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Wnt/β-catenin signal alteration and its diagnostic utility in basal cell adenoma and histologically similar tumors of the salivary gland

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ABSTRACT

Differential diagnosis among basal cell adenoma (BCA), basal cell adenocarcinoma (BCAC), adenoid cystic carcinoma (ACC) and pleomorphic adenoma (PA) of the salivary gland can be challenging due to their similar histological appearance. Although frequent nuclear β-catenin expression and *CTNNB1* mutations have been reported in BCA, further details of the Wnt/β-catenin signal alterations are unclear. The aim of this study was to assess the diagnostic utility of Wnt/β-catenin signal alteration in BCA and morphological mimics. We performed immunohistochemical staining for β-catenin and mutation analysis for Wnt/β-catenin-related genes (*CTNNB1*, *APC*, *AXIN1* and *AXIN2*) in BCA (n = 34), BCAC (n = 3), ACC (n = 67) and PA (n = 31). We also analyzed ACC-specific *MYB* and *MYBL1* gene rearrangements by fluorescence in situ hybridization (FISH). Nuclear β-catenin expression (≥3%) was present in 32/34 cases (94.1%) of BCA, and the nuclear β-catenin labeling index was significantly higher than in other tumor types (p = < 0.0001). In BCA, we found mutations in *CTNNB1*, *APC* and *AXIN1* genes (41.1%, 2.9% and 8.8%, respectively). In BCAC, nuclear β-catenin expression with *CTNNB1* mutation was present in 1/3 cases (33.3%). As for ACC, nuclear β-catenin expression was observed in 3/67 cases (4.4%), but all 3 cases harbored either *MYB* or *MYBL1* gene rearrangement. The results suggest that nuclear β-catenin immunoreactivity with appropriate criteria may be helpful to distinguish BCA from histologically similar tumors. However, a minor subset of ACCs with nuclear β-catenin expression require careful diagnosis. In addition, Wnt/β-catenin signal alteration may play a role in the pathogenesis of BCA and BCAC.

1. Introduction

Basal cell adenoma (BCA) is a benign tumor accounting for 1–3% of salivary gland epithelial tumors [1,2], and is histologically characterized by basaloid cell proliferation in an anastomosing jigsaw puzzle-like solid or trabecular pattern with peripheral palisading [3]. Other kinds of salivary gland tumors with basal cell differentiation can share morphologic features with BCA. Such tumors include basal cell adenocarcinoma (BCAC), adenoid cystic carcinoma (ACC), and pleomorphic adenoma (PA). BCAC is the malignant counterpart to BCA, and has a relatively high recurrence rate but favorable prognosis [4–6]. BCA and ACC show similar histological appearance in terms of their focal or extensive cribriform structures [7,8]. ACC is generally indolent, but the long-term prognosis is poor due to persistent local recurrence and distant metastases. Thus, differential diagnosis of these tumors is clinically very important, but sometimes challenging. Precise diagnosis can be particularly difficult for cases in which only biopsy or other small samples are available for analysis.

Characteristic molecular abnormalities have been identified in salivary gland tumors. β-catenin is one of the canonical Wnt signaling pathway components regulating the expression of Wnt target genes. β-catenin is usually maintained at a low level in the cytoplasm due to phosphorylation by a multiprotein complex containing APC, Axin, GSK3β and PP2A. Mutations of *CTNNB1*, *APC* or *AXIN* result in nuclear β-catenin accumulation linked with abnormal activity of Wnt/β-catenin signaling pathway [9], and play a role in the pathogenesis of various diseases [10–12]. Nuclear β-catenin expression is observed in most cases of BCA, and activating mutations of *CTNNB1* have been identified

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in about half of BCAs [13–15]. However, the molecular mechanisms causing nuclear accumulation of β-catenin, other than *CTNNB1* mutation, remain to be clarified. Although nuclear β-catenin expression has been reported in some cases of basal cell adenocarcinoma (BCAC), the pathogenesis of BCAC has been unclear [14].

As for ACC, *MYB-NFIB* fusion gene has been identified in approximately 60% to 70% of cases [16–19]. In a recent study, the presence of *MYBL1* gene rearrangement was reported in about 25% of ACCs without *MYB* gene rearrangement [20]. In a previous report about Wnt/β-catenin signal alteration in ACC, mutations in *CTNNB1*, *APC* or *AXIN1* were found in some populations of ACCs [21]. However, the relationship between gene rearrangement in *MYB* or *MYBL1* and Wnt/β-catenin signal alteration has not been clarified in ACC.

The aim of this study was to further elucidate the Wnt/β-catenin signal alterations in BCA and the diagnostic utility of these alterations in the differentiation between BCA and morphological mimics.

2. Materials and methods

2.1. Case selection

We examined 135 cases of salivary gland tumors, including BCA (n = 34), BCAC (n = 3), ACC (n = 67) and PA (n = 31). These cases were selected from the institutional database of Kyushu University Hospital and its affiliated hospitals, dating from 1983 to 2014. BCA, BCAC and PA occurred in parotid gland (n = 33, n = 3 and n = 22, respectively) and submandibular gland (n = 1, n = 0 and n = 9, respectively). Forty-seven cases of ACC occurred in parotid gland (n = 9), submandibular gland (n = 13), sublingual gland (n = 3) and nasal/paranasal cavity (n = 22). The remaining twenty cases occurred in other sites of head and neck (Table 1). This study was approved by the Institutional Review Boards of Kyushu University (no. 26–185, 29–200) and the National Kyushu Cancer Center (no. 2015-5).

