

RESEARCH ARTICLE

Target Gene Activation of the Wnt Signaling Pathway in Nuclear β -Catenin Accumulating Cells of Adamantinomatous Craniopharyngiomas

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Abstract

Activating β -catenin (*CTNNB1*) mutations can be identified in the majority of adamantinomatous craniopharyngiomas (adaCP), suggesting an aberrant Wnt signaling pathway in this histopathologically peculiar tumor entity. However, there is no proven evidence that nuclear translocation of β -catenin is associated with *CTNNB1* mutations and target gene activation. We performed a laser-microdissection-based study comparing β -catenin accumulating vs. non-accumulating tumor cells. Mutational analysis and gene expression profiling using real-time polymerase chain reaction were conducted in adamantinomatous and papillary tumor specimens. Target gene activation, that is, over-expression of Axin2 could be detected in adaCP, especially in tumor cells with nuclear β -catenin accumulation. In addition, increased expression of BMP4 was identified in the accumulating cell population, which supports the hypothesis of an oral ectodermal origin. Interestingly, accumulating and non-accumulating tumor cell populations carried *CTNNB1* mutations within exon 3. We extended the analysis, therefore, towards genetic regions encoding for membrane linkage and active/passive nuclear transport mechanisms (exon 4 and exon 8–13), but could not detect any alteration. This is the first report demonstrating an association between nuclear β -catenin accumulation and target gene activation in adaCP. The results confirm the Wnt signaling pathway as molecular basis of the distinct and challenging clinical and morphological phenotype of adaCP.

INTRODUCTION

Craniopharyngiomas (CP) are rare, benign epithelial tumors of the sellar region, to be distinguished by their clinical occurrence, outcome and histopathology into an adamantinomatous (adaCP) and a rare papillary (papCP) variant. AdaCP constitute about 10% of childhood intracranial neoplasms, whereas papCP occur predominantly in adults. Moreover, papCP are characterized by well-differentiated, solid squamous epithelium-lacking cystic areas, palisaded cells and regressive changes, that is, keratin nodules (wet keratin), cholesterol clefts as well as calcifications. The latter represent histological hallmarks of the adamantinomatous subtype (9, 12). AdaCP seem to arise from remnants of Rathke's pouch or misplaced enamel organ as indicated by striking histological similarities to odontogenic tumors and cysts (27, 32). Recently, nuclear β -catenin accumulation was shown as a highly significant finding in adaCP (10). As a key player of the canonical Wnt signaling pathway, β -catenin plays an important role during development,

cellular proliferation, differentiation and migration (23). It is located either at the cell membrane establishing adherens junctions, or in the cytoplasm tagged for proteosomal degradation when Wnt signaling is inactive (16). The cytoplasmic amount is strictly regulated by a multiprotein-complex containing the tumor suppressor adenomatous polyposis coli (APC), the scaffold proteins Axin and Axin2, and the phosphokinases GSK3 β and CK1. Following binding to this multiprotein-complex, β -catenin becomes phosphorylated and tagged for its β -transducing repeat-containing protein driven ubiquitinylation and subsequent proteosomal clearance (1, 2, 29, 30). Noteworthy, N-terminal phosphorylation sites are essential for β -catenin functional regulation and encoded by exon 3 of the β -catenin gene (*CTNNB1*). Activation of Wnt signaling leads to inhibition of GSK3 β , which in turn, effects cytoplasmatic stabilization and subsequent nuclear translocation of β -catenin (22). Within the nucleus, β -catenin initiates gene expression via interaction with transcription factors of the Lef1/TCF family (3). Aberrant β -catenin stabilization can be the result of an impaired degradation

process and causes carcinogenesis. In many malignancies, genes encoding for the multiprotein complex components, that is, *APC*, *Axin* and *Axin2*, exhibit genetic loss of function alterations, whereas medulloblastomas, hepatoblastomas, hepatocellular carcinomas, pilomatricomas and ovarian carcinomas reveal activating mutations in *CTNNB1* (4, 8, 14, 28, 37, 39). These mutations are thought to entail cytoplasmatic and corresponding nuclear accumulation of the protein by its stabilization. Such activating mutations within the region encoding the phosphorylation sites in exon 3 of the *CTNNB1* gene have also been described for adaCP (7, 15, 25, 33, 34). However, the relation between activating mutations in exon 3, nuclear β -catenin accumulation and resultant target gene activation remains to be shown, and may involve active and passive nuclear transport mechanisms. A divergent interaction between members of the canonical Wnt signaling pathway is challenged by the histopathological heterogeneity. For that, neoplastic cells with nuclear accumulation of β -catenin are the clear minority and appear not equally distributed throughout adaCP (7). In addition, until now there is no proven evidence that nuclear β -catenin accumulation activates target genes of the Wnt signaling cascade in affected tumor cells of adaCP.

