Expression of β-catenin in carcinoma in pleomorphic adenoma, pleomorphic adenoma and normal salivary gland: An immunohistochemical study

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ABSTRACT

Objetives. Pleomorphic adenomas are the most frequent type of epithelial salivary gland neoplasms, and their malignant counterpart, the carcinoma in pleomorphic adenomas, is much less common. β-catenin is a cell adhesion molecule associated with the invasion and metastasis of carcinomas of the head and neck, esophagus. The objective of this study was to detect the expression of β-catenin in pleomorphic adenomas, carcinomas in pleomorphic adenomas and normal salivary glands to discuss its role in the development of these two lesions.

Study Design. The expression of β-catenin (BD Transduction Laboratories) was analyzed by immunohistochemistry in formalin-fixed, paraffin embedded specimens by the avidin-biotin-peroxidase complex method in 16 pleomorphic adenomas (12 from minor salivary glands), 3 carcinomas in pleomorphic adenomas (all from palate) and 10 normal salivary glands as control group (5 from major and 5 from minor salivary glands).

Results. All cases of glands, adenomas and carcinomas in pleomorphic adenomas have membranous and cytoplasmic immunostaining. Nuclear β-catenin immunostaining was not observed. The antibody presented a fine granular arrangement in the cytoplasm and cellular membrane of duct and acinic cells. Higher β-catenin index rates were seen mainly in salivary gland ducts and in ductal structures in the adenomas and carcinomas in pleomorphic adenomas. There was protein loss in pleomorphic adenomas and cytoplasmic accumulation in carcinoma in pleomorphic adenomas.

Conclusions. The present study showed participation of the loss of β-catenin adhesion molecule in the development of pleomorphic adenoma, and that the cytoplasmic accumulation of the molecule takes part in the malignant transformation of the pleomorphic adenoma into carcinoma in pleomorphic adenoma.

Key words: *Pleomorphic adenoma, Carcinoma in pleomorphic adenoma, β-catenin, immunohistochemistry.*

INTRODUCTION

Pleomorphic adenomas are the most frequent type of salivary gland neoplasms; however, their malignant counterpart, the malignant mixed tumor, is much less common (1,2). β-catenin was originally identified on the basis of its association with cadherin adhesion molecules and it is now widely recognized as an essential element of the wingless WNT signaling cascade (3). β-catenin is significantly associated with the invasion and metastasis of carcinomas of the head and neck, esophagus, stomach, colon, liver, lung, breast, female genitalia, prostate, bladder, pancreas and melanomas (4-9). Although it is generally assumed that β-catenin may play major roles not only in malignant transformation but also in the regulation of physiological functions, the expression of this adhesion molecule in human salivary gland neoplasms has been investigated (10-12).

In the present study, in an attempt to clarify the possible role of cell adhesion in oncogenesis and/or cytodifferentiation of pleomorphic adenomas and carcinomas in pleomorphic adenomas of the salivary glands, the expression of β-catenin was immunohistochemically examined in these lesions as well as in normal salivary glands, to analyze the expression of the protein from the viewpoint of malignant transformation.

MATERIALS AND METHODS

The cases (10 normal salivary glands, 16 pleomorphic adenomas, and 3 carcinomas in pleomorphic adenomas) analyzed in the study were obtained from the archives of the Department of Pathology at Bauru Dental School, University of Sao Paulo. Formalin-fixed, paraffin embedded specimens were used for the immunohistochemical analysis by the avidin-biotin-peroxidase complex method. Briefly, 3μm sections of tumors and glands were dewaxed and rehydrated prior to antigen retrieval, which was performed in a steamer at 100°C for 40min. These preparations were incubated in H_2O_2 for 5 min to block endogenous peroxidase. Then, they were incubated overnight at 4°C with primary mouse monoclonal antibody β-catenin (Clone: β-Catenin-14, 1:125; BD Transduction Laboratories - USA). Chromogenic detection was performed with 3,3-diaminobenzidine (DAB). Counterstaining was briefly performed with Mayer's hematoxylin. Negative controls for immunostaining were obtained by substituting the primary antibodies for PBS. Gastric fibromatosis was used as a positive control and there was immunopositivity on ducts of salivary glands and sometimes mucosal epithelium. Each cluster of cells was recorded as positive or negative. Clusters which exhibited fine brown granules of chromogen were considered mild stainging. The presence of thick granules characterized the moderate and intense expression. The percentage of cells staining positively for the antibody was assessed and graded: - , negative; + (0-33% of cells with mild staining); ++ (0-33% of cells with moderate to intense staining and 33-100% of cells with mild staining); +++ (33-100% of cells with moderate staining); ++++ (33-100% of cells with intense staining). The immunohistochemical results were analyzed by Student's t test. Differences were regarded as significant if $p<0.05$.

