Regulation of Wnt/β-catenin pathway may be related to Regγ in benign epithelial odontogenic lesions

Hellen Bandeira de Pontes Santos, DDS, MSc,[a] Hianne Cristinne de Morais Medeiros, DDS, MSc,[a] Rodrigo Porpino Mafra, DDS, MSc,[a] Márcia Cristinada Costa Miguel, DDS, MSc, PhD,[b] Hébel Cavalcanti Galvão, DDS, MSc, PhD,[b] and Lélia Batista de Souza, DDS, MSc, PhD[b]

Objectives. The aim of this study was to analyze and compare the immunoexpressions of Regγ, Wnt-1, and β-catenin in ameloblastomas, adenomatoid odontogenic tumors (AOTs), and odontogenic keratocysts (OKCs).
Study Design. Thirty solid ameloblastomas, 20 AOTs, and 30 OKCs were selected for analysis of the immunoexpression of Regγ, Wnt-1, and β-catenin. Each case was semiquantitatively evaluated in the epithelial component and in their different cellular compartments (membrane, cytoplasm, and nucleus).
Results. Ameloblastomas displayed higher cytoplasmic and nuclear Regγ expression compared with AOTs and OKCs, as well as higher membrane and cytoplasmic Wnt-1 expression ($P < .05$). β-catenin membrane expression was higher in OKCs compared with ameloblastomas and AOTs ($P < .05$). Nuclear β-catenin expression was higher in ameloblastomas and AOTs than in OKCs ($P < .05$). Cytoplasmic and nuclear Regγ expression in AOTs were positively correlated with nuclear β-catenin expression ($P < .05$).
Conclusions. The marked expressions of Regγ, Wnt-1, and β-catenin suggest the participation of these proteins in the pathogenesis of the studied lesions. The greater expressions of Regγ, Wnt-1, and nuclear β-catenin in ameloblastomas may be related to their more aggressive behavior. Pro-tumor effects of nuclear β-catenin may be counterbalanced by inhibitory pathways in AOTs, justifying their low aggressiveness. (Oral Surg Oral Med Oral Pathol Oral Radiol 2019;128:43−51)

Odontogenic cysts and tumors are exclusive gnathic bone lesions that display heterogeneous biological behavior.[1] Ameloblastoma is the most prevalent benign odontogenic neoplasm, characterized by aggressive biologic behavior and high recurrence potential.[1,2] However, adenomatoid odontogenic tumor (AOT) is characterized by poorly aggressive biologic behavior and indolent growth, and its recurrence is very rare.[1,3,4] Odontogenic keratocyst (OKC) is one of the most frequent odontogenic cysts in gnathic bones,[5] representing a distinct form of odontogenic developmental cyst that deserves special attention because of its histopathologic characteristics, aggressive clinical behavior, and high relapse rate.[6] Several studies are currently being performed to elucidate the molecular mechanisms involved in the differentiated behavior of this cyst.[7-9]

The etiopathogenesis of benign epithelial odontogenic lesions has not yet been fully elucidated. In this perspective, several studies have investigated signaling pathways involved in odontogenesis, which may also be related to the development of these lesions.[7,9,10] The Wingless type (Wnt) pathway is linked to embryonic development, including during odontogenesis. The human Wnt family is composed of 19 different cysteine-rich glycoproteins acting as ligands for greater than 15 receptors or co-receptors, and the activation and inactivation of these components can occur in 3 ways: canonical pathway, noncanonical planar cell polarity pathway, and noncanonical Wnt/calcium pathway. With regard to the canonical pathway, the Wnt ligand, especially Wnt-1, binds to Frizzled (FZD) receptors as well as to LRP5/6 co-receptors (low-density lipoprotein receptor−related protein [LRP] 5/6) to initiate intracellular signaling via β-catenin nuclear translocation.[11,12] This process is closely related to regulation of cell proliferation and differentiation processes during odontogenesis.[13]

In addition, interactions between the Wnt pathway and β-catenin, which also acts alongside other components, such as the Sonic hedgehog (Shh) pathway, coordinate the expression of inhibitory and stimulatory odontogenesis factors. Activation of the Wnt/β-catenin pathway triggers proliferative stimuli in mesenchymal cells in relation to the development of dental tissues and has been linked to the progression of odontogenic lesions.[14]

The Wnt/β-catenin pathway activity can be regulated by the Reg protein family.[15] Some studies have

Statement of Clinical Relevance

The findings of this study may contribute to a greater understanding of the role of Regγ as a transcriptional regulator in the Wnt pathway and its relationship to the pathogenesis of odontogenic lesions. Thus, our findings may lead to further studies to investigate new therapeutic strategies.