To address this issue, we conducted reverse transcription polymerase chain reaction (RT-PCR) analysis of *Axin2* and *bone morphogenetic protein 4* (*BMP4*) using total cDNA from snap frozen tissue samples of adaCP and papCP as well as cDNA from laser-microdissected formalin fixed and paraffin embedded tumor cell fractions with and without nuclear β -catenin accumulation. Whereas *Axin2* is a well-recognized inhibitor and target gene of β -catenin (13), *BMP4* is described to be enhanced in tumors with oncogenic β -catenin elevation (17, 35). In addition, *BMP4* plays a crucial role in tooth development, which seems to be imitated in adaCP (24, 27, 38). To proof the concept of genotypic heterogeneity within adaCP as potential cause of differential nuclear β -catenin accumulation, we submitted laser-microdissected cell clusters with or without accumulation also to a mutational analysis of exon 3 of *CTNNB1*. Besides exon 3, we extended our genetic analyses to exclude further alterations in regions of *CTNNB1* encoding the ability to shuttle between the nucleus and the cytoplasm. Phosphorylation sites regulating dissociation from adherens junctions are encoded partly by exon 4 (20). In contrast, exon 8 to 13 encodes the armadillo-repeats, which are not only crucial for the passive nuclear transport of β -catenin, but represent also interaction sites to members of the destruction complex (eg, *APC*, *Axin2*), TCFs and cadherins (18, 40).

MATERIALS AND METHODS

Surgical specimens from 37 patients with histopathologically confirmed adaCP, and eight patients with papCP, were retrieved from the archives of the Departments of Neurosurgery and Neuropathology at the University of Erlangen-Nuremberg, International Neuroscience Institute in Hannover, and the Evangelisches Krankenhaus in Bielefeld. There were 21 males, 16 females with adaCP, four males, and four females with papCP. The age ranged between two and 77 years in the group of adaCP (mean age 34, 4 years) and between 19 and 57 years in papCP (mean age 42, 4 years). Each specimen was classified according to World Health Organization guidelines using Haematoxylin and eosin as well as immunohistochemistry (IHC) (10).

IHC

Surgical samples were fixed overnight in 4% formaldehyde and routinely processed into liquid paraffin. Sections were cut at 4 μ m with a microtome (Microm, Heidelberg, Germany) and mounted on positively charged slides (Menzel, Braunschweig, Germany). The slides were air dried in an incubator at 37°C overnight. Microwave pretreatment was performed in Citrate-buffer at pH 6.0. β -catenin immunoreactions were performed as described elsewhere (6). Immunohistochemical staining for *Axin2* was performed at room temperature for 2 hours, using a polyclonal rabbit antibody (1:200; Abcam, Cambridge, UK). For detection of *BMP4*, we used the monoclonal mouse antibody clone 3H2 (1:50; Novocastra Laboratories Ltd, Newcastle, UK). *BMP4* staining was operated at room temperature for 30 minutes. Both reactions were followed by avidin-biotin labeling detected with diaminobenzidine (Dako, Hamburg, Germany). Serial paraffin sections (1 μ m) of tumor specimens were prepared and stained, either with antibodies against β -catenin, *Axin2* or *BMP4*, to examine intratumoral coexpression patterns.

DNA and cDNA preparation

For DNA extraction, snap frozen tissue samples were retrieved from our tissue bank (-80°C). Total cellular RNA was extracted from 10 patients (5 adaCP and 5 papCP). From all tissues, frozen sections were microscopically reviewed to confirm tumor tissue content. DNA was extracted using the DNeasy® tissue kit (Qiagen, Hilden, Germany). DNA from laser-microdissected formalin fixed paraffin embedded material was applied using the QIAamp® DNA Micro kit (Qiagen) according to the manufacturer's instructions. The RNeasy® extraction kit (Qiagen) was used for RNA isolation. Subsequently, digestion with RNase-free DNase I and purification via RNeasy® columns (Qiagen) was followed by reverse transcription using SuperScript™ First-strand synthesis system for RT-PCR (Invitrogen™, Carlsbad, California, USA) with oligo (dT) primers.

For RNA extraction of laser-microdissected probes, cell fractions were collected in lyses buffer (36) and digested 16 h at 56°C with Proteinase K® (Qiagen). Using the TRIzol® reagent (Invitrogen™), RNA was isolated following the manufacturer's instructions. Digestion with RNase-free DNase I and purification was followed by reverse transcription using SuperScript™ First-strand synthesis system for RT-PCR (Invitrogen™) with random hexamer primers.