RESULTS

All cases of glands, adenomas and carcinomas in pleomorphic adenomas in this study had membranous and cytoplasmic immunostaining. It was not possible to distinguish membranous from cytoplasmic immunostaining.

Normal Salivary Gland

The salivary glands were included in the control group. There were 5 cases derived from major salivary glands (3 submandibular and 2 sublingual) and 5 cases derived from minor salivary glands (4 lower lip and 1 upper lip). Higher β-catenin index rates were seen in intercalated, striated and excretory duct cells and nerve fibers. There were some cells in the surface of acinic cells morphologically similar to myoepithelial cells that showed immunopositivity. The mucosal epithelium, when present, was also strongly positive. The inflammatory, acinic, vascular and muscle cells did not demonstrate expression of β-catenin.

Pleomorphic Adenoma

The present investigation included 12 women and 4 men with pleomorphic adenomas. The mean age was 44 years and six months (Table 1). This information was not available for three cases. The glands most commonly affected were located in the palate. The mean tumor size was three centimeters. Seventy-one percent of the pleomorphic adenomas showed some atypical feature on histological examination. These atypical features were histopathologically demonstrated as neoplastic cells with bizarre appearance, hypercellularity and hyperchromatism. The bizarre cells had an irregular shape and large nuclei with or without hyperchromatism, although their nucleoli were small and mitotic figures were few. These bizarre cells were seen in proliferating tumor cells in solid areas of sheets or strands between tubular structures, areas of tightly packed so-called hyaline cells or plasmocytoid cells and areas of loosely arranged stellate or polyhedral cells (Table 1). On immunohistochemical analysis, summarized in Table 2, the tubulo-ductal structures ranged from mild to intense positivity of β-catenin. Some clusters with or without squamous metaplasia were also positive. The tubular areas containing tubulo-ductal structures composed of two-layer cells, which may contain amorphous eosinophilic substances in their lumens, also showed moderate positivity for β-catenin. There were few positive cells on the sheets, yet there was no immunopositivity on mixoid, chondroid, osteoid, mucoid, and fibrohyaline stromas. The hyaline, clear and spindle-shaped cells (myoepithelial cells) were positive in some cases (6,3%, 12,5%, 12,5% respectively). Those cells with intermediate myoepithelial differentiation were not included in the present observations.

Carcinoma in Pleomorphic Adenoma

This sample comprised two women and one man with carcinoma in pleomorphic adenomas. The mean age was 62 years and one month. The tumors were located in the

palate and, histopathologically, the malignant component presented the phenotype of squamous cell carcinoma in all cases. The malignant features of carcinoma were histopathologically demonstrated as epithelial cells with increased nuclear ratio, prominent nucleoli and hyperchromatic nuclei with numerous mitoses. Higher β-catenin index rates were found in duct-like tumor cells of remaining benign pleomorphic adenoma. The malignant counterpart showed few cells positive to β-catenin, which were atypical squamous cells with pearl formation (Table 2 and Figure 2).

Comparison of two independent groups with the Student t test, as follows: salivary glands vs. pleomorphic adenomas, salivary glands vs. carcinoma in pleomorphic adenomas, and pleomorphic adenomas vs. carcinomas in pleomorphic adenomas, revealed statistically significant difference only between pleomorphic adenomas and salivary glands with p=0.008, but the reduced number of carcinoma in pleomorphic adenomas could have altered the result when this group was included in the comparison.

Table 1. Clinical and atypical histopathological features of pleomorphic adenomas.