[a]PhD student, Postgraduate Program in Oral Pathology, Federal University of Rio Grande do Norte, Natal, RN, Brazil.
[b]Professor, Postgraduate Program in Oral Pathology, Federal University of Rio Grande do Norte, Natal, RN, Brazil.
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suggested that high Regγ expression may be related to the development and progression of several malignant neoplasms.[16,17] To date, no studies evaluating the association between the Regγ and Wnt/β-catenin pathways in benign epithelial odontogenic lesions have been reported in the literature (PubMed database). In this context, the aim of the present study was to analyze the immunoexpressions of Regγ, Wnt-1, and β-catenin in ameloblastomas, AOTs, and OKCs to elucidate the participation of these proteins in the biologic behavior of these lesions.

MATERIAL AND METHODS

Eighty tissue specimens, including 30 solid ameloblastomas, 20 AOTs, and 30 OKCs, archived in the files of the Oral Pathology Department of the Federal University of Rio Grande do Norte, Brazil, were selected for this study. Only tissue specimens previously formalin-fixed (10%) and paraffin-embedded with sufficient biologic material for immunohistochemical analyses were selected. The histopathologic diagnoses of each case were reviewed by 2 pathologists, according to World Health Organization criteria.[1] Ameloblastoma and OKC cases submitted to the marsupialization technique before the biopsy, as well as the cases of OKC associated with Gorlin syndrome, were not included. This retrospective study was independently reviewed and approved by the Research Ethics Committee of Federal University of Rio Grande do Norte, Natal, Brazil (No. 2.356.485). All procedures were conducted in full accordance with the tenets of the Declaration of Helsinki.

Immunohistochemistry

Histologic sections (3 μm) were obtained from the paraffin-embedded material and mounted on glass slides previously prepared with organosilane (3-aminopropyltriethoxysilane; Sigma Chemical Co., St. Louis, MO) used as the adhesive. The sections were deparaffinized, rehydrated, and submitted to antigen retrieval with Trilogy (1:100; Cell-Marque, Rocklin, CA) in a Pascal pressure cooker (Dako, Carpinteria, CA) for 30 minutes and then immersed in 10 volumes of hydrogen peroxide solution to block endogenous peroxidase. The tissue sections were then washed in phosphate-buffered saline (PBS). Subsequently, the tissue sections were incubated overnight in a moist chamber, with the following primary antibodies: Regγ (clone: 20H9L19; dilution: 1:1000; ThermoFisher Scientific, Waltham, MA), Wnt-1 (clone: Ab15201; dilution: 1:200; Abcam, Cambridge, MA), and β-catenin (clone: 17C2; dilution: 1:50; Santa Cruz Biotechnology, Dallas, TX). Sections were then washed twice in PBS and incubated in the HiDef visualization system (HiDef Detection, HRP Polymer System; Cell-Marque, Rocklin, CA) at room temperature. The reactions were developed with 0.03% diaminobenzidine (Liquid DAB + Substrate; Dako, Carpinteria, CA), resulting in a brown reaction product. Finally, tissue sections were counterstained with Mayer's hematoxylin and coverslipped on Erv-mount (EasyPath, Brazil).

As positive controls for the reactions, mammary carcinoma specimens were used for the Regγ labeling and human tonsils fragments for the anti-Wnt-1 and anti-β-catenin antibodies. The negative control consisted of bovine serum albumin in PBS without antibody.

Immunohistochemical analyses

Immunoexpression of Regγ, Wnt-1, and β-catenin was analyzed semiquantitatively by 2 independent and previously calibrated examiners. Immunoreactivities were evaluated by light microscopy (Olympus CH30; Olympus Co., Tokyo, Japan) at × 100 and × 400 magnifications in the parenchymal cells of ameloblastomas and AOTs, as well as in the OKC epithelial lining. Each case was classified according to the percentage of immunopositive cells, with the following scores: 0 (no staining); 1 (1%−25% stained cells); 2 (26%−50% stained cells); 3 (51%−75% stained cells); 4 (>75% stained cells) (adapted from Nascimento et al.[18]). It is noteworthy that all the markers used can be expressed in different locations, resulting in activation of distinct functions. Therefore, Regγ immunoexpression was evaluated separately in both cytoplasmic and nuclear compartments, and Wnt-1 expression was analyzed in both the cytoplasm and the membrane. In turn, β-catenin expression was evaluated in the cytoplasmic, membrane, and nuclear compartments.

The results were analyzed by using the Statistical Package for Social Sciences software for Windows version 20.0 (SPSS Inc., Chicago, IL). The nonparametric Kruskal-Wallis (KW) and Mann-Whitney (U) tests were performed to compare the immunoexpression scores between the groups of lesions. Correlations among Wnt-1, β-catenin, and Regγ expressions were evaluated by the Spearman (r) correlation test. The level of significance for all statistical tests was set at 5% ($P < .05$).