Laser-microdissection

Serial sections of 4 μ m were prepared from eight different formalin fixed and paraffin embedded (FFPE) tissue tumor samples, harboring a substantial amount of β -catenin accumulating cell clusters (Figure 1A indicated as +). Immunohistochemical staining procedure for β -catenin was conducted as described above. Cell fractions of interest were separated using the laser-microdissection microscope P.A.L.M. MicroBeam from Carl Zeiss (P.A.L.M. Microlaser Technologies GmbH, Bernried, Germany). DNA and RNA were isolated from up to four sections of each tumor sample (approximately 50.000 cells) as described above.

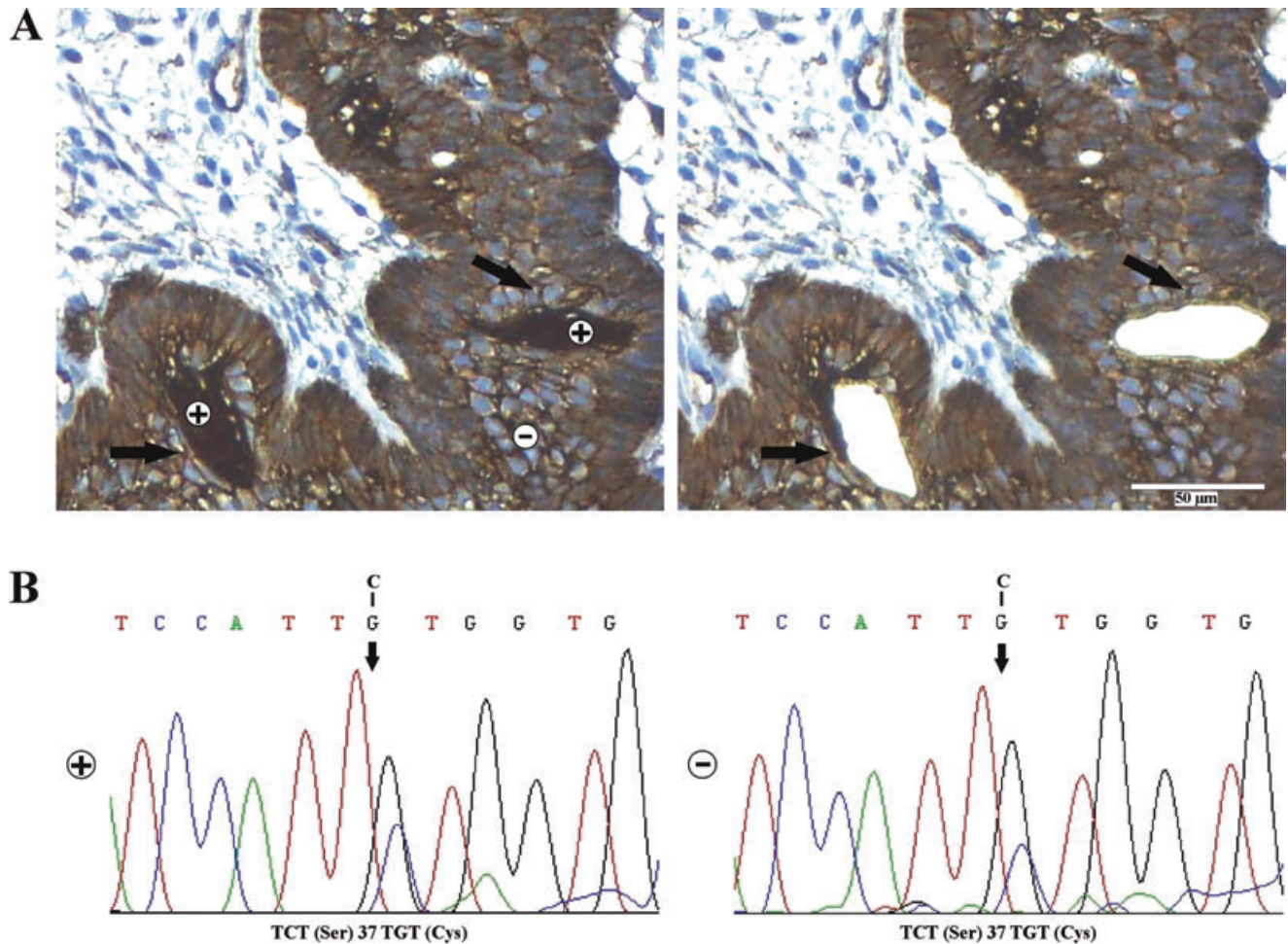


Figure 1. Molecular-genetic analysis of laser-microdissected tumor cells. For mutational analysis, we separated nuclear β -catenin accumulating (+) vs. non-accumulating (-) tumor cell fractions using laser microdissection. The arrows indicate accumulating cell clusters before and

after dissection (A). Direct sequencing of exon 3 of CTNNB1 revealed a missense mutation at codon 37 encoding for a serine residue and phosphorylation site of GSK-3 β kinase in both fractions [accumulating (+) and non-accumulating (-)] (B).

