UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE ODONTOLOGIA

PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

NÍVEL MESTRADO

ÁREA DE CONCENTRAÇÃO PATOLOGIA BUCAL

ESTUDO DA VIA DE SINALIZAÇÃO BDNF/TrkB EM CERATOCISTOS ODONTOGÊNICOS, AMELOBLASTOMAS, CARCINOMAS AMEOBLASTICOS.

FLORENCIA MARIANA LAMELA DORNELLES

Porto Alegre
2018

FLORENCIA MARIANA LAMELA DORNELLES

ESTUDO DA VIA DE SINALIZAÇÃO BDNF/TrkB EM CERATOCISTOS ODONTOGÊNICOS, AMELOBLASTOMAS, CARCINOMAS AMELOBLASTICOS

Dissertação apresentada ao programa de Pós-Graduação em Odontologia, nível Mestrado, da Universidade Federal do Rio Grande do Sul, como pré-requesito final para a obtenção do título de Mestre em Odontologia
Área de concentração: Patologia Bucal.
Linha de pesquisa: Diagnóstico de Afecções Buco-Faciais

Orientadora: Profa. Dra. Manoela Domingues Martins

Porto Alegre
2018

CIP - Catalogação na Publicação

DORNELLES, FLORENCIA MARIANA LAMELA
Estudo da via de sinalização BDNF/TrkB em Ceratocistos Odontogênicos, Ameloblastomas e Carcinomas Ameloblasticos. / FLORENCIA MARIANA LAMELA DORNELLES. -- 2018.
42 f.
Orientadora: Manoela Domingues Martins.

Dissertação (Mestrado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Odontologia, Programa de Pós-Graduação em Odontologia, Porto Alegre, BR-RS, 2018.

1. lesões odontogênicas. 2. BDNF. 3. TrkB. 4. m-TOR. 5. Akt. I. Domingues Martins, Manoela, orient. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

DEDICATÓRIA

Em primeiro lugar ao meu pai, dono de cada uma das minhas conquistas, por me ensinar a ser alegre, forte, perseverante e lutadora.

Para minha mãe por me apoiar incondicionalmente e junto com meu pai me ensinar os valores que eu tenho hoje.

Para minha irmã por ser minha companheira e exemplo a seguir.

Aos meus sobrinhos Sandino e Josefina, eles são o nosso futuro.

AGRADECIMENTOS

À minha orientadora, **Profa. Manoela Domingues Martins** pela sua grande dedicação, compromisso, paciência e amor pelo seu trabalho. Por me receber e me guiar sempre com um sorriso e ser generosa com seus conhecimentos.

À sua equipe colaboradora, em especial a **Dra. Vivian Petersen Wagner**, por sua importante colaboração no desenvolvimento desta dissertação.

Aos profesores **Dra. Veronica Beovide, Dr. Ronell Bologna, Dra Laura Cosetti, Dra Susana Vazquez** por serem meus grandes inspiradores e referências de patologia oral no Uruguai.

À **Faculdade de Odontologia da UFRGS (PPG em Odontologia)** e **Faculdade de Odontologia da UDELAR** por ter me dado a oportunidade de fazer o mestrado.

À **Fundação de Incentivo à Pesquisa do HCPA (FIPE/GPPG)** e PPG em estomatopatologia da **FOP-UNICAMP** pelo apoio financeiro para a realização das reações imunoistoquimicas e aquisição de anticorpos, respectivamente..

RESUMO

DORNELLES, Florencia Mariana Lamela. **Estudo da via de sinalização BDNF/TrkB em Ceratocistos Odontogênicos, Ameloblastomas e Carcinomas Ameloblasticos.** 38f. Dissertação de Mestrado apresentado ao Programa de Pós-Graduação em Odontologia da Universidade Federal do Rio Grande do Sul, área de concentração Patologia Bucal – Faculdade de Odontologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2018.