RESULTS

All ameloblastomas, AOTs, and OKCs showed cytoplasmic and nuclear Regγ immunoexpression (Figure 1) (Supplementary Table I). The nonparametric KW test demonstrated significantly higher Regγ expression in ameloblastomas compared with AOTs and OKCs, both at the cytoplasmic ($P < .001$) and at nuclear ($P = 0.002$) levels. No statistically significant differences in cytoplasmic or nuclear Regγ expressions were observed between AOTs and OKCs (Table I).

Wnt-1 membrane immunoexpression was evidenced in all ameloblastoma cases, in 10 (33.3%) OKC cases,

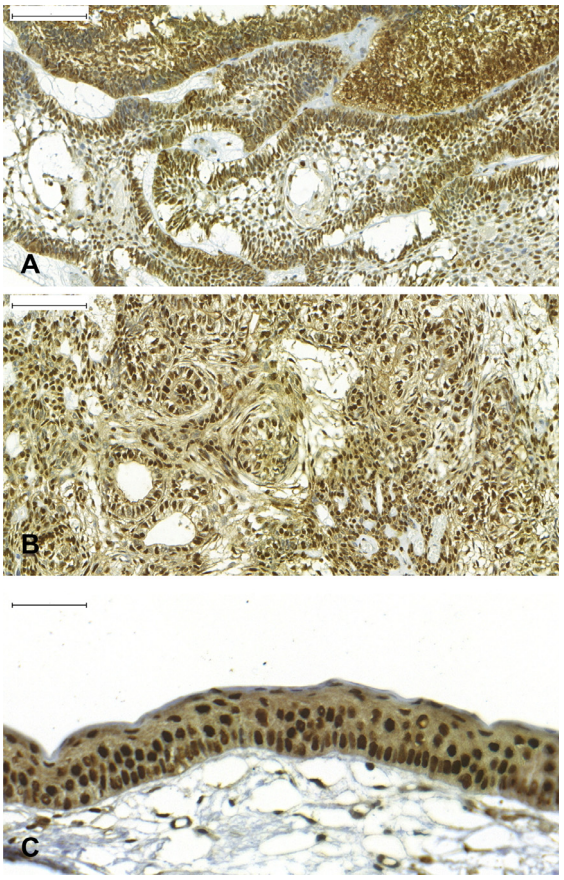

Fig. 1. Nuclear and cytoplasmic immunoexpression of Regγ in the odontogenic lesions. **(A),** Ameloblastoma showing expression in central and peripheral cells of the neoplastic islands (HiDef; scale = 50 μm). **(B),** Adenomatoid odontogenic tumor showing positivity in duct-like structures and epithelial cords (HiDef; scale = 50 μm). **(C),** Odontogenic keratocyst showing diffuse expression in lining epithelium (HiDef; scale = 50 μm).

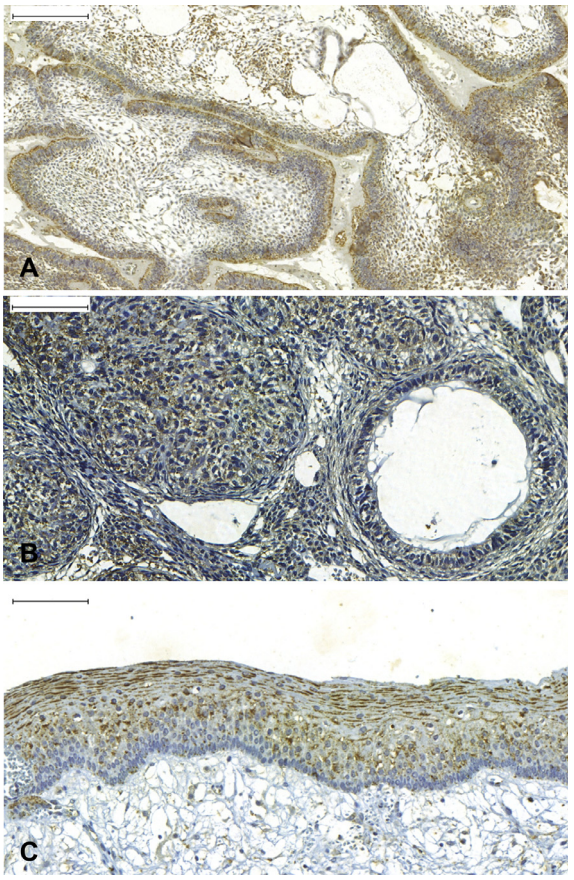

Fig. 2. Immunohistochemical reactivity for Wnt-1 in the odontogenic lesions. **(A),** Ameloblastoma showing cytoplasmic and membrane expression mainly in peripheral cells of epithelium islands (HiDef; scale = 100 μm). **(B),** Adenomatoid odontogenic tumor showing cytoplasmic expression in duct-like structures and whorled masses of cells (HiDef; scale = 100 μm). **(C),** Odontogenic keratocyst showing expression in epithelial suprabasal layers (HiDef; scale = 100 μm).