Mutational analysis

Exons 3, 4, 8, 9, 10, 11, 12 and 13 of the β -catenin gene were amplified by PCR using genomic DNA extracted from fresh frozen tumor samples, for subsequent single-strand conformation polymorphism (SSCP) analysis. PCR reactions were performed in a total volume of 10 μ L with 10 ng genomic DNA in a PCR buffer containing, in a final concentration of 1.5 mM MgCl₂ (Qiagen), 200 μ M of each dNTP, 200 nM of forward and reverse primer (Table 1) and 0.25 U Taq polymerase (Qiagen).

The amplified PCR products were denatured for 10 minutes at 95°C. SSCP electrophoresis of exons and exon fragments was conducted on polyacrylamide gels with different concentrations (10% and 14%) and varying bis-acrylamide/acrylamide ratios (1:10, 1:29, 1:49, 1:99), with or without 5% glycerol in 0.5 \times tris-borate-EDTA-bufer (TBE; Merck, Darmstadt, Germany) at room temperature. The single and double strands of the PCR products were visualized by silver staining, as described previously (5). The shifted bands were excised from the wet gel, eluted and reampli-

Table 1. Primer used for mutation analysis of CTNNB1.

CTNNB1		
Exon	Forward primer 5'-3'	Reverse primer 5'-3'
3	gatttgatggagttggacatgg	tggtcttgagtgaaggactgag
4-a	aggtaaatgctgaactgtgg	gatctgcatgccctcatcta
4-b	tagatgagggcatgcagatc	gtcattgcttacctgtcct
8	gtggaatgcaagcttagga	tctgaaccagaagctgaac
9-a	aactggtgccatgggaatag	cacttggcagaccatcatct
9-b	gttcagctctctgggtcaga	gtcagatgacgaagagcaca
9-c	gacaggggaagacatcactga	agccatccaacagctagaga
10	gttgagttgtatgccagttc	agatagccaggtatcactgt
11	tacagaggagaatgccctgt	cacttcacttaaacatacctgc
12	gtgtttctccttagctgct	ctgtaaaggcttggttcc
13	ctcctgtctcttagcgaca	tgctcacatttccctacctc

fied. DNA purification and sequencing was done by GATC Biotech AG (Konstanz, Germany) and the DNA sequencing facility of the Department of Human Genetics, University Hospital Cologne, Germany. Primers used for mutational screening are listed in Table 1.

For *CTNNB1* gene exon 3 analysis of laser-microdissected samples (β -catenin accumulating vs. non-accumulating cells), PCR amplicons were purified from agarose gels using the high pure PCR product purification kit (Roche, Mannheim, Germany) and cloned into the Topo TA cloning vector (Life Technologies) according to the manufacturer's instructions. Ten white colonies were picked, as blue white screening indicates plasmid integration. To confirm β -catenin content, a colony PCR was conducted. Amplified plasmid-DNA was extracted employing the plasmid midi kit® (Qiagen) and submitted to sequence analysis.

RT-PCR analysis

TaqMan® gene expression assays (Applied Biosystems, Darmstadt, Germany) were employed for relative quantification. Analyses were performed with the applied Biosystems 7500 fast RT-PCR system. The RNA isolated from laser-microdissected FFPE cells, was transcribed in cDNA and preamplified using the TaqMan® PreAmp Master Mix Kit (Applied Biosystems) as recommended. Uniformity of all assays used was checked previously as described in the manufacturer's instructions.

Relative quantification analysis of β -catenin target genes *Axin2/Conductin* (Hs01063170_m1) and *BMP4* (Hs00370078_m1) was performed using total tumor cDNA from adaCP (n = 5) and papCP (n = 5). To analyze whether nuclear β -catenin accumulation in adaCP correlates with target gene activation, we analyzed the expression of *Axin2/Conductin* within accumulating and non-accumulating cell fractions of three representative tumor samples. As endogenous control served *ubiquitin* (Hs00824723_m1) and *β -2-microglobulin* (Hs99999907_m1) using the letter for analysis. The experiment was replicated three times, whereas a non-template control was carried along and always revealed a negative result.

RESULTS

AdaCP show target gene activation of the Wnt signaling pathway, particularly in cell clusters with nuclear β -catenin accumulation

Total cDNAs obtained from snap frozen papCP (n = 5) as well as adaCP (n = 5) were analyzed employing TaqMan *Axin2/Conductin* and *BMP4* gene expression assays. Samples were normalized to *β -2-microglobulin*, used as housekeeping control gene. Relative quantification of *Axin2/Conductin* and *BMP4* expression in adaCP was performed using the average of papCP as calibrator. The expression of *Axin2/Conductin* was enhanced in adaCP between 28.8 fold (case 3239) and 288.4 fold (case 3598) compared with papCP. *BMP4* expression was increased in a range of 1.3 fold (case 3239) to 4.3 fold (case 1551) in adaCP vs. papCP (Figure 2A).

