BDNF/TrkB/Akt Signaling Pathway Epithelial Odontogenic Tumors and Keratocyst: An Immunohistochemical Study Comparative With Dental Germs

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Abstract

Odontogenic lesions (OL) are an important group of oral and maxillofacial diseases represented by odontogenic cysts, benign, and malignant tumors. The brain-derived neurotrophic factor (BDNF)/ tropomyosin receptor kinase B (TrkB) signaling pathway has multiple biological actions and has been identified as an important pathway in the proliferation, invasion, and survival of different epithelial tumors. Its role in the development of OL, however, has so far been unexplored. Our aim was to evaluate the BDNF/TrkB/Akt/p-RPS6 signaling pathway in OL of epithelial origin. This cross-sectional study comprised 3 cases of tooth germs, 25 cases of odontogenic keratocyst (OK), 29 cases of ameloblastoma (Am), and 6 cases of ameloblastic carcinoma. Immunohistochemical staining for BDNF, TrkB, p-Akt, and p-RPS6 was performed. OLs were evaluated according to the pattern of immunohistochemical expression in epithelial cells and by semiquantitative scores that considered the intensity of staining and percentage of positive cells. BDNF stromal expression was also assessed. No significant differences were observed with respect to the percentage of positive cases for all markers. Regarding the immunoreactive scores, BDNF and p-RPS6 expressions were similar in the odontogenic epithelium of all OL. However, TrkB and p-Akt were overexpressed in OK compared with ameloblastic carcinoma. In Am, epithelial BDNF was significantly higher compared with stromal expression. In conclusion, BDNF seems to participate in the development of cystic, benign, and malignant odontogenic epithelium to similar degrees. The acquisition of the invasive or malignant

phenotype in odontogenic neoplasms is not associated with alterations in the BDNF/TrkB/Akt/RPS6 axis, which could be implicated in the differentiation process.

Keywords: odontogenic lesions; growth factor; signaling pathway; mTOR; BDNF; TrkB

Odontogenic lesions (OLs) are a heterogenous group of disorders derived from the tooth-forming apparatus or its remnants.[1,2] The vast majority of cystic and neoplastic lesions derived from odontogenic epithelium are intraosseous and thus require invasive and sometimes extensive surgeries.[3] Moreover, recurrences can occur, leading to the necessity for new surgical procedures and further morbidity to the patients.[4,5] Some OLs stand out because of their higher prevalence and more aggressive clinical behavior. Odontogenic keratocyst (OK) and ameloblastoma (Am) are derived from odontogenic epithelial components and are among the most common OLs.[1,4,5] OK has a more aggressive growth compared with other odontogenic cysts and higher recurrence rates after conservative therapy.[5] Am is a benign odontogenic tumor with infiltrative growth, leading to a significant local aggressiveness and the requirement for wide surgical resections to prevent tumor relapse.[4] The malignant counterpart of Am is referred to as ameloblastic carcinoma (AmC) and shows an ameloblastic epithelium with malignant features. Although AmC is considered a rare lesion, it represents the most frequent malignant OL and is associated with high recurrence and metastatic rates.[6] Some transcriptional alterations have been identified as involved with the development and progression of such lesions.[7] However, further mechanisms that could reveal differences in the proliferative and invasive capacity of OL epithelial cells need to be better understood.

Growth factors and their receptors are key regulators of cell differentiation, survival, metabolism, mobility, and cell growth in different types of benign and malignant lesions.[8] Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, was originally known to play an important role in controlling cell survival, differentiation, and death in the nervous system. The major ligands for BDNF are the cell membrane tropomyosin receptor kinase (Trk), particularly TrkB.[9] BDNF binding to TrkB elicits its dimerization through conformational changes and autophosphorylation of tyrosine residues in the intracellular environment.[9] Mechanistically, stimulation of the BDNF/TrkB signaling pathway promotes the activation of signaling molecules such as Akt, STAT3, Src, ERK, and MAPK, resulting in increased cell proliferation, resistance to apoptosis, invasion, metastasis, and resistance to chemotherapy in several types of cancer. Thus, increased BDNF/TrkB expression has been associated with more aggressive behavior of different malignant tumors and worse prognosis.[10,11]