and in only 1 (5%) AOT case (Figure 2) (Supplementary Table II). Ameloblastomas exhibited higher Wnt-1 membrane expression compared with AOTs and OKCs ($P < .001$). In addition, Wnt-1 membrane expression was higher in OKCs compared with AOTs ($P = .018$).

At the cytoplasm level, Wnt-1 was expressed in all cases. Cytoplasmic expression scores were significantly higher in ameloblastomas compared with AOTs and OKCs ($P = .036$), whereas cytoplasmic AOT and OKC scores were not statistically different (Table II).

Table I. Parameters used for the calculation of the Kruskal-Wallis (KW) test for the evaluation of the scores of cytoplasmic and nuclear immunoexpression of Regγ in ameloblastomas, adenomatoid odontogenic tumors, and odontogenic keratocysts

Location/ lesion	*n*	*Median*	$Q_{25}-Q_{75}$	*Mean of ranks*	*KW*	*P*
Cytoplasm						
Ameloblastoma	30	4.00	3.00–4.00	53.85	18.865	<.001
Adenomatoid odontogenic tumor	20	3.00	2.00–3.00	29.20		
Odontogenic keratocyst	30	3.00	2.00–4.00	34.68		
Nucleus						
Ameloblastoma	30	4.00	4.00–4.00	51.28	12.980	.002
Adenomatoid odontogenic tumor	20	3.00	2.25–4.00	31.40		
Odontogenic keratocyst	30	3.00	3.00–4.00	35.78		

Table II. Parameters used for the calculation of the Kruskal-Wallis (KW) test for the evaluation of the scores of the membrane and cytoplasmic immunoexpression of Wnt-1 in ameloblastomas, adenomatoid odontogenic tumors, and odontogenic keratocysts

Location/ lesion	*N*	*Median*	$Q_{25}-Q_{75}$	*Mean of ranks*	*KW*	*P*
Membrane						
Ameloblastoma	30	1.00	1.00−1.00	59.33	43.215	<.001
Adenomatoid odontogenic tumor	20	0.00	0.00−0.00	21.83		
Odontogenic keratocyst	30	0.00	0.00−1.00	34.12		
Cytoplasm						
Ameloblastoma	30	3.00	2.00−4.00	48.48	6.674	.036
Adenomatoid odontogenic tumor	20	2.00	1.00−3.00	32.93		
Odontogenic keratocyst	30	2.00	2.00−3.00	37.57		

β-catenin was expressed in the epithelial component of all ameloblastoma, AOT and OKC cases in the membrane, cytoplasmic and nuclear compartments (Figure 3) (Supplementary Table III). Membrane β-catenin expression was significantly higher in ameloblastomas compared with AOTs and OKCs ($P < .001$). Higher β-catenin membrane expression was detected in OKCs compared with AOTs ($P < .001$). Cytoplasmic β-catenin expression did not differ significantly among the assessed lesions ($P = .454$). Ameloblastomas exhibited higher nuclear β-catenin expression compared with OKCs ($P < .001$). In addition, greater β-catenin nuclear expression in AOTs compared with OKCs was detected ($P < .001$) (Table III).

Possible correlations between Wnt-1, β-catenin, and Regγ expression scores were analyzed for the 3 lesions (Table IV). In ameloblastomas, a significant positive correlation was observed between membrane and cytoplasmic Wnt-1 expressions ($r = 0.448$; $P = .013$), as well as between cytoplasmic and nuclear β-catenin expressions ($r = 0.463$; $P = .010$). At the cytoplasmic level, Wnt-1 expression also correlated positively with both cytoplasmic β-catenin ($r = 0.448$; $P = .013$) and Regγ ($r = 0.443$; $P = .014$).

With regard to AOT, nuclear β-catenin expression was positively correlated with Regγ expression at the cytoplasmic ($r = 0.485$, $P = .030$) and nuclear ($r = 0.449$, $P = .047$) levels. Cytoplasmic β-catenin expression was also positively correlated with nuclear ($r = 0.652$; $P = .002$) and cytoplasmic ($r = 0.620$; $P = .040$) Regγ expressions. In addition, nuclear and cytoplasmic Regγ expression scores were positively correlated ($r = 0.750$; $P < .001$) (see Table IV).