2.2. Immunohistochemical staining for β-catenin

Immunohistochemical staining was performed using 4-μm-thick, formalin-fixed, paraffin-embedded (FFPE) tissue sections and the primary antibodies for β-catenin (clone 14, mouse monoclonal; dilution × 200; BD Biosciences, San Jose, CA). A biotin-free, horseradish peroxidase enzyme-labeled polymer method (EnVision+ system; Dako, Carpinteria, CA) was used. All tissue sections were counterstained with hematoxylin.

We evaluated at least 500 cells in the hot spot, then assessed the labeling index of nuclear immunoreactivity of β-catenin in neoplastic cells. We drew a ROC curve to calculate a cut-off value of the nuclear β-catenin labeling index for BCA compared with other tumors (BCAC, ACC and PA), and found that 3% was the most appropriate value (see Results).

Table 1
Clinical data of BCA, BCAC, ACC and PA.

	BCA	BCAC	ACC	PA
	n = 34	n = 3	n = 67	n = 31
Age (years)[a]	60 (18–78)	61 (30–77)	61 (22–81)	54 (13–84)
Sex				
Male	12 (35.3%)	1 (33.3%)	28 (41.8%)	8 (25.8%)
Female	22 (64.7%)	2 (66.7%)	39 (58.2%)	23 (74.2%)
Site				
Parotid gland	33 (97.1%)	3 (100%)	9 (13.4%)	22 (71.0%)
Submandibular gland	1 (2.9%)		13 (19.4%)	9 (29.0%)
Sublingual gland			3 (4.5%)	
Nasal/Paranasal cavity			22 (32.8%)	
Other head and neck regions[b]			20 (29.9%)	

Abbreviation: BCA, basal cell adenoma; BCAC, basal cell adenocarcinoma; ACC, adenoid cystic carcinoma; PA, pleomorphic adenoma; M, male; F, female; N.A., not available data.
[a] The data are shown by median with the range in the parenthesis.
[b] Other head and neck regions include tongue (n = 7), palate (n = 1), orbit (n = 3), lacrimal duct (n = 2), external ear (n = 4), nasopharynx (n = 2) and trachea (n = 1).

2.3. Mutational analysis for CTNNB1, APC and AXIN

We extracted genomic DNA from FFPE tissue sections by using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. To detect mutations in *CTNNB1* (exon3), *APC* (exon15), *AXIN1* (exon5, 7, 10) and *AXIN2* (exon1, 5, 7) [10], we performed a polymerase chain reaction (PCR) followed by Sanger sequencing, as previously described [22]. The primer sequences are summarized in **Supplementary** Table 1. The PCR products were subjected to 2% agarose gel electrophoresis, and the sequence was confirmed by the direct sequencing method using an ABI Prism 310 sequence analyzer (Applied Biosystems, Foster City, CA, USA).

2.4. MYB and MYBL1 fluorescence in situ hybridization (FISH) and evaluation

Fluorescence in situ hybridization (FISH) was done on FFPE tissue sections of ACC to detect *MYB* or *MYBL1* gene rearrangements. We used commercially available break-apart probes for the *MYB* gene (*MYB* Split Dual Color FISH Probe; GSP Laboratory, Kobe, Japan) and *MYBL1* gene (*MYBL1* Split Dual Color FISH Probe; GSP Laboratory, Kobe, Japan) in accordance with the manufacturer's instructions. In the *MYB* FISH probe design, the 5′ *MYB* signal was labeled with spectrum green, and the 3′ *MYB* signal was labeled with spectrum red. In the *MYBL1* FISH probe design, the 5′ *MYBL1* signal was labeled with spectrum red, and the 3′ *MYBL1* signal was labeled with spectrum green. The hybridized slides were reviewed on a fluorescent microscope (Olympus BX53; Olympus, Tokyo, Japan) under a × 100 objective lens with oil immersion using a DAPI/Green/Red triple band-pass filter set. The results were scored by evaluating 100 non-overlapping tumor cells with hybridization signals. Tumor cells lacking hybridization signals were excluded from evaluation. A split signal was defined by 5′ and 3′ signals observed at a distance larger than the signal size, and signals separated by a distance smaller than the signal size were regarded as fused signals. When the ratio of tumor cells having split signals or isolated 5′ signals was more than 20%, the samples was considered to be positive for gene rearrangement.

2.5. Statistical analysis

All calculations were done using JMP Statistical Discovery Software (version 11.0.0; SAS, Cary, NC). We used the Wilcoxon rank sum test to evaluate the comparisons between basal cell tumors and mimics. The results were considered significant when the p-value was < 0.05.

3. Results

3.1. Histological findings and immunohistochemical results for β-catenin

BCA showed diverse histological appearances, including predominantly tubulotrabecular (n = 28) (Fig. 1**A**), cribriform (n = 1) (Fig. 1**B**), solid (n = 4) and membranous (n = 1) patterns. Among these 34 cases of BCA, 10 cases (29.4%) had at least focally cribriform or membranous structure similar to ACC. In most BCAs (n = 32), strong nuclear β-catenin immunoreactivity was present in abluminal cells, but not in luminal cells (Fig. 1**C**). One case of membranous-type BCA with focal tubulotrabecular component showed nuclear β-catenin expression. Two cases without nuclear β-catenin expression were tubulotrabecular-type, but not membranous-type.

BCAC resembled BCA cytologically but exhibited infiltrative growth