Case	Gender	Age	Size (cm)	Location	Atypical Feature
1	F	58	5	Palate	Bizarre Cells
2	F	63	5	Submandibular	Absent
3	F	28	2	Palate	Absent
4	F	48	2	Parotid	Hypercellularity; Hyperchromatism
5	F	49	9	Palate	Hypercellularity; Hyperchromatism
6	M	39	-	-	Absent
7	F	12	-	Upper Lip	Absent
8	F	-	-	Palate	Hypercellularity; Hyperchromatism
9	F	38	1,5	Oral Mucosa	Hypercellularity; Hyperchromatism
10	M	38	1	Palate	Hypercellularity
11	F	36	2	Parotid	Absent
12	F	43	3	Palate	Hypercellularity; Hyperchromatism
13	M	-	2	Oral Mucosa	Hypercellularity
14	M	51	4	Palate	Hypercellularity; Hyperchromatism
15	F	44	-	Palate	Hypercellularity; Hyperchromatism; Pleomorphism
16	F	78	1	Palate	Hypercellularity; Hyperchromatism

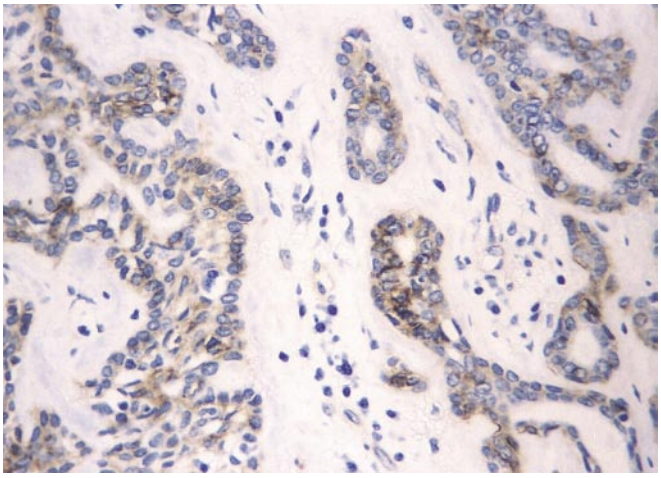

Fig. 1. Light micrograph showing the ductal structures of the pleomorphic adenoma immunostained with anti-mouse β-catenin. These structures presented positivety pattern with heterogeneous staining with mixed negative and positive cells. There was moderate to intense positivity. The stromal cells were stained negatively.(β-catenin, original magnification X400).

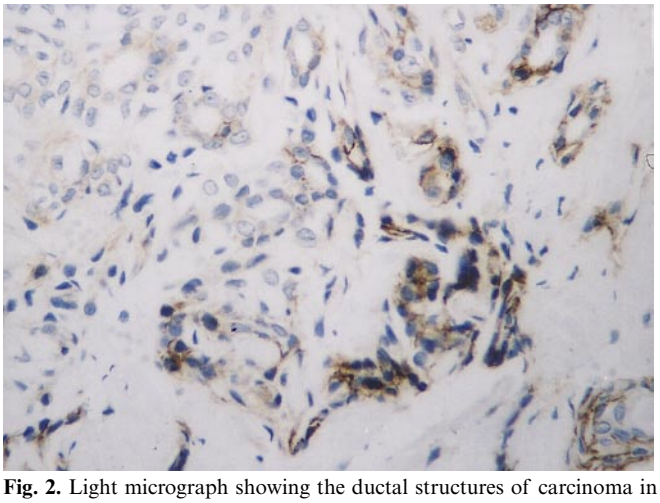

Fig. 2. Light micrograph showing the ductal structures of carcinoma in pleomorphic adenoma immunostained with anti-mouse β-catenin. There was intense positivity in some of these structures with heterogeneous staining with mixed negative and positive cells. The negative were mainly spindle-shaped cells and the positive were the ductal cells.(β-catenin, original magnification X400).

Table 2. Immunohistochemical findings of β-catenin in pleomorphic adenomas (case 1-16) and carcinoma in pleomorphic adenomas (case 1-3).