During odontogenesis, neurotrophic factors may have multiple functions, such as the establishment of the dental innervation and modulating epithelial-mesenchymal interactions during tooth morphogenesis.[12–14] Nevertheless, the pattern of BDNF/TrkB signaling pathway activation in cystic and neoplastic OL has yet to be elucidated. The evaluation of growth factors expression in normal and pathologic conditions has the potential to contribute to the establishment of new diagnostic indicators, prognostic biomarkers, and might also provide important basic knowledge for the further evaluation of

target therapies. Herein we evaluate the expression of BDNF/TrkB and 2 downstream targets of this pathway—Akt and ribosomal protein S6 (RPS6)—in human tooth germs, OK, Am, and AmC. By comparing the expression of these proteins between a normal condition, a pathologic nontumoral condition, benign and malignant tumors, our aim was to uncover if BDNF/TrkB has a key role in odontogenic tumorigenesis.

MATERIALS AND METHODS

This cross-sectional observational study was approved by the Ethics Committee on Human Research (approval No. 95687218.6.0000.5327).

Study Population

A retrospective search was performed at the Laboratory of Oral Pathology at the Federal University of Rio Grande do Sul, School of Dentistry and the Histology Department at the Universidad de la Republica. All specimens of OK, Am, and AmC diagnosed between 2000 and 2019 were included. Inclusion criteria consisted of an adequate amount of material for the analysis of specimens. The patients' records were manually evaluated, and information concerning gender, age, and site was collected. Three tooth germs were retrieved from the Molecular Pathology Area and Histology laboratory from Facultad de Odontologia, Universidad de la Republica for analysis of the odontogenic epithelium during odontogenesis. Sixty cases of OL were included. The sample comprised 25 cases of OK (41.66%), 29 cases of Am (48.33%), and 6 cases of AmC (10%). The clinic-demographic features of each diagnosis are described in Table 1.

TABLE 1 - Clinicodemographic Features of OL

	OK (n = 25)	Am (n = 29)	AmC (n = 6)
Sex (n, %)			
Male	11 (44%)	13 (44.8%)	4 (80%)
Female	14 (56%)	16 (55.2%)	1 (20%)
Missing	—	—	1
		$P = 0.313$*	
Age (mean, SD)	34.80 (± 19.62)	38.03 (± 17.44)	61.80 (± 28.16)
		$P = 0.104$†	
Site (n, %)			
Mandible	14 (60.9%)	26 (89.7%)	4 (80%)
Maxilla	9 (39.1%)	3 (10.3%)	1 (20%)
Missing	2	—	1
		$P = 0.048$*	

*Chi-square test.
†Kruskal-Wallis test.
Am indicates ameloblastoma; AmC, ameloblastic carcinoma; OK, odontogenic keratocyst; OL, odontogenic lesions.

Histopathologic Analysis

Slides of the incisional biopsies were reviewed by 2 blinded pathologists and diagnosed according to the more recent World Health Organization Histological Classification of Head and Neck Tumors.[3] The presence of intense inflammatory infiltrate was considered to be an exclusion criterium during the histopathologic evaluation because it is known that the inflammatory process can modify the immunoexpression of some proteins and also the epithelial behavior. Am were not subclassified on the basis of the histologic pattern once we

evaluated incisional biopsies, which could lead to a misdiagnosis. The absence of cellular atypia was included as diagnostic criteria for AM. Most cases had radiographic information, which corroborated to define the final diagnosis.

Immunohistochemistry

Immunohistochemical reactions were performed at the Experimental Pathology Unit at the Hospital das Clínicas at Porto Alegre. Briefly, paraffin-embedded tissues were sectioned (3 μm) and placed on silanized slides. Then, they were subsequently deparaffinized in xylene and hydrated in descending grades of ethanol. Antigen retrieval was performed for 18 hours in a citrate buffer solution heated to 90°C in a water bath. Endogenous peroxidase activity was blocked using 10% hydrogen peroxide in 5 baths for 5 minutes each. The slides were then incubated with the primary antibodies: BDNF (1:750, EPR1292, Abcam), TrkB (1:1000, Polyclonal, Abcam), p-Akt s473 (1:200, EP2109Y, Abcam), and p-RPS6 S235+S236 (1:200, Polyclonal, Abcam). All slides were then exposed to avidin-biotin complex and horseradish peroxidase reagents (LSAB Kit; Dako Cytomation). The reactions were revealed with diaminobenzidine tetrahydrochloride (Novocastra, Newcastle, UK) and counterstained with Mayer's hematoxylin. Negative controls were obtained through incubation with nonimmune serum instead of primary antibodies. Positive controls for BDNF, TrkB, p-Akt, and p-RPS6 were human brain tissue, cervical carcinoma, human pancreas tissue, and human brain tissue, respectively. Only a brown color, regardless of its intensity, was considered as positive staining.