In OKC, a positive and statistically significant relationship was observed between cytoplasmic β-catenin expression and nuclear Regγ expression ($r = 0.373$; $P = .042$). Membrane and nuclear β-catenin expression were not significantly related to the expression of the other analyzed proteins in OKC ($P > .05$) (see Table IV).

DISCUSSION

The tumorigenesis process originates from multiple stages that culminate in phenotypic changes in neoplastic

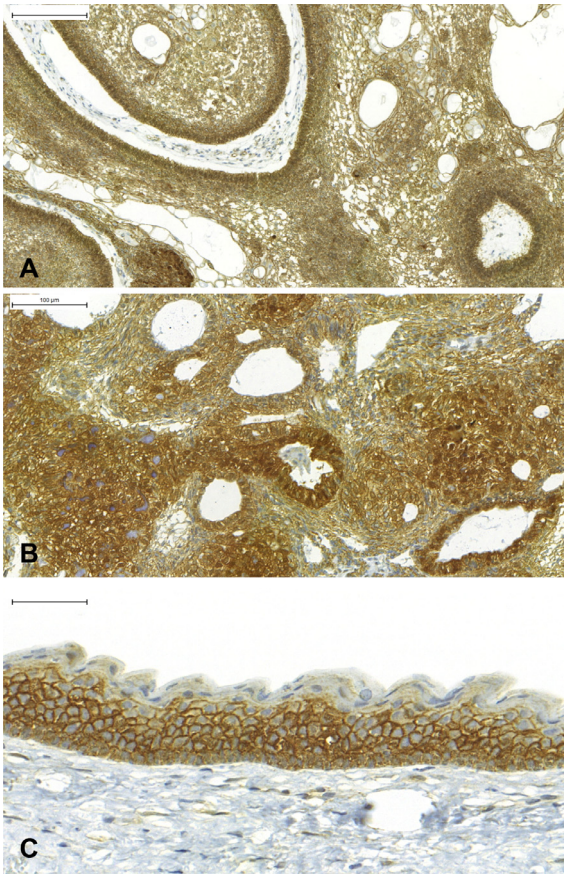

Fig. 3. Immunoexpression of β-catenin in the odontogenic lesions. **(A),** Ameloblastoma showing marked nuclear and cytoplasmic expression in peripheral cells of a neoplastic island (HiDef; scale = 100 μm). **(B),** Adenomatoid odontogenic tumor showing predominantly cytoplasmic and membrane positivity in epithelial cells (HiDef; scale = 50 μm). **(C),** Odontogenic keratocyst showing cytoplasmic and membrane positivity in basal and suprabasal epithelial layers (HiDef; scale = 50 μm).

Table III. Parameters used for the calculation of the Kruskal-Wallis (KW) test for the evaluation of the scores of membrane, cytoplasmic and nuclear immunoexpression of β-catenin in ameloblastomas, adenomatoid odontogenic tumors, and odontogenic keratocysts

Location/ lesion	*n*	*Median*	$Q_{25}-Q_{75}$	*Mean of ranks*	*KW*	*P*
Membrane						
Ameloblastoma	30	2.00	1.00−3.00	35.58	36.167	<.001
Adenomatoid odontogenic tumor	20	1.00	1.00−2.00	20.78		
Odontogenic keratocyst	30	4.00	3.00−4.00	58.57		
Cytoplasm						
Ameloblastoma	30	4.00	3.00−4.00	36.97	1.581	.454
Adenomatoid odontogenic tumor	20	4.00	3.00−4.00	41.83		
Odontogenic keratocyst	30	4.00	3.00−4.00	43.15		
Nucleus						
Ameloblastoma	30	2.00	1.00−3.00	46.48	20.583	<.001
Adenomatoid odontogenic tumor	20	2.00	1.00−3.00	51.40		
Odontogenic keratocyst	30	1.00	1.00−1.00	27.25		

cells and molecular modifications in the intercellular environment, giving rise to a microenvironment favorable for the growth and survival of these cells. These modifications are modulated by genetic and epigenetic events, which result in the activation or inhibition of signaling pathways that regulate vital cellular functions, such as cell differentiation, proliferation, and death.[19] The study of these alterations, as well as the identification of molecules involved in this process, are extremely important in understanding the pathogenesis of odontogenic cysts and tumors, as well as in the development of more specific therapies for these lesions. To the best of our knowledge, this is the first study to comparatively evaluate Regγ expression in ameloblastomas, AOTs, and OKCs, as well as to assess their possible relationship to Wnt/β-catenin pathway proteins.

