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Immunohistochemical expression of WNT5A and MMPs in odontogenic epithelial tumors and cysts

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ABSTRACT

Objective: The aim of this study was compare the expression of WNT5A and MMP2, 7 and 20, in frequent benign odontogenic tumors and odontogenic cysts, since these lesions have a different biological behavior.
Materials and methods: Eighty-one paraffin-embedded specimens of odontogenic tumors, including ameloblastoma and keratocystic odontogenic tumor, and thirty-two odontogenic cysts were used for immunohistochemical analysis.
Results: The expression of WNT5A in odontogenic tumors and inflammatory cyst was higher than in developmental odontogenic cyst. There was no statistical difference ($p < 0.05$) in the expression of WNT5A when comparing the analyzed tumors. The expression of MMP7 was lower in RC with a statistical difference when compared with all tumors and cysts. Statistical differences also occurred when comparing glandular odontogenic cyst (GOC) to keratocyst odontogenic tumor (KOT) and calcifying cystic odontogenic tumor (CCOT). MMP20 expression was higher in ameloblastoma when compared to adenomatoid odontogenic tumor (AOT), DC and GOC. The expression of MMP20 was lower in CCOT when compared to all tumors and cysts.
Conclusions: The expression of WNT5A in a group of odontogenic lesions suggests the participation of a non-canonical WNT signaling pathway in the progression and maintenance of these lesions. These molecules are possibly involved in the biological differences between odontogenic tumors and cysts. Considering previous studies, WNT5A may help promote the calcification seen in AOT, CCOT and CEOT by activating MMP7.

1. Introduction

Odontogenic cysts and tumors are a group of benign and malignant lesions that may occur at any age. Histologically, the odontogenic tumors are related to the remnants of the odontogenic epithelium, which includes the dental lamina, the enamel organ, and the root sheath of Hertwig (Kumamoto, 2006; Reichart and Jundt, 2008). The development and progression of these tumors is a complex and poorly understood process that may cause maxillofacial deformities. Aberrant function of the pathways involved in tooth formation, such as Sonic hedgehog (SHH), bone morphogenetic protein (BMP), HGF, FGF and WNT signaling, are associated with odontogenic tumors formation (Sarkar and Sharpe, 1999; Tucker and Sharpe, 1999; Jernvall and Thesleff, 2000).

WNT proteins are a large family of cysteine-rich secreted molecules, with at least 19 members identified in humans, triggering distinct signaling pathways (Wodarz and Nusse, 1998). WNT signaling is required in most embryonic developmental processes in both invertebrates and vertebrates (Yang, 2012). Abnormal WNT signaling has been reported in some tumors such as lung, lips, breast and prostate cancer (Huang et al., 2005; Xavier et al., 2009; Lu et al., 2012; Green et al., 2013).

There are three pathways related with WNT ligands: canonical WNT/β-catenin, and non-canonical "WNT/Ca2+" or "WNT/JNK". WNT5a is considered a non-canonical WNT family member that activates the β-catenin-independent pathway. WNT5A is implicated in several processes during embryogenesis including cell fate specification, tissue patterning, cell differentiation, and migration (Danielson et al., 1995). In odontogenesis, the expression of WNT5a

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Table 1
Clinical information of the 70 cases studied.

Tumor	Age (years)	Gender		Site	
		M	F	Md	Mx
Ameloblastoma	36 (14–72)	17 (57%)	13 (43%)	29 (97%)	1 (3%)
UA	25 (25–45)	7 (70%)	3 (30%)	10 (100%)	0
KOT	34.6 (8–65)	8 (72%)	3 (8%)	9 (82%)	2 (18%)
AOT	23 (11–56)	5 (50%)	5 (50%)	5 (50%)	5 (50%)
CCOT	43 (16–70)	7 (70%)	3 (30%)	5 (50%)	5 (50%)
CEOT	28 (7–64)	4 (40%)	6 (60%)	6 (60%)	4 (40%)
DC	29.6 (9–67)	7 (70%)	3 (3%)	7 (70%)	3 (3%)
GOC	51.9 (36–70)	5 (50%)	5 (50%)	10 (100%)	0
RC	50.6 (33–87)	8 (67%)	4 (33%)	6 (50%)	6 (50%)

Abbreviations: UA: unicystic ameloblastoma; KOT: keratocystic odontogenic tumor; AOT: adenomatoid odontogenic tumor; CCOT: calcifying cystic odontogenic tumor; CEOT: calcifying epithelial odontogenic tumor; DC: dentigerous cyst; GOC: glandular odontogenic cyst; RC: residual cyst.; Md: mandible; Mx maxilla.

begins in the bud stage and is prolonged until the enamel and dentin formation stage. WNT5A is also involved in the activation of several transcription factors related with tooth formation. Furthermore, in WNT5A-deficient mice, the teeth appear smaller and mis-patterned showing a delayed odontoblast differentiation (Sarkar and Sharpe, 1999; Peng et al., 2010).