Semiquantitative Analysis

All immunohistochemical analyses were performed in a blind manner. Two experienced and previously trained pathologists performed a semiquantitative analysis. The final score was established by a consensus. For each case, an immunoreactive score (IRS) at the epithelial and stromal tissues was determined. The IRS was calculated by multiplying the percentage of positive (PP) cells (stained 0 to 4) by staining intensity (SI) (stained 0 to 3). The PP was scored as follows: 0—0% of stained cells; 1—1% to 20% of stained cells; 2—21% to 50% of stained cells; 3—51% to 80% of stained cells; 4—81% to 100% of stained cells. SI was scored as follows: 0—no staining; 1—weak staining; 2—moderate staining; and 3—strong staining. The final IRS value represents a product of points and ranged from 0 to 12. The IRS system was initially proposed by Remmele and Stegner[15] and has been previously used for the study of OLs.[16,17]

The arrangement of positive expression within cystic and tumoral epithelium was also evaluated using previously described expression patterns: (1) prostromal pattern—positivity of peripheral/basal cells and negativity of central/suprabasal cells; (2) antistromal pattern—negativity of peripheral/basal cells and positivity of central/suprabasal cells; (3) full pattern—positivity of both peripheral/basal and central/suprabasal cells.[18] Periphery cells were considered as the most external layer of cells in close contact with the ectomesenchymal tissue (basal cells). All other layers including suprabasal and cells at the core of the island were considered central.

Statistical Analysis

The immunohistochemical data were analyzed using SPSS software (IBM Corporation, Armonk, NY), version 18.0. Initially, a descriptive analysis of clinicopathologic features was performed. A χ^2 test was used to compare gender and site between diagnoses, whereas differences in age were assessed by the Kruskal-Wallis test. Results from immunohistochemistry were compared using a χ^2 test (positive vs. negative expression) and Kruskal-Wallis test followed by a Dunn's post hoc test adjusted for Bonferroni error correction (IRS score). A Wilcoxon test was used to compare epithelial and stromal expression within each diagnosis. For all tests, $P \leq 0.05$ was considered indicative of statistical significance.

RESULTS

Study Population

The clinicodemographic features of the 60 cases of OL are described in Table 1. The overall male:female ratio was 1:1.10, and the majority (77.2%) of cases were located at the mandible. The mean age at diagnosis was 38.96 years, ranging from 11 to 86 years. A significant difference was encountered between sites of OL, in which OKs were more frequent at the maxilla compared with Am and AmC, which occurred predominantly in the mandible. No differences were observed concerning age.

Immunohistochemical Results

Dental Germ

Analysis of dental germs revealed that epithelial remnants from the dental lamina expressed mainly BDNF and TrkB, and both proteins were also highly expressed in the ectomesenchymal tissue surrounding the epithelial islands. BDNF expression was more evident in central cells within the epithelial islands, whereas TrkB was mainly expressed at the periphery. p-Akt was weakly expressed in the epithelial remnants from the dental lamina and was negative in the adjacent connective tissue. p-RPS6 was overall negative in epithelial remnants from the dental lamina and mesenchymal cells (Supplementary Fig. 1, Supplemental Digital Content 1, https://links.lww.com/AIMM/A266). In tooth germ, inner enamel epithelium highly expressed BDNF. Interestingly, cells at the cervical loop lost BDNF expression, and the outer enamel epithelium was also negative. Stellate reticulum expressed TrkB, particularly cells in proximity to the dental papilla. The outer enamel epithelium was mostly negative for all proteins analyzed, with surrounding ectomesenchymal cells being positive for BDNF and TrkB. Moreover, dental papilla was also positive for BDNF and TrkB. p-Akt and p-RPS6 were negative in all components of the tooth germ (Supplementary Fig. 1, Supplemental Digital Content 1, https://links.lww.com/AIMM/A266).