Table IV. Sample size, Spearman's correlation coefficient (*r*) and statistical significance (*P*) of the immunoexpression scores for Wnt, β-catenin, and Regγ in the epithelial component of ameloblastomas, adenomatoid odontogenic tumors, and odontogenic keratocysts

Comparison	*Ameloblastoma (n = 30)*		*Adenomatoid odontogenic tumor (n = 20)*		*Odontogenic keratocyst (n = 30)*	
	r	*P*	*r*	*P*	*r*	*P*
Wnt-1 (membrane) × Wnt-1 (cytoplasm)	0.448	.013*	0.251	.286	0.331	.074
β-catenin (membrane) × β-catenin (cytoplasm)	0.075	.695	0.207	.380	0.148	.436
β-catenin (membrane) × β-catenin (nucleus)	0.216	.251	0.271	.248	0.087	.647
β-catenin (cytoplasm) × β-catenin (nucleus)	0.463	.010*	0.154	.516	−0.215	.254
Regγ (cytoplasm) × Regγ (nucleus)	0.301	.106	0.750	<.001*	0.205	.277
Wnt-1 (membrane) × β-catenin (membrane)	0.006	.974	0.254	.280	0.329	.076
Wnt-1 (membrane) × β-catenin (cytoplasm)	0.328	.077	0.166	.483	0.206	.274
Wnt-1 (membrane) × β-catenin (nucleus)	0.168	.376	0.000	1.000	0.263	.161
Wnt-1 (cytoplasm) × β-catenin (membrane)	0.346	.061	0.214	.365	0.341	.065
Wnt-1 (cytoplasm) × β-catenin (cytoplasm)	0.448	.013*	0.066	.782	0.111	.560
Wnt-1 (cytoplasm) × β-catenin (nucleus)	0.218	.200	0.162	.494	0.335	.070
Wnt-1 (membrane) × Regγ (cytoplasm)	0.235	.212	0.083	.727	0.070	.714
Wnt-1 (membrane) × Regγ (nucleus)	0.194	.304	−0.021	.929	0.054	.775
Wnt-1 (cytoplasm) × Regγ (cytoplasm)	0.443	.014*	0.197	.405	0.253	.177
Wnt-1 (cytoplasm) × Regγ (nucleus)	0.109	.568	0.145	.541	0.028	.885
β-catenin (membrane) × Regγ (cytoplasm)	0.190	.315	0.241	.306	0.330	.075
β-catenin (membrane) × Regγ (nucleus)	0.121	0.525	0.273	.244	0.215	.255
β-catenin (cytoplasm) × Regγ (cytoplasm)	0.226	0.229	0.620	.004*	0.169	.371
β-catenin (cytoplasm) × Regγ (nucleus)	0.135	0.478	0.652	.002*	0.373	.042*
β-catenin (nucleus) × Regγ (cytoplasm)	0.171	0.365	0.485	.030*	−0.222	.238
β-catenin (nucleus) × Regγ (nucleus)	−0.039	0.838	0.449	.047*	−0.084	.660

*Correlation is statistically significant ($P < .05$).

Regγ, also called *PA28* or *PSME3*, is an activating 11S subunit, which has the ability to bind to and activate the 20S proteasome, triggering specific protein degradation processes associated with the modulation of cellular functions, such as cell cycle inhibition and apoptosis.[20-22] To date, no reports on Regγ expression in benign odontogenic lesions have been published in the literature. Nevertheless, studies have pointed to Regγ as an oncogene related to the tumorigenesis of malignant neoplasms, including oral squamous cell carcinoma.[16,17,23] Li et al.[17] demonstrated that high Regγ expression is related to a worse prognosis and lower survival in patients diagnosed with oral squamous cell carcinoma. The present study indicates higher Regγ expression in ameloblastomas compared with AOTs and OKCs.

It is believed that transcriptional Regγ regulation occurs by means of a negative feedback mechanism regulated by the p53 tumor suppressor. Wan et al.[24] demonstrated structural affinity of nuclear Regγ and p53 by co-immunoprecipitation assays. The authors verified a transcriptional relationship, in which greater expression of p53 protein would be directly related to greater Regγ expression. In turn, Wang et al.[25] emphasized that mutation of the *p53* gene may trigger higher Regγ expression, resulting in increased potential for proliferation, migration, and invasion of malignant cells. It is worth mentioning that investigations have demonstrated that ameloblastomas exhibit higher p53 protein expression compared with AOTs[26] and OKCs.[27] In view of this, it is possible to hypothesize that greater ameloblastoma proliferative potential is related to higher Regγ expression compared with the other odontogenic lesions evaluated in our study.