To examine the impact of nuclear β -catenin accumulation on target gene expression, we performed the same TaqMan analysis using cDNA of laser-microdissected fractions including β -catenin accumulating vs. non-accumulating epithelial tumor cells of three

representative tumor samples (case numbers 2874, 3239 and 3598). *β -2-microglobulin* was used to equalize the samples. The β -catenin accumulating cell fraction revealed an up-regulation of *Axin2/Conductin* in a range of 3.9 fold (case 2874) to 24.6 (case 3239) compared with the non-accumulating cells. The expression of *BMP4* was enhanced between 1.2 fold (case 3239) and 20.9 (case 2874) in accumulating vs. non-accumulating tumor cell mRNA (Figure 2B).

Correlation of Axin2 as well as BMP4 protein distribution and gene expression

Axin2, as well as *BMP4* protein distribution, was immunohistochemically analyzed and compared with nuclear β -catenin staining in adaCP using serial sections. As depicted in Figure 3, there was a distinct association between elevated Axin2 and *BMP4* content and tumor cells with nuclear β -catenin accumulation. In surrounding tumor cells without nuclear β -catenin accumulation, both proteins were detectable, but were expressed obviously lower (data not shown) as indicated by RT-PCR.

Mutations in exon 3 (CTNNB1) are not sufficient to induce nuclear β -catenin accumulation in CP per se

Eight different FFPE tumor samples showing a representative number of cells with distinct nuclear β -catenin accumulation (>50.000), as detected by IHC, were selected for mutational analysis. To have the possibility to analyze accumulating vs. non-accumulating epithelial tumor cell clusters independently, both fractions were separated using laser-microdissection (Figure 1A). Subsequent mutational analysis within the region encoding the phosphorylation sites (exon 3 of *CTNNB1*) revealed activating missense mutations in both fractions without discovering a significant pattern associated with nuclear accumulation (Figure 1B, Table 2). Experiments were repeated twice to control for paraffin-induced aberrations.

Mutational analysis of exon 4 as well as exon 8–13 (CTNNB1) revealed no genetic alterations in CP

We used SSCP analysis in 32 adaCP and four papCP to detect mutations in exon 4 of the *CTNNB1* gene encoding phosphorylation sites regulating dissociation of β -catenin from adherens junctions. In addition, we examined exon 8–13 encoding the armadillo-repeats which are essential for the passive nuclear im- and export of β -catenin. No mutations were identifiable in these gene regions (data not shown).

DISCUSSION

Constitutive Wnt signaling activation is recognized as an important pathomechanism in cancer origin and progression (4). Distinct nuclear β -catenin accumulation represents a histopathological hallmark of adaCP, differentiating this variant from other cystic lesions in the sellar region (10). In addition, these tumor cells show specific morphologic features with arrangement in circumscribed whirl-like clusters and simple epithelial differentiation (6). Activating