Odontogenic Keratocyst

OK was positive for BDNF, TrkB, p-Akt, and p-RPS6 markers in 83.3%, 100%, 100%, and 90.9% of cases, respectively (Fig. 1, Table 2). All markers were detected in the cytoplasm of

OK epithelial cells. The IRS mean values for BDNF, TrkB, p-Akt, and p-RPS6 expression were 5.27, 10.13, 9.76, and 10.77, respectively (Fig. 2). Aiming to evaluate whether the activation of TrkB was more dependent on paracrine or autocrine mechanisms in epithelial cells, we evaluated the expression of BDNF in the stroma of OL. In OK, the stromal BDNF IRS mean was 5.11 (Fig. 2B). No significant difference between epithelial and stromal BDNF IRS score was observed (Fig. 2C). Concerning the pattern of expression analysis, OK showed a predominance of the full pattern of all markers (Table 3).

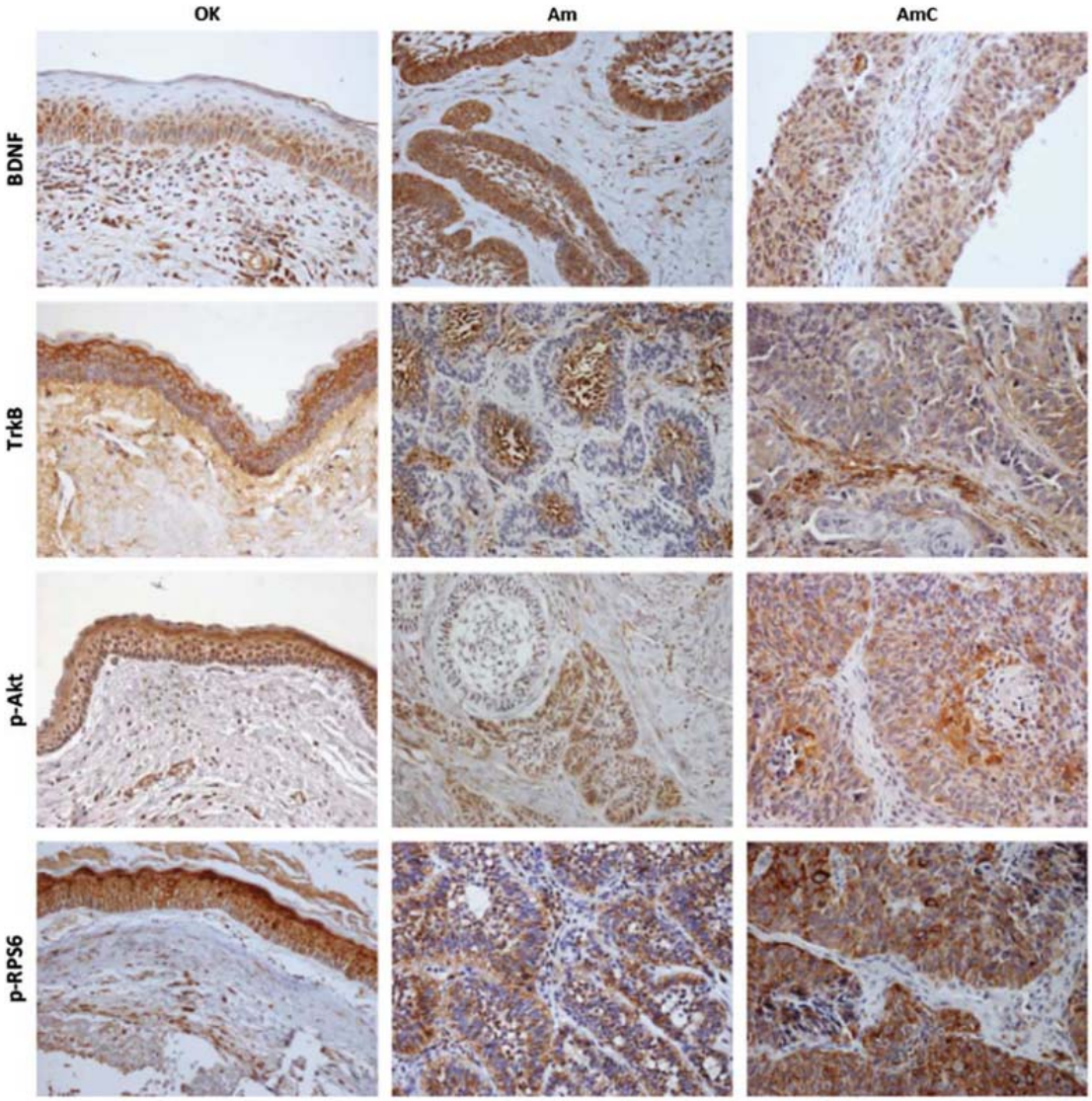

FIGURE 1: A, Representative images of BDNF, TrkB, p-Akt, and p-RPS6 immunohistochemical expression in OK, Am, and AmC. Am indicates ameloblastoma; AmC, ameloblastic carcinoma; BDNF, brain-derived neurotrophic factor; TrkB, tropomyosin receptor kinase B; OK, odontogenic keratocyst.

TABLE 2 - Percentage of Positive and Negative Cases

	OK (%)	Am (%)	AmC (%)	*P**
BDNF				
Positive expression	83.3	82.1	100	0.537
Negative expression	16.7	17.9	0	
TrkB				
Positive expression	100	80.8	100	0.094
Negative expression	0	19.2	0	
p-Akt				
Positive expression	100	96.0	100	0.625
Negative expression	0	4.0	0	
p-RPS6				
Positive expression	90.9	92.0	100	0.751
Negative expression	9.1	8.0	0	

*$\chi 2$ test.

Am indicates ameloblastoma; AmC, ameloblastic carcinoma; BDNF, brain-derived neurotrophic factor; TrkB, tropomyosin receptor kinase B; OK, odontogenic keratocyst.

