wall of the nasal cavity and the nasopharynx and is characterized by irregular vessels embedded in a fibrous stroma. Palisaded myofibroblastoma of lymph node represents a rare benign myofibroblastic neoplasm restricted to lymph nodes, mainly of the inguinal region. Thus, SN-HPC represents the fourth documented example of a β-catenin-driven mesenchymal neoplasm and the first one of possible/probable perivascular myoid cell origin.

Distinct Types of CTNNB1 Mutations in β-Catenin—Driven Mesenchymal Neoplasms Correlate with Different Local Recurrence Rates

Interestingly, CTNNB1 mutations in desmoid-type fibromatoses are mainly restricted to serine 45 and threonine 41 with <3% of the cases harboring mutations at positions 33, 34, and 37. In contrast, CTNNB1 mutations in nasopharyngeal angiofibromas and palisaded myofibroblastoma of lymph node cluster within codons 32 to 37, whereas no mutations at serine 45 and threonine 41 have been documented. In the current series of six SN-HPCs, we observed a similar pattern comparable to the latter two entities with predominant mutations of amino acids 33 to 35, and only one case displaying a mutation affecting serine 45. We cannot explain these interesting differences in the affected amino acid positions, but differential activation of components of Wnt signaling network and the β-catenin destruction complex in the probable different cells of origin of these distinct tumor entities may contribute to these different mutation signatures. Interestingly, site-specific different mutation frequencies have been reported comparing intra-abdominal (mesenteric) with extra-abdominal (desmoid-type) fibromatoses, and mutations involving serine 45 have been recently introduced as an independent prognostic marker for local recurrence. Accordingly, different mutation sites within the amino-terminal region of CTNNB1 may contribute to the morphological and biological heterogeneity of the well-characterized β-catenin—driven mesenchymal neoplasms ranging from fully benign lesions (intranodal palisaded myofibroblastoma) to non-metastasizing tumors of varying intermediate biological features, with low (SN-HPC), intermediate (nasopharyngeal angiofibroma), or high (desmoid fibromatosis) local recurrence rates.

Diagnostic and Prognostic Relevance of CTNNB1 Mutation Analysis and β-Catenin Immunostaining in SN-HPC

Well-documented distant metastasis from SN-HPCs is almost nonexistent. Among the initial series with the first description of SN-HPC, none of 23 cases metastasized, with 4 (17%) local
recurrences. In the largest published series of 104 well-characterized SN-HPCs, 17 (17%) of 101 cases with follow-up developed local recurrence after resection, but no distant metastasis was reported. In that study, three patients (3%) died with local disease, including two cases with bone invasion, after one to eight local recurrences. Regarding the hereby introduced CTNNB1 mutations in SN-HPCs and integrating these tumors among other β-catenin—driven mesenchymal neoplasms with varying potential for local recurrence but absence of any metastatic potential, the few reported examples of highly aggressive SN-HPCs might have represented rare cases of sinonasal SFTs.

From a differential diagnostic point of view, β-catenin IHC may emerge as an adjunct diagnostic tool in better defining and recognizing SN-HPCs compared with other mesenchymal neoplasms occurring in the sinonasal tract on the one hand. On the other hand, frequent CTNNB1 mutations and β-catenin reactivity in SN-HPCs, particularly their collagen-rich (fibrous) variants, should be distinguished from rare cases of extra-abdominal desmoid-type fibromatoses originating within the sinonasal tract. In this regard, the classic histopathological features of the two neoplasms are readily distinguishable if sufficient tissue is obtained. Most important, a strong nuclear β-catenin staining in limited or crushed biopsy specimen of a HPC-like sinonasal lesion should warrant diagnosis of SN-HPCs.

Conclusion

In summary, we report herein, for the first time, recurrent CTNNB1 missense mutations associated with diffuse and strong nuclear accumulation of β-catenin in SN-HPCs, pointing to these findings as the most likely initiating molecular driver event in this uncommon site-specific tumor. The potential targeting of these molecular alterations in cases with unresectable disease remains to be assessed in future studies. SN-HPCs must be distinguished from other β-catenin—positive lesions, such as desmoid-type fibromatosis, which may rarely originate at this anatomical site.

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References