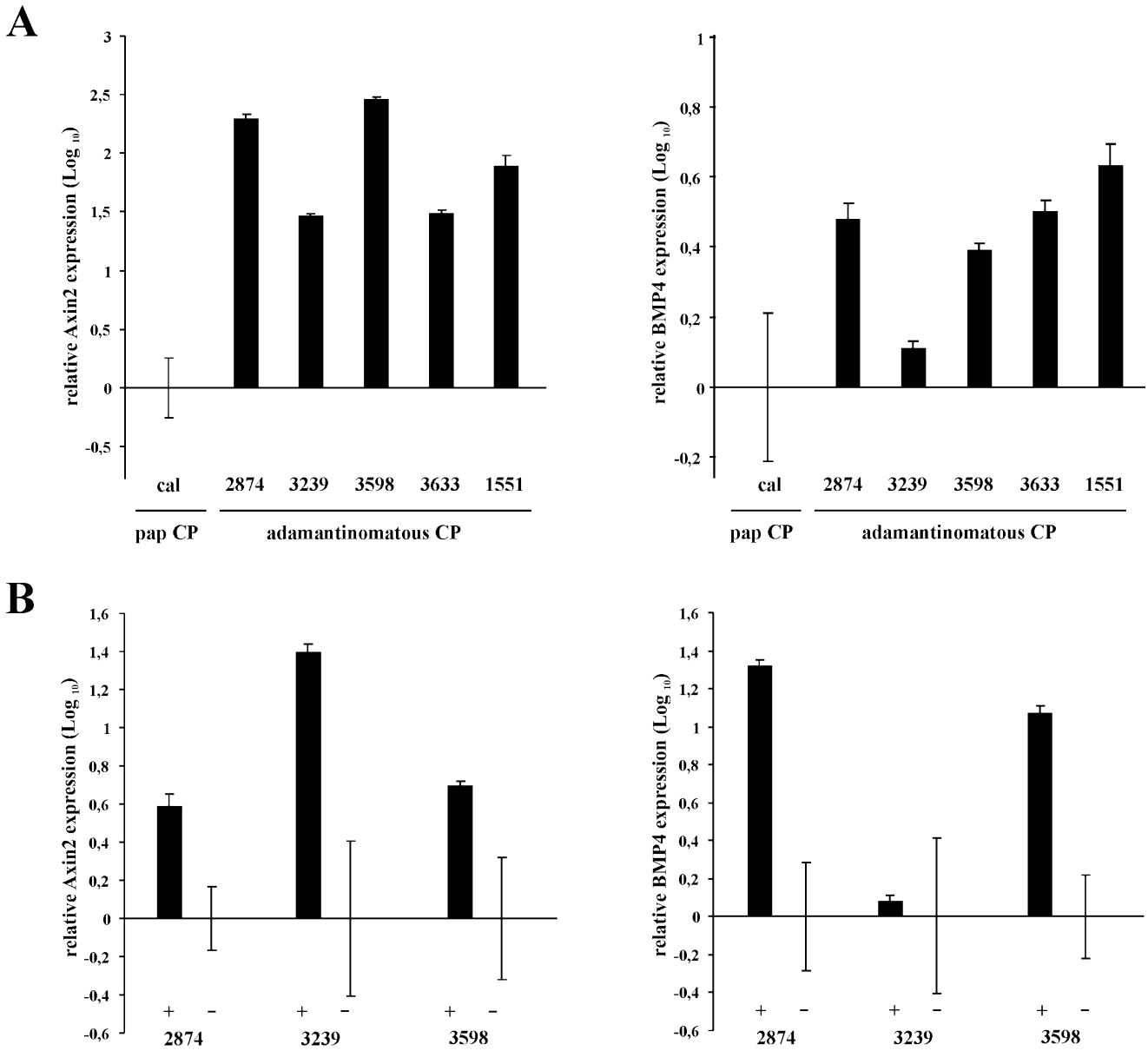


Figure 2. Elevated levels of Axin2/conductin and BMP4 in total tumor cDNA of adaCP vs. papCP as well as in accumulating vs. non-accumulating tumor cell mRNA. **A.** Relative quantification TaqMan analysis of Axin2/Conductin and BMP4 cDNA obtained from snap frozen and histologically confirmed papCP (n = 5) and adaCP (n = 5), harboring a sufficient amount of β -catenin accumulating tumor cell clusters. β -2-microglobulin served as endogenous control and papCP were used as

calibrator. All analyses were performed in quadruplicates of each sample. **B.** mRNA expression of β -catenin target genes Axin2/Conductin as well as BMP4 is increased in laser-microdissected tumor cell clusters with nuclear β -catenin accumulation (+) in relation to non-accumulating cells (-) (TaqMan analysis). The experiments were conducted three times.

mutations within exon 3 of the β -catenin gene (*CTNNB1*) were likely to cause this nuclear β -catenin translocation as a consequence of disordered degradation and intra-cytoplasmatic accumulation (7, 33). However, molecular-genetic studies demonstrated a broad range of missense mutations within *CTNNB1* in adaCP, primarily affecting codons 32, 33, 37, 41, 43 and 45 (7, 15, 33). To the best of our knowledge, confirmation of target gene activation in adaCP as well as the heterogeneous distribution of tumor cells with *CTNNB1*

mutations and nuclear β -catenin accumulation remains to be shown. To address this intriguing issue, we conducted gene expression and mutational analyses. Our study design reliably identifies elevated levels of well-accepted β -catenin target genes, that is, Axin2 and BMP4 in adaCP compared with papCP. Furthermore, we could demonstrate that elevated gene expression levels were associated with nuclear β -catenin accumulating tumor cell clusters and corresponding protein accumulation, as shown by IHC.