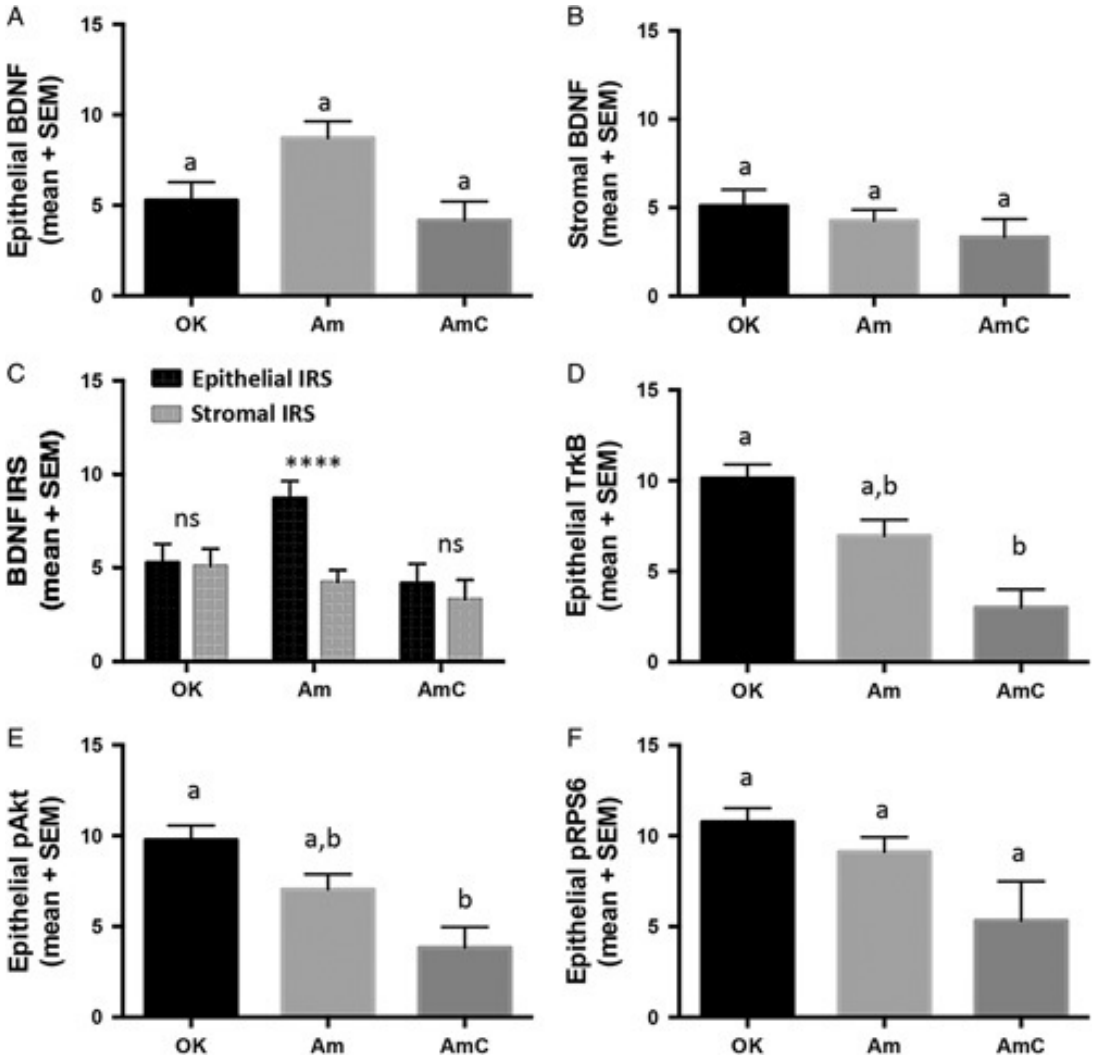

FIGURE 2: A, Epithelial BDNF IRS (mean±SEM) according to each diagnostic entity (Kruskal-Wallis test). B, Stromal BDNF IRS (mean±SEM) according to each diagnostic entity (Kruskal-Wallis test). C, Epithelial and stromal BDNF IRS (mean±SEM) within each diagnostic entity (Wilcoxon test, ****denote a *P*<0.0001). D, Epithelial TrkB IRS (mean±SEM) according to each diagnostic entity (Kruskal-Wallis test, followed by Dunn's

post hoc test adjusted for Bonferroni error correction). E, Epithelial p-Akt IRS (mean±SEM) according to each diagnostic entity (Kruskal-Wallis test, followed by Dunn's post hoc test adjusted for Bonferroni error correction). F, Epithelial p-RPS6 IRS (mean±SEM) according to each diagnostic entity (Kruskal-Wallis test). Different lowercase letters denote the presence of a significant difference (P<0.05). Am indicates ameloblastoma; AmC, ameloblastic carcinoma; BDNF, brain-derived neurotrophic factor; IRS, immunoreactive score; ns, not significant; OK, odontogenic keratocyst; SEM, standard error deviation; TrkB, tropomyosin receptor kinase B.

TABLE 3 - Pattern of BDNF, TrkB, p-Akt, and p-RPS6 Expression

Pattern of Expression	OK (%)*	Am (%)*	AmC (%)*
BDNF			
Prostromal	46.66	8.69	14.28
Antistromal	0	4.34	42.85
Full	53.33	86.95	42.85
TrkB			
Prostromal	21.05	0	0
Antistromal	26.31	91.30	0
Full	52.63	8.69	100
p-Akt			
Prostromal	5.55	4.34	0
Antistromal	0	17.39	0
Full	94.44	78.26	100
p-RPS6			
Prostromal	0	0	0