Several studies suggest that WNT5A has oncogenic properties stimulating the migration and invasiveness in some cancer cells such as melanoma, breast cancer, lung cancer and gastric cancer (Huang et al., 2005; Kikuchi et al., 2012; Zhao et al., 2014). The aggressiveness caused by WNT5A is related with increased expression of matrix metalloproteinases (MMPs) 1, 2, 9 and 13. MMPs are zinc-containing endopeptidases that mediate cell surface-associated dissociation and degradation of the extra-cellular matrix (ECM). These proteins act with ECM components, favoring the invasion and proliferation of tumors cells (O'Sullivan et al., 2015).

Few studies report the expression of WNT5A in odontogenic lesions. Sukarawan et al. (2010) showed the participation of WNT5A in ameloblastoma progression. Since WNT5A is an important molecule in odontogenesis, and reported as being expressed in the dental epithelium, this study analyzed the expression of this molecule in frequent benign odontogenic tumors and cysts. Since WNT5A and metalloproteinases are related in different diseases, the expression of MMP2, 7 and 20 was also analyzed in this group of odontogenic lesions.

2. Material and methods

An experienced pathologist reviewed all cases for compliance to the WHO Classification of Tumors. Only representative cases were selected for immunohistochemistry. Thirty paraffin-embedded specimens of ameloblastoma, ten unicystic ameloblastoma, ten calcifying cystic odontogenic tumor (CCOT), ten calcifying epithelial odontogenic tumor (CEOT), ten adenomatoid odontogenic tumor (AOT), eleven cases of keratocyst odontogenic tumor (KOT), ten cases of dentigerous cyst (DC), ten glandular odontogenic cyst (GOC) and twelve residual cyst (RC) were used for immunohistochemical analysis. For comparison, 10 samples of normal oral parakeratinized epithelium tissue were included. Selected cases were received for routine diagnosis from 2008 to 2013. Age, gender, and localization of each tumor are represented in Table 1. The Ethics Committee of the School of Dentistry at University of São Paulo approved this study.

Sections 3 μm thick were cut and mounted on silanized slides then deparaffinized in xylene, rehydrated in a graded alcohol series, and washed in tap water. For antigen-retrieval, sections were immersed in citric acid solution 0.1 mM, pH 6.0, and microwaved for 15 min. Endogenous peroxidase activity was blocked with methanol containing 0.3% hydrogen peroxide. The optimized dilution for each antibody was: WNT5A (cat# ab72583, rabbit polyclonal, Abcam, Cambridge, MA, EUA) 1:12,000, MMP2 (cat# ab3158, Mouse monoclonal, Abcam) 1:100, MMP7 (cat# ab3205, mouse monoclonal, Abcam), MMP20 (cat# sc26926, goat polyclonal, Santa Cruz, Santa Cruz, CA, USA). All antibodies were incubated at 4 °C overnight. This procedure was followed by ADVANCED HRP (cat# K406987, Dako, Carpinteria, USA) polymer conjugate and Dako Liquid DAB plus Substrate Chromogen System TM (330-diaminobenzidine) (cat# k3468, Dako) incubation for antigen–antibody complex visualization. All sections were counterstained with Mayer hematoxylin. A human breast tumor was used as a positive control group. As a negative control, the primary antibodies were omitted from the reaction sequence.

Immunohistochemical analysis to WNT5A was performed semi-quantitatively by using the following scores: negative (0, not detectable), 1 (detectable but less than 50% of stained cells), 2 (more than 50% and less than 75% stained cells), and 3 (more than 75% of stained cells). MMP2, 7 and 20 immunohistochemical analysis was also performed semi-quantitatively by using the following scores: 0 (<10% of positive tumor cells), 1 (10–50% of positive cells), 2 (>50% of positive cells) (Xavier et al., 2009; Ribeiro et al., 2009; Kurayoshi et al., 2006; Fregnani et al., 2009; Leonardi et al., 2010). Two authors independently analyzed the slides until a consensus was reached (Xavier et al., 2009). Analysis of variance (ANOVA) and *T*-test were performed to evaluate the difference between tumors and homogeneity variance. Pearson's correlation was used for statistical analysis for correlation between proteins. A p value lower than 0.05 ($p < 0.05$) was considered statistically significant. Statistical analysis was performed using GraphPad Prism 6 software (GraphPad Software Inc, La Jolla, CA USA).

3. Results

The results for WNT5A expression are summarized in Table 2. In the normal oral mucosa the expression of WNT5A was absent in all epithelial layers (Fig. 1a). However, the WNT5A was expressed in tumor cells' cytoplasm within all of the odontogenic tumors. Ameloblastomas consisted of thirty-five solid/multicystic, three unicystic, one desmoplastic and one peripheral type. In solid/multicystic ameloblastomas the expression was observed in the peripheral and central cells of odontogenic epithelium, of plexiform, follicular, acanthomatous and granular histopathological types (Fig. 1b–e, respectively). The same was observed in unicystic and peripheral types (Fig. 1f and g, respectively). Desmoplastic ameloblastoma did not show WNT5A expression in odontogenic epithelial islands (Fig. 1h). In 42.5% of cases, the positive cells ranged from 50 to 75%, and 57.5% of cases expressed this protein in more than 75% (Table 2).

The negative control to WNT5A is represented in Fig. 2a. WNT5A was positive in all cases of KOT with an expression in more than 50% of the cells, mainly at the basal and suprabasal layer of the