In this study, we demonstrated a variable percentage of labeled cells and localization of Regγ, Wnt-1, and β-catenin expression in odontogenic tumors. Several studies have demonstrated the modulatory function of Regγ proteasome in the Wnt/β-catenin pathway during tumorigenesis processes in malignant neoplasms.[17,28,29] Wnt proteins comprise a large family of glycoproteins that act as extracellular signaling molecules. After activation of the canonical pathway, Wnt ligands bind to FZD-type transmembrane receptors, resulting in cytoplasmic β-catenin accumulation and subsequent translocation to the nucleus.[30] Thus, one may hypothesize that Regγ is involved in the modulation of the Wnt canonical pathway and has a role in the pathogenesis of the odontogenic lesions evaluated because the lesions that presented higher nuclear Regγ expression also had higher β-catenin expression in the same location.

Wnt-1 represents the first discovered protein belonging to this family, acting as a growth factor that regulates intercellular adhesion.[31] The Wnt-1 protein is located in the cytoplasm and has affinity with FZD receptors on the membrane, forming a stable complex, along with LRP, Disheveled, and AXIN. This complex can inhibit glycogen synthase kinase-3 beta, thereby reducing phosphorylation/proteolytic destruction of β-catenin, resulting in its translocation to the nucleus.[32,33] In our study, membrane and cytoplasmic Wnt-1 expression were observed in the assessed odontogenic lesions. Similar results were reported by Siar et al.[34] and Dutra et al.,[35] who observed heterogeneous Wnt-1 labeling in ameloblastomas. However, Hakim et al.[36] demonstrated intense nuclear labeling for Wnt-1 in OKCs. In the present study, higher Wnt-1 expression was observed in ameloblastomas compared with the other odontogenic lesions, in accordance with the results reported by Dutra et al.[35] Sklavos et al.[37] investigated the biologic behavior of hepatocellular carcinoma in Wnt-$1^{-/-}$ knockout mice and demonstrated that the absence of the expression of this protein is related to the suppression of the Cyclin D1, FOXM1, and nuclear factor -κB pathways, resulting in decreased proliferation of malignant cells.

The β-catenin function is directly modulated by the canonical Wnt pathway. When the pathway is inactive, this protein is located in the membrane, playing an important role in cell adhesion, alongside E-cadherin.[38] When the pathway is activated, β-catenin translocates to the nucleus and couples with lymphoid enhancer−binding factor 1 to form a transcription factor that can act as a transcriptional mediator during tumor progression, invasion, and metastasis.[38-40] In our study, the odontogenic lesions showed variable β-catenin expression. Ameloblastoma demonstrated higher membrane Wnt-1 expression as well as greater nuclear β-catenin expression. In contrast, OKC demonstrated lower membrane Wnt-1 expression and higher membrane β-catenin expression. These findings may indicate participation of the canonical Wnt pathway in the pathogenesis of epithelial odontogenic lesions. Higher membrane Wnt-1 expression concomitant with the higher nuclear β-catenin expression may represent greater activation of this pathway in more aggressive lesions with higher proliferative index, such as ameloblastoma.

Miyake et al.[41] elucidated the presence of specific mutations in the *CTNNB1* gene encoding β-catenin in ameloblastomas, resulting in strong nuclear and cytoplasmic expression of this protein in these lesions. In contrast, Dutra et al.[35] observed only cytoplasmic and membrane labeling for β-catenin in ameloblastomas. In the present study, membrane, cytoplasmic, and nuclear labeling of this protein were observed in the epithelial component of the studied odontogenic lesions. Dutra et al.[35] did not find nuclear β-catenin

expression in solid ameloblastomas, which could be attributed to their lower cohort (n = 17) or the role of Wnt5a, a constant positive marker capable of inhibiting the Wnt/β-catenin pathway, consequently promoting lower nuclear accumulation of this protein in that study. Nuclear expression in ameloblastomas found in our study may be related to tumor aggressiveness because when this protein is located in the nucleus, it may exhibit considerable transcriptional activity, regulating several genes associated with proliferation, migration, and other biologic processes. Ameloblastomas presented higher membrane and nuclear β-catenin expression compared with OKCs and AOTs. Considering that ameloblastomas display more aggressive biologic behavior compared with the other odontogenic lesions,[1,26] it is possible that the higher nuclear β-catenin expression in ameloblastomas is also related to Wnt-1 and Regγ expression, indicating the active participation of this pathway in the development of these tumors.

AOTs are known to display more indolent biologic behavior compared with such tumors as ameloblastomas, which display aggressive behavior.[42] In this study, higher nuclear β-catenin expression was observed in AOTs compared with OKCs. Other studies have shown β-catenin immunopositivity in both OKCs[43] and AOTs.[44] Reichart et al.[45] have suggested that molecular AOT alterations indicate a more hamartomatous than of a neoplastic nature of the tumor. Although nuclear β-catenin expression may be associated with lesions displaying greater proliferative potential,[38-40] we suggest that AOTs may present inhibitory signaling pathways that would counterbalance the effects triggered by the nuclear expression of this protein, which could justify the less aggressive behavior of these tumors.