Antistromal	43.75	42.85	0
Full	56.25	57.14	100

*Percentage of cases among positive ones.
Am indicates ameloblastoma; AmC, ameloblastic carcinoma; BDNF, brain-derived neurotrophic factor; TrkB, tropomyosin receptor kinase B; OK, odontogenic keratocyst.

Am

Odontogenic epithelial cells of Am were positive BDNF, TrkB, p-Akt, and p-RPS6 in 82.1%, 80.8%, 96%, and 92% of cases, respectively (Fig. 1, Table 2). The IRS mean values for BDNF, TrkB, p-Akt, and p-RPS6 expression were 8.71,.6.96, 7.04, and 9.12, respectively (Fig. 2). The stromal BDNF IRS mean was 4.25 (Fig. 2B). This resulted in Am exhibiting a significantly higher BDNF epithelial expression compared with stromal expression (Fig. 2C). In Am, the full pattern was also more commonly observed; however, TrkB seemed like an exception, with a marked tendency for antistromal expression (Table 3).

AmC

All cases of AmC presented positive expression for BDNF, TrkB, p-Akt, and p-RPS6 in the malignant odontogenic epithelium (Fig. 1, Table 2). Despite being positive, the SI and PP cells were lower compared with the other lesions as revealed by the IRS. In AmC, the IRS mean values for BDNF, TrkB, p-Akt, and p-RPS6 expression were 4.16, 3.00, 3.83, and 5.33, respectively (Fig. 2). The stromal BDNF IRS mean was 3.33 (Fig. 2B). No significant difference between epithelial and stromal BDNF IRS score was observed (Fig. 2C). In AmC, the full pattern of expression was observed as the most prevalent for all markers (Table 3).