Case	Sheets	Clusters	Ductal Structures	Myoepithelial Cells	Squamous Metaplasia	Pearl Formation	Positive control
1	-	++	++	-	++	-	+
2	+	-	+	++	-	-	+
3	-	++	++	-	-	-	+
4	-	++++	++++	++++	-	-	+
5	-	-	+++	-	-	-	+
6	-	-	-	-	-	-	+
7	-	-	+	+++	-	-	+
8	-	+	-	-	-	-	+
9	+	-	+	-	-	-	+
10	-	-	+++	-	++	-	+
11	-	++	+	-	-	-	+
12	-	-	+	++	-	-	+
13	-	-	+	-	-	-	+
14	-	++	++	-	-	-	+
15	++	-	+++	-	-	-	+
16	-	+++	++++	++++	-	-	+
1	++	-	++++	++	-	++	+
2	++	-	+++	++	-	+	+
3	+	-	+++	-	-	-	+

DISCUSSION

The malignant potential of a pleomorphic adenoma increases with the duration of proliferation and increasing tumor size, so that pleomorphic adenomas could represent an early (pre)malignant neoplastic lesion (13-16). The present mean size of the samples of pleomorphic adenomas was three centimeters, yet there was a case with nine centimeters in diameter. The mean size of carcinomas in pleomorphic adenomas was one centimeter. The possibility of malignancy increased if the tumors had a diameter over five centimeters. There is a tendency for carcinoma in pleomorphic adenomas to occur more frequently in patients above 50 years (17).

A statistical difference was observed in the β-catenin expression between the salivary glands and pleomorphic adenomas groups. The gene of β-catenin is the CTNNB1, which has been mapped to chromosome 3p21 (18). β-catenin has been implicated in signal transduction (Wingless/Wnt) and specification of cell fate during embryogenesis (19). Recent studies have shown that β-catenin directly interacts with members of the Tcf/Lef family of architectural transcription factors involved in the activation of specific target genes (20).

There are several subgroups of genetic defects in pleomorphic adenomas mainly characterized by structural deviations and, in particular, reciprocal translocations (21). The largest subgroup is characterized by rearrangements involving the band 8q12. The gene on chromosome 8q12 is a novel, developmentally regulated zinc fingers gene, designated PLAG1 (21). The t(3;8)(q21;q12) translocation results in promoter swapping between PLAG1 and the constitutively expressed gene for β-catenin (CTNNB1)22.

This may be functionally significant, as it could have a substantial impact on the stability and/or translatability of the resulting mRNAs fusion and consequently also on the concentrations of PLAG1 and β-catenin[22]. This study confirms the reduction of expression of the adhesion molecule in neoplastic cells of the tumor if compared with duct gland cells (Figure 1). It can probably be associated with these translocations between PLAG1 and CTNNB1. There was a tendency of the neoplastic cells organized in ductal structures to continue expressing β-catenin (Figure 1) while the cells in strands, clusters and sheets lost this protein, so the protein can participate in the morphological variation of pleomorphic adenoma.

The membranous loss of the E-cadherin-catenin complex and nuclear translocation of β-catenin are early events of gastric carcinogenesis and participate in the adenoma-carcinoma sequence[6]. Some studies on salivary gland tumors have been conducted[23,24]. Abnormal E-cadherin-catenin adhesion system is a common phenomenon in mucoepidermoid carcinoma. Abnormal β-catenin expression is significantly correlated with histological grade and clinical stage, and among the cadherins and catenins, the expression of β-catenin is the best for prediction of patient clinical outcome[23]. The neoplastic cells of adenoid cystic carcinoma express E-cadherin for use in intercellular adhesion, but probably the cadherin-catenin complex might not operate properly, which is the cause of neoplastic cell dissociation, followed by invasion and metastasis[24]. The loss of function of the E-cadherin-catenin complex may result in an increased invasiveness and metastatic ability of malignant cells[5].

A strong and disorganized immunopositivity was observed in

ductal neoplastic cells of the benign counterpart of the carcinoma in pleomorphic adenoma (Figure 2). It seems to denote that the cytoplasmic accumulations of β-catenin by β-catenin gene mutations/translocations, or APC gene mutations can participate in the malignant transformation of pleomorphic adenoma. Many other genetic and cytogenetic subgroups of alterations with rearrangements and clonal changes have been associated with the malign transformation of pleomorphic adenomas, such as abnormalities of chromosome 17 and p53 gene deletion[25], mutations on c-erbB2 gene[26], gains seen in 13q1-2 and 15q1[27] and alterations in protoncogenes associated with c-myc, ras p21 and p53[28].

This study demonstrated that loss of the β-catenin adhesion molecule can be one among many other events in the development of pleomorphic adenoma. It was also observed that the cells expressing the protein could be organized in ductal structures, whereas cells that cannot express it are located in strands and sheets. The cytoplasmic accumulation of the molecule may take part in the malignant transformation of pleomorphic adenoma.

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