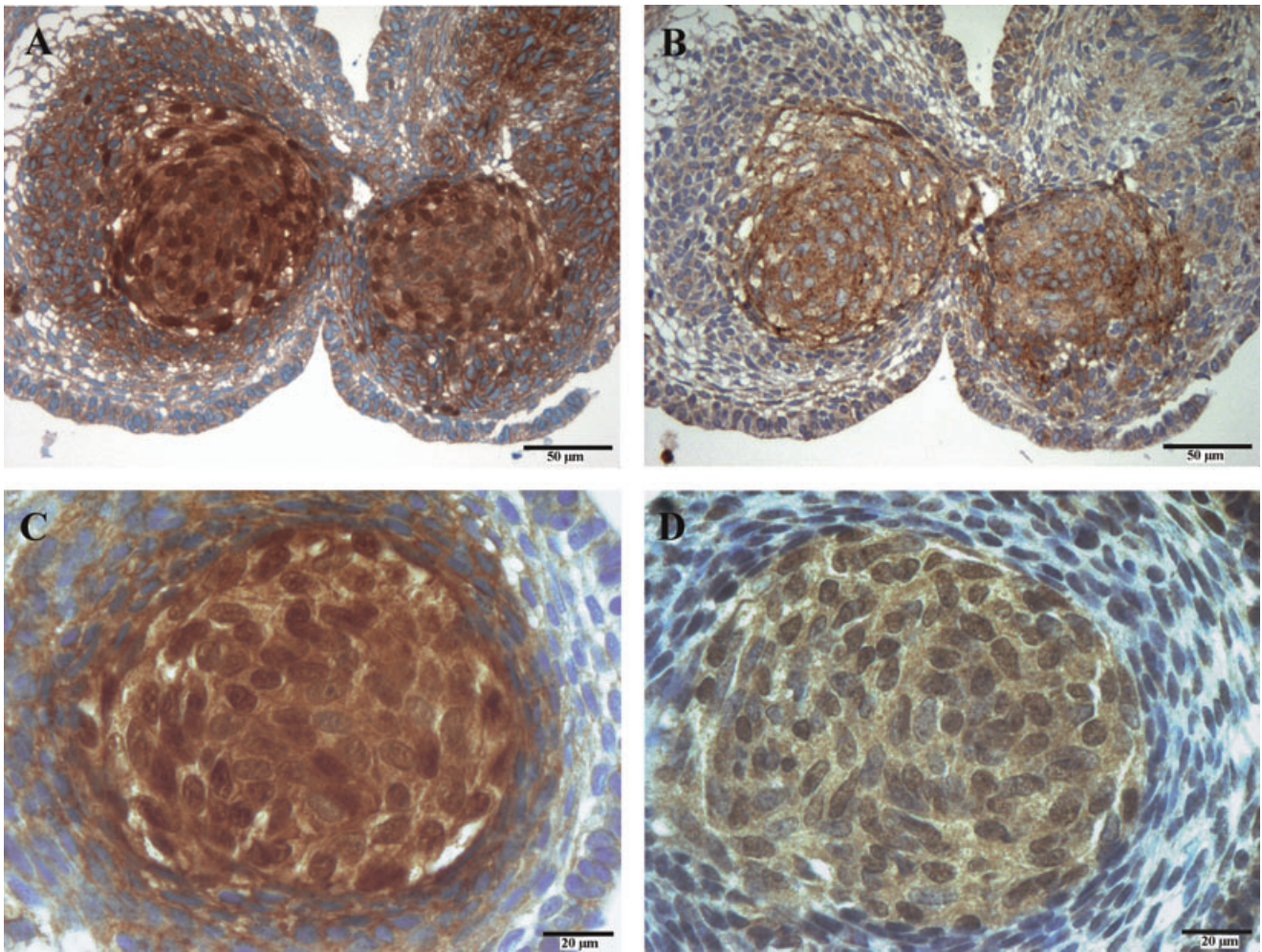


Figure 3. Immunohistochemical co-localization of Axin2/BMP4 and β -catenin. Using serial sections, whorl-like tumor cell clusters showing

nuclear β -catenin accumulation (A,C), displayed elevated levels of Axin2 (B) and BMP4 (D). Magnification is 200x for A,B and 400x for C,D.

Noteworthy, this is the first report indicating *BMP4* as a β -catenin target gene in adaCP. *Lef1* expression is activated by *BMP4*, and oncogenic β -catenin is required for *BMP4* expression in human cancers (17, 19). *BMP4* is important in many developmental processes, particularly during early tooth development

where its expression occurs downstream of Wnt signaling (21, 35, 38). In contrast to papCP, adaCP express various tooth specific genes and exhibit an odontogenic morphological appearance (27, 34). Elevated expression levels of *BMP4* in adaCP, further confirm its probable oral ectodermal origin and that developmental pro-

Case	Age (years)/gender	Mutations in accumulating cells	Mutations in cells without accumulation
adaCP 1	48/F	TCT(Ser)37TGT(Cys)	GAC(Asp)32CAC(His)
adaCP 2	9/F	TCT(Ser)37TGT(Cys)	TCT(Ser)37TGT(Cys)
adaCP 3	9/F	ACC(Thr)41ATC(Ile)	ACC(Thr)41ATC(Ile)
		GAC(Asp)32AAC(Asn)	
adaCP 4	3/F	TCT(Ser)37TGT(Cys)	TCT(Ser)37TGT(Cys)
adaCP 5	25/F	GAC(Asp)32CAC(His)	GAC(Asp)32CAC(His)
		ACC(Thr)41ATC(Ile)	TCT(Ser)37TGT(Cys)
adaCP 6	2/F	TCT(Ser)37TGT(Cys)	TCT(Ser)33TGT(Cys)
adaCP 7	14/F	GAC(Asp)32CAC(His)	TCT(Ser)37TGT(Cys)
adaCP 8	35/M	TCT(Ser)37TGT(Cys)	TCT(Ser)37TGT(Cys)