Compairison Between Diagnoses

BDNF expression was similar in OK, Am, and AmC with respect to both the PP cases and the IRS analysis (Fig. 2A and Table 2). The percentage of cases with positive TrkB, p-Akt, and p-RPS6 expression was also similar between all OL (Table 2). The IRS analysis, however, revealed that TrkB and p-Akt differed between lesions. TrkB and Akt were significantly more expressed in odontogenic epithelial cells of OK compared with AmC (Figs. 2D, E). The p-RPS6 IRS did not differ between OK, Am, and AmC (Fig. 2F).

DISCUSSION

Several studies have been conducted aiming to identify deregulated proteins and signaling pathways in odontogenic cysts and tumors.[3,18,19] Growth factors are recognized as major regulators in determining the fate of cells in both normal and pathologic conditions. In OL, some growth factors, such as transforming growth factor (TGF)-β,[20] have been previously evaluated. The role of BDNF, however, had never been assessed until the present time. Herein, we identified a high percentage of OK, Am, and AmC cases with BDNF expression in the odontogenic epithelial cells, suggesting that this growth factor might participate in the development of such lesions. Unexpectedly, OK exhibited the higher levels of TrkB and p-Akt. In other types of epithelial neoplasms, such as head and neck, lung, and breast cancer, the BDNF/TrkB/Akt axis has been associated with the acquisition of a malignant phenotype[10,21]. We believe that the downregulation of TrkB and Akt in Am and AmC indicates that the invasive and malignant phenotypes are not associated with activation of the BDNF/TrkB/Akt/RPS6 axis.

BDNF is involved in the survival and differentiation of neurons and neurotransmission mechanisms[9,22]; however, several non-neural cells are able to produce and secrete BDNF and express its receptor TrkB, including odontogenic cells during tooth development.[12–14] Herein, we analyzed for the first time the expression of all pathway components in dental lamina remnants and tooth germs. Because of a limited number of cases, we performed a qualitative analysis for protein expression during odontogenesis. The overall analysis revealed a relatively consistent pattern of expression among tooth germs in different stages such as bell and cap, and therefore no subdivisions for analysis were performed. Our results corroborate with the hypothesis that the BDNF/TrkB pathway is associated with epithelial-mesenchymal interactions. In tooth germs, for example, BDNF is mainly expressed by the inner enamel epithelium, and TrkB is highly expressed in the stellate reticulum and dental papilla, suggesting a paracrine mechanism of activation within epithelial cells and toward mesenchymal cells. Expressions in epithelial remnants of the dental lamina and surrounding ectomesenchymal tissue also support that this pathway plays a role in tooth development. Interestingly, this function does not occur through Akt and RPS6 activation because both proteins were mainly negative in tooth germs. Other downstream targets of TrkB are probably triggered, and further investigations are required to clarify this matter.

Our results suggest that the vast majority of OK, Am, and AmC odontogenic epithelial cells produced BDNF. Odontogenic cysts and tumors originate from odontogenesis cell remnants, and it is proposed that these cells maintain a genetic or epigenetic “memory.” Herein, we could observe that epithelial cells of OL maintained the capacity to produce BDNF, such as

inner enamel epithelium and dental lamina remnants during odontogenesis. Our results suggest that the BDNF/TrkB pathway is involved in the development of odontogenic cysts and tumors; however, it is nondiscriminatory pertaining to lesion behavior. OL epithelial cells can produce BDNF, and the expression of other growth factors has already been demonstrated in different OL, such as TGF-β[20] and fibroblast growth factor-8.[23]

Growth factors can be released by either parenchyma cells or stromal components.[8] In physiological processes, the paracrine mode of activation of growth factors usually dominates. In neoplasms, however, it is common for tumoral cells to have the capacity of self-stimulation, leading to autocrine loops and constitutive pathway activation.[8] Therefore, we considered it important to evaluate whether TrkB activation in odontogenic epithelial cells was more dependent on stromal stimulation or self-stimulation. We detected that in OK and AmC, epithelial and stromal BDNF expression was similar, suggesting an equal paracrine and autocrine activation of TrkB in these lesions. In Am, in contrast, BDNF in epithelial cells was significantly higher compared with stromal BDNF, indicating a predominance in autocrine mechanisms of TrkB activation. Previous studies have demonstrated the presence of epithelial expression of other growth factors in Am, such as TGF-β[17] and fibroblast growth factor-8.[23] Combined, these results suggest that epithelial cells of OL might acquire the capacity to produce and secrete growth factors, triggering autocrine stimulus. Further mechanistic studies should be performed to confirm this hypothesis.