Investigations have shown that Regγ is highly expressed in several malignant neoplasms.[16,17,46] In experimental knockout mice models, Li et al.[17] observed that the absence of this protein resulted in longer time intervals for the development of neoplasms compared with control groups constituted by wild-type mice. Furthermore, the authors suggested, through in vitro molecular assays, that Regγ expression would be related to the degradation of the enzyme glycogen synthase kinase-3 beta and that this event directly results in the activation of the Wnt/β−catenin pathway, in addition to increased levels of c-Myc and Cyclin D1. Thus, Li et al. pointed to Regγ as a possible regulatory protein of the Wnt/β-catenin pathway and the tumorigenesis process. In the present study, significant positive correlations were found between cytoplasmic and nuclear Regγ expressions and between nuclear and cytoplasmic β-catenin expressions in AOTs, suggesting a possible role for these proteins in the biologic events of these tumors.

The Wnt/β-catenin signaling pathway exerts an important influence on the modulation of several cellular events, such as cell proliferation, migration, and death. The aberrant expression of its proteins has been reported in several lesions, including odontogenic cysts and tumors.[34,35,47,48] In the present study, a positive correlation was observed between cytoplasmic Wnt-1 and β-catenin expression in ameloblastomas, which are remarkable lesions among benign odontogenic tumors because they commonly present locally aggressive behavior. Moreover, a positive significant correlation between cytoplasmic and β-catenin nuclear expression in ameloblastomas was also detected, suggesting that the cytoplasmic increase of β-catenin leads to greater translocation of this protein to the nucleus, generating responses that contribute to the aggressiveness, such as local invasiveness. The findings of our study are similar to those reported by Siar et al.[34], Siar et al.[47] and Dutra et al.,[35] who proposed that the high expression of molecules of this pathway in ameloblastomas may contribute to the migration of the tumor cells, thus promoting local invasion. Taken together, these findings suggest that the Wnt/β-catenin signaling pathway molecules could participate in biologic events related to ameloblastoma pathogenesis. In the context of current scientific literature, information on the role of β-catenin in the biologic behavior of AOTs is still scarce. An interesting finding of the present study was the detection of a positive and significant correlation between β-catenin and Regγ expression in AOTs, at both cytoplasmic and nuclear levels. This reinforces the theory that increased Regγ expression plays a greater role in the activation of the Wnt/β-catenin pathway in these lesions.

Similarly, a positive correlation was also observed between nuclear Regγ expression and cytoplasmic β-catenin expression in OKCs, which are cystic lesions notable for their aggressive behavior, high recurrence rate, and genetic alterations.[8] β-catenin has been shown to modulate cell-to-cell adhesion by stabilizing binding to E-cadherin, a cell adhesion protein.[49] Deregulation of cytoplasmic β-catenin and E-cadherin expression leads to a decrease in cell-to-cell adhesion and rupture of tissue morphogenesis, which is correlated with invasion of neoplastic cells [36,49] and, possibly, OKC epithelial cells.[36] The results reported here suggest possible interaction between Regγ and β-catenin in OKCs. However, further molecular studies are required to elucidate the actual biologic influence of the Regγ protein on the Wnt/β-catenin pathway in these odontogenic lesions.

CONCLUSIONS

The high expression of Regγ, Wnt-1, and β-catenin in ameloblastomas, OKCs, and AOTs suggests their

participation in the development and biologic events of these odontogenic lesions. The greater expression of Regγ, Wnt-1, and β-catenin in ameloblastomas may be related to the more aggressive behavior of these tumors. In addition, certain correlations found in our study suggest that Regγ expression may contribute to activation of the Wnt/β-catenin pathway in the studied lesions. Our findings suggest that Regγ may contribute to the increase in nuclear β-catenin expression in AOTs, but not in ameloblastomas or OKCs. Moreover, the protumor effects of nuclear β-catenin in AOTs may be counterbalanced by inhibitory pathways in AOTs, explaining the low aggressiveness of these lesions.

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SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.oooo.2018.12.019.

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Reprint requests:

Lélia Batista de Souza
Universidade Federal do Rio Grande do Norte
Departamento de Odontologia
Programa de Pós-Graduação em Patologia Oral
Av. Senador Salgado Filho, 1787, Lagoa Nova
Natal-RN, CEP: 59056-000
Brazil
Leliabsouza@gmail.com