As lesões odontogênicas (LO) constituem um importante grupo de patologias bucomaxilofaciais representadas por cistos odontogênicos, tumores benignos e malignos. A via de sinalização BNDF / TrkB possui múltiplas ações biológicas e tem sido identificada como uma importante via na proliferação, invasão e sobrevivência de diferentes tumores epiteliais. Seu papel no desenvolvimento do LO, no entanto, permanece totalmente inexplorado. O objetivo deste estudo foi avaliar a via de sinalização BDNF / TrkB / Akt / pRPS6 em LO de origem epitelial. O estudo incluiu 25 casos de ceratocisto odontogênico (CO), 29 casos de ameloblastoma (Am) e 6 casos de carcinoma ameloblástico (CAM). A coloração imuno-histoquímica para BDNF, TrkB, pAkt e pRPS6 foi realizada. As lâminas foram avaliadas de acordo com o padrão de expressão nas células epiteliais e pelos escores imunoreativos semiquantitativos que consideraram a intensidade de coloração e porcentagem de células positivas. A expressão estromal do BNDF também foi avaliada.Não foram observadas diferenças significativas quanto à porcentagem de casos positivos para todos os marcadores. Em relação aos escores imunorreativos, as expressões de BDNF e pRPS6 foram semelhantes no epitélio odontogênico de todos os LO. No entanto, CO expressou mais TrkB e pAkt em comparação com AmC. No Am, a expressão epitelial do BNDF foi significativamente maior em comparação com a expressão estromal. O BDNF parece participar no desenvolvimento de epitélio odontogênico cístico, benigno e maligno em níveis semelhantes. Além disso, a aquisição do fenótipo invasivo ou maligno em neoplasias odontogênicas não está associada à ativação do eixo BDNF / TrkB / Akt / RPS6.

Palavras-chave: lesões odontogênicas, BDNF,TrkB, m-TOR, Akt

ABSTRACT

DORNELLES, Florencia Mariana Lamela. **Study of BDNF/TrkB signaling pathway in Odontogenic Keratocyst, Ameloblastoma, Ameloblastic Carcinoma.** 38p. Dissertation presented to the Postgraduate Program in Dentistry of the Federal University of Rio Grande do Sul, area of concentration: Oral Pathology – Faculty of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, 2018.

Odontogenic lesions (OL) are an important group of oral and maxillofacial pathologies represented by odontogenic cysts, benign and malignant tumors. The BNDF/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. It's role in the development of OL, however, remains utterly unexplored. The aim of this study was to evaluate the BDNF/TrkB/Akt/pRPS6 signaling pathway in OL of epithelial origin. The study comprised 25 cases of odontogenic keratocyst (OK), 29 cases of ameloblastoma (Am) and 6 cases of ameloblastic carcinoma (AmC). Immunohistochemical staining for BDNF, TrkB, pAkt and pRPS6 were performed. The slides were evaluated according to the pattern of expression in epithelial cells and by semi-quantitative immunoreactive scores that considered the intensity of staining and percentage of positive cells. BNDF stromal expression was also assessed. No significant differences were observed concerning the percentage of positive cases for all markers. Concerning the immunoreactive scores, BDNF and pRPS6 expressions were similar in the odontogenic epithelium of all OL. However, OK expressed more TrkB and pAkt compared to AmC. In Am, BNDF epithelial expression was significantly higher compared to stromal expression. BDNF appears to participate in the development of cystic, benign and malignant odontogenic epithelium in similar levels. Moreover, the acquisition of the invasive or malignant phenotype in odontogenic neoplasms is not associated with activation of BDNF/TrkB/Akt/RPS6 axis.

Keywords: odontogenic lesions, BDNF,TrkB, m-TOR, Akt

LISTA DE QUADROS E TABELAS

Lista de figuras da introdução

Lista de tabelas e figuras do artigo

SUMÁRIO

1. INTRODUÇÃO

As lesões odontogênicas (LOs) compreendem um importante grupo de patologias orais e maxilofaciais (Al-Rawi et al., 2013; Grossmann et al 2014; Wright, Vered 2017). Estas lesões originam-se dos remanescentes epiteliais e mesenquimais do desenvolvimento dentário ou dos tecidos de sustentação dos dentes, tendo como exemplos os restos epiteliais de Malassez, a lâmina dentária e o órgão do esmalte (Urrutia et al, 2010; Baghaei et al, 2014). As LOs, representadas pelos cistos e tumores odontogênicos, são entidades incomuns que somam menos de 2-3% dentre todos os espécimes enviados para análise histopatológica em serviços de patologia oral (Imran et al., 2016).

Os cistos odontogênicos (COs) desenvolvem-se do epitélio derivado do aparato odontogênico ou de seus remanescentes aprisionados no interior do osso ou dos tecidos gengivais (Mosqueda et al., 2002). De acordo com a sua etiopatogênese, são classificados em cistos inflamatórios, cujos fatores etiológicos provém de uma inflamação, ou cistos de desenvolvimento, dos quais os fatores desencadeantes são ainda desconhecidos (Arce et al., 2002; Sharifian et al., 2012). Geralmente, os COs são assintomáticos e apresentam crescimento lento e expansivo (Ochsenius et al., 2007; Souza et al., 2010). Entretanto, alguns tipos podem demonstrar crescimento rápido e sintomatologia clínica (Souza et al., 2010; Ferreres et al., 2016). Radiograficamente, os COs são, em sua maioria, lesões radiolúcidas bem delimitadas (Urrutia et al., 2010). O diagnóstico final das lesões é feito por meio da análise histopatológica, sendo o cisto radicular e o cisto dentígero os exemplos majoritariamente diagnosticados em diversos estudos acerca do tema (Jones et al., 2006; Meningaud et al., 2006; Tortorici et al., 2008).

Os tumores odontogênicos (TOs) compõem um grupo heterogêneo de lesões que derivam de elementos epiteliais e/ou mesenquimais de remanescentes odontogênicos (Lee et al., 2015). As lesões podem variar desde proliferações hamartomatosas, neoplasias benignas até neoplasias malignas com potencial metastático (Costa et al., 2012; Alsheddi et al., 2015). Seu desenvolvimento pode ocorrer centralmente, no interior do osso, ou perifericamente, em regiões extra-ósseas, e sua etiopatogenia permanece incerta (Alsheddi et al., 2015; Deepthi et al. 2016; Iyogun et al., 2016). Os TOs compreendem lesões com diferentes aspectos clínicos e histopatológicos, as quais podem apresentar-se como um crescimento assintomático lento e expansivo ou lesões cujo desenvolvimento é rápido, infiltrativo e sintomático (Barnes et al.,

2005; Pereira et al., 2010; Osterne et al., 2011). Os TOs são lesões relativamente raras, compreendendo cerca de 12.6% de todos os tumores dos ossos gnáticos (Goteti 2016).
Os ceratocistos comprenden 10-20% de todos os cistos odontogenicos, Os carcinomas ameloblasticos em China comprenden for 2% os ameloblastomas (El Naggar 2017)
e os tipos que apontam as maiores taxas de prevalência em diversos estudos prévios são o ameloblastoma e o odontoma (Sekerci et al., 2015; Goteti et al., 2016).

Atualmente existe uma vasta literatura relatando o papel dos fatores de crescimento e seus receptores na ativação das vias de sinalização intracelulares envolvidas na regulação da diferenciação, sobrevivência, metabolismo, mobilidade e crescimento celular nos diferentes tipos de lesões benignas e malignas. Isto porque, a desregulação dos fatores de crescimento, de seus receptores e das diversas vias de sinalização por eles estimuladas são responsáveis pela aquisição do fenótipo neoplásico, da capacidade de invasão e metástase dos tumores malignos (Alyasiri et al. 2012; Thomaz et al. 2015). Estes estudos são fundamentais para compreender a patogênese das neoplasias, identificação de marcadores biológicos de progressão tumoral e para o desenvolvimento de terapias alvo que atuem na regulação dessas vias. Neste sentido, a via do fator neurotrófico derivado do cérebro (BDNF, do inglês Brain-derived neurotrophic factor) e seu receptor trompomiosina quinase B (TrkB) vem sendo estudada em câncer de pâncreas, pulmão, colón, próstata, mama, bexiga, neuroblastoma, meduloblastoma, mieloma múltiplo, entre outros (Lai et al. 2010; Roesler et al. 2011; de Farias et al. 2012; Yin et al. 2015; Akil, Perraud et al. 2016) e pouco explorada em câncer de cabeça e pescoço (Kupferman et al. 2010; Yilmaz et al. 2010; Lee et al. 2012; Sasahira et al. 2013; Jia et al. 2015).

O BDNF é um membro da família das neurotrofinas que são fatores de crescimento que participam da diferenciação, proliferação e sobrevivência de células neurais e da neurogênese nos sistemas nervosos central e periférico. O principal ligante para o BDNF são os receptores de membrana celular trompomiosina quinase (Trk) (Schecterson, Bothwell, 2010; Akil, Perraud, et al., 2016), em especial o TrkB. A ligação do receptor de BDNF no TrkB desencadeia a sua dimerização através de mudanças conformacionais e autofosforilação de resíduos de tirosina no ambiente intracelular (Liu, Chan, Ye, 2016). Em câncer, a estimulação da via de sinalização do BDNF/TrkB promove a ativação de moléculas de sinalização como Akt (Figura 1), STAT3, Src, ERK e MAPK resultando no aumento da proliferação celular, resistência a apoptose, invasão, metástase e resistência a quimioterapia. Desta forma, o aumento da expressão de BDNF/TrkB tem sido associado com comportamento mais agressivo de diferentes tumores malignos e pior prognóstico, devido ao seu papel nos processos de

angiogênese, invasão, metástase e a resistência à quimioterapia (Fujikawa et al. 2012; Kupferman et al. 2010; Guo et al. 2011; Roesler et al. 2011; Okamura et al. 2012; Sasahira et al. 2013; Jia et al. 2015; Kim et al. 2015).

A via de sinalização da PI3K é crucial em numerosos aspectos celulares envolvidos no crescimento e sobrevivência celular.

É estimulada fisiologicamente em consequência da ativação dos receptores de membrana tirosina quinase, que fosforilam e formam automaticamente o substrato de insulina (IRS) que fosforila a subunidade p85 de PI3K. O que produz uma mudança de conformação da dita proteína levando à ligação da subunidade catalítica (p 110) a PI3K ativa, fosforila a (PIP2) convertendo-a no segundo mensageiro (PI3P), que a jusante leva à ativação da proteína. Proteína Akt..

Akt (Proteína quinase B) é o homólogo humano do oncogene viral v-Akt (retrovírus Akt 8) (Pinzon 2009) A ativação anormal dessa via leva a uma resposta proliferativa e antiapoptótica que está relacionada ao desenvolvimento de múltiplos tipos de câncer. (Pinzon 2009)

TrkB é uma proteína transmembrânica e que é codificada pelo proto-oncogene Trk humano, o qual tem a capacidade para adquirir propriedades oncogênicas. Este proto-oncogene, através de um rearranjo cromossômico adquire a capacidade de oncogênese participando nos processos de transformações malignas.

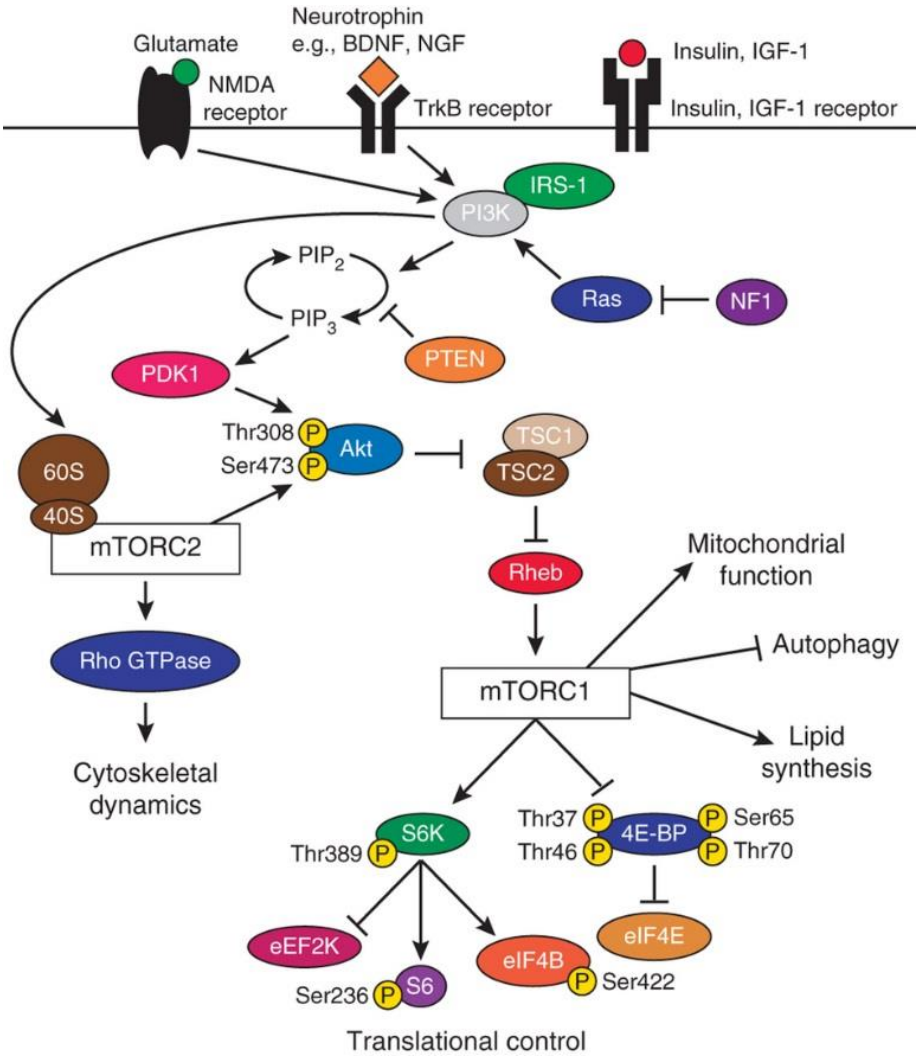


Figura 1.Ilustração da via de sinalização BDNF/TrkB/PI3K/Akt/m-TOR .

Em câncer de cabeça e pescoço, apesar de poucos estudos terem sido realizados, o BDNF/TrkB aparece altamente expresso nas células tumorais (Zhu et al. 2007; Yilmaz et al. 2010; Jia et al. 2015), além de ter demonstrado relação com pior prognóstico, aumento da angiogênese e linfangiogênese (Sasahira et al. 2013), e de participar do processo de transição epitelial mesenquimal (EMT), que é considerado um processo biológico chave na invasão tumoral e metástase de tumores epiteliais (Jia et al. 2015). A relação da ativação da via de sinalização do BDNF/TrkB em CECP com a agressividade tumoral e senescência entretanto ainda necessitam ser elucidadas (de Moraes et al. 2017). Além disso, não há estudos que avaliam o papel desta via nas lesões benignas e malignas odontogênicas.

2. OBJETIVOS

2.1 Objetivo Geral

Estudar a via de sinalização BDNF/TrkB/Akt em CO, Am e Cam.

2.2 Objetivos Específicos

Correlacionar os tipos histológicos de CO, Am, Cam com imunomarcação de BNDF, TrkB, pS6 (mTORC1), p-Akt 473 (mTORC2).

3. ARTIGO CIENTÍFICO

Este trabalho será submetido ao periódico Journal of Oral Pathology and Medicine (Qualis Capes 2013- A2, ISSN 1079-2104, fator de impacto 2.237). Já estando formatado conforme as normas da revista citada.

BDNF/TrkB/Akt signaling pathway in epithelial odontogenic lesions

Running title: BDNF/TrkB/Akt axis odontogenic lesions

Florencia Mariana Lamela Dornelles[1,2], Vivian Petersen Wagner[3], Felipe Paiva Fonseca[3,4], Carla Ariotti[1], Vinicius Coelho Carrard[1], Pablo Agustin Vargas[3], Veronica Beovide[1,2], Ronell Bologna-Molina[2], Manoela Domingues Martins[1,3]

[1]Department of Oral Pathology, School of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.
[2]Molecular Pathology Area, School of Dentistry, Universidad de La Republica (UDELAR), Montevideo, Uruguay
[3]Department of Oral Diagnosis, Piracicaba Dental School, University of Campinas, Piracicaba, Brazil
[4]Department of Oral Surgery and Pathology, School of Dentistry, Federal University of Minas Gerais, Belo Horizonte, Brazil
[7]Department of Pharmacology, Institute for Basic Health Sciences, Federal University of Rio Grande do Sul, Porto Alegre, Brazil
[8]Department of Oral Pathology, School of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

Corresponding author:
Manoela Domingues Martins
Universidade Federal do Rio Grande do Sul - Faculdade de Odontologia
Rua Ramiro Barcelos, 2492, sala 503
CEP: 90035-003
Santana, Porto Alegre RS, Brazil
Phone: 55-51-33085011
manomartins@gmail.com

Abstract

Background: Odontogenic lesions (OL) are an important group of oral and maxillofacial pathologies represented by odontogenic cysts, benign and malignant tumors. The BNDF/TrkB signaling pathway has multiple biological actions and has been identified as an important pathway involved with proliferation, invasion and survival of different epithelial tumors. It's role in the development of OL, however, remains utterly unexplored. The aim of this study was to evaluate the BDNF/TrkB/Akt/pRPS6 signaling pathway in OL of epithelial origin.

Methods: The study comprised 25 cases of odontogenic keratocyst (OK), 29 cases of ameloblastoma (Am) and 6 cases of ameloblastic carcinoma (AmC). Immunohistochemical staining for BDNF, TrkB, pAkt and pRPS6 were performed. The slides were evaluated according to the pattern of expression in epithelial cells and by semi-quantitative immunoreactive scores that considered the intensity of staining and percentage of positive cells. BNDF stromal expression was also assessed.

Results: No significant differences were observed concerning the percentage of positive cases for all markers. Concerning the immunoreactive scores, BDNF and pRPS6 expressions were similar in the odontogenic epithelium of all OL. However, OK expressed more TrkB and pAkt compared to AmC. In Am, BNDF epithelial expression was significantly higher compared