We unexpectedly observed a higher expression of TrkB and p-Akt in OK compared with AmC. Moreover, despite no significant difference being observed, OK also presented elevated expression of these proteins compared with Am. OK is known for its increased growth capacity and a higher chance of recurrence compared with other odontogenic cysts. Moreover, OK has a considerably high proliferative index.[24] Am and AmC, however, have a true neoplastic nature; hence, they are associated with a more aggressive phenotype, including invasive growth.[2] In Am, for example, epithelial cells have the capacity to degrade the extracellular matrix by matrix metalloproteinase production.[25] OK, in contrast, grows as most cysts because of the differences in the osmotic pressure inside the cavity associated with bone resorption through compression.[26] Because several studies have demonstrated the association of TrkB and Akt activation with an invasive and migratory phenotype,[22] we expected to observe an upregulation of these proteins in Am and especially AmC. The inverse findings of the present study indicate that in odontogenic neoplasms, particularly, the acquisition of the invasive or malignant phenotype is not associated with the activation of the TrkB/Akt axis. It could also be hypothesized that Akt activation might play a role in odontogenic epithelium differentiation or specialization into a keratin secreting phenotype and this would explain its higher expression in OL. Yet, is important to stress some limitation of our study such as retrospective nature and limited AmC sample size. Thus, prospective studies with a more representative sample of OL might bring new evidence concerning the role of Akt in odontogenic carcinogenesis.

The activation of PI3K/Akt and its downstream proteins regulate many cellular functions. Following PI3K phosphorylation, different targets can be activated, including mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2).[27] The RPS6, evaluated here, is a downstream target of Akt/mTORC1. Previously, Chaisuparat et al[28] observed that OK and Am are positive

for both p-Akt Ser473 and p-RPS6. Yet, the authors did not evaluate the differences between the diagnoses. Kumamoto and Ooya[29] identified a similar expression of p-Akt in Am and AmC, which is in agreement with our findings. Yet, we also demonstrated that Akt phosphorylation is significantly increased in OK compared with AmC. This finding had not been previously described in the literature. Akt can induce aggressive cell behaviors, however, it can also stimulate keratinocyte differentiation in other contexts.[30] OK is assumed to originate from remnants of the primordial odontogenic epithelium that maintains the potential of keratinization inherited from its parent tissue, the stomadeal.[31] Our hypothesis, as stated before, is that Akt activation in OK is associated with the need for differentiation of primordial odontogenic epithelial cells in keratin-producing cells. More studies are required to confirm this theory. Concerning the downstream pathway of Akt, we observed no significant differences in p-RPS6 between OK and AmC, as observed for Akt. This suggests that Akt activation does not stimulate mTOR1 in OK and other downstream proteins should be evaluated.

In the present study, we evaluated the pattern of expression of different markers in each lesion and the TrkB pattern of expression in Am stood out. Clearly, this protein was more highly expressed in the central cells of islands, cords, and nests of Am and was less expressed in the peripheral cells. Jaafari-Ashkavandi et al[24] demonstrated a higher expression of proliferative markers MCM3 and Ki67 in the peripheral ameloblast-like cells of Am. Combining these findings, we can infer that the less proliferative cells of Am, located at the center of tumor islands, are the ones that express more TrkB. This finding reinforces the suggestion that TrkB activation is not associated with increased proliferation in OL.

In conclusion, our results suggest the participation of the BDNF/TrkB pathway in the development of odontogenic pathologic conditions such as OK, Am, and AmC, independently of the lesion nature or behavior. The higher TrkB and Akt expression in OK suggests that the BDNF/TrkB/Akt/RPS6 axis is not associated with the invasive or malignant phenotype of OLs. Rather, we hypothesize that this axis might be associated with the cell differentiation process.

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