Table 2. Mutational analyses of exon 3 (*CTNNB1*) in accumulating vs. non-accumulating tumor cells. DNA was extracted from laser-microdissected, FFPE tissue pre-stained immunohistochemically for β -catenin (Figure 2). PCR product was cloned and subsequently sequenced. Abbreviations: F = female; M = male; FFPE = formalin fixed and paraffin embedded.

cesses mimicking tooth formation occur in adaCP. In particular, nuclear β -catenin accumulating tumor cell clusters show an increased expression of the enamel knot marker p21 and are characterized by a specialized cytoarchitecture and differentiation resembling the early tooth anlage (6, 21).

Compared with *Axin2* cDNA expression levels, the difference of *BMP4* expression between the CP subtypes is significantly lower. A possible explanation for this observation could be that *Axin2* was identified not only as a β -catenin target gene but also being involved in cytoplasmatic β -catenin destruction as a component of the multiprotein-degradation-complex (2, 13). In the latter role, increased levels of *Axin2* could be an indicator for an elevated negative feedback loop to opposite the activated Wnt signaling pathway in adaCP.

After demonstrating target gene activation in nuclear β -catenin expressing cell clusters, we were interested to characterize alterations involved in nuclear protein accumulation. Herein, we were able to demonstrate that both cell populations, β -catenin accumulating as well as non-accumulating cells, harbor activating mutations in exon 3 of the *CTNNB1* gene, without identifying a cell specific mutation pattern. This implicates that alterations in β -catenin gene regions encoding for phosphorylation sites crucial for protein degradation may not be sufficient to cause nuclear accumulation per se. This assumption is supported by a recent study of Sekine *et al* describing β -catenin mutations within the mesenchymal component of adaCP, displaying no nuclear β -catenin accumulation (33). In contrast, Kato *et al* were not able to verify mutations beyond the epithelial tumor cell component (15). Previous studies also identified multiple mutations in a single CP sample, which could suggest an increased genetic instability. However, chromosomal instability appears unlikely in CP (7, 41). Genetic heterogeneity within one tumor refers rather to a polyclonal origin of tumor cells and data from our, and previous, mutational analysis indicate that nearly all aberrations were heterozygous (31).

Cytoplasmatic β -catenin levels and corresponding nuclear accumulation is not only determined by activating mutations. Many other mechanisms are likely to play a role, that is, E-cadherin binding as well as active and passive nuclear shuttling processes. Nuclear localization and transcriptional activity of β -catenin is prevented by binding to E-cadherin (26). The membranous binding of β -catenin to the intracellular part of cadherins and α -catenins is regulated by phosphorylation of tyrosine residues Y142 encoded by exon 4, as well as Y489 and Y654 encoded by exons 9 and 13 of the *CTNNB1* gene, the latter being partly identical to the armadillo-repeat sequence (20). The armadillo repeat represents a core region of β -catenin forming a super helix with a positively charged groove for protein binding, thus, regulating its activity (11). Identified binding partners include TCF-family transcription factors, Axin2 and APC (11, 40). In addition, armadillo repeat units 10–12 are essential for the passive nuclear-pore-complex translocation of β -catenin (18). Although compromised nuclear shuttling of β -catenin may lead to nuclear accumulation, we were neither able to verify mutations within the *CTNNB1* region encoding for the armadillo repeats (exon 8–13), thus indicating an intact passive nuclear transport machinery, nor in additional gene regions (exon 4) encoding interaction sites for membrane anchorage (20).

In conclusion, target genes of the Wnt signaling pathway are activated in adaCP, especially in cells with aberrant nuclear β -catenin accumulation. The increased expression of inhibitors of

the Wnt signaling pathway, that is, *Axin2/Conductin* indicate an activated negative feedback loop to opposite Wnt signaling in adaCP, whereas, elevated levels of *BMP4* point to an oral ectodermal differentiation. Activating mutations in exon 3 of *CTNNB1* are not restricted to nuclear β -catenin accumulating tumor cells. Lack of additional alterations in the *\beta*-catenin gene affecting important phosphorylation and interaction sites, points towards additional mechanisms being involved in the molecular trapping of β -catenin within the nucleus. Further studies are mandatory to clarify the role of important components of the Wnt signaling pathway, that is, APC to be involved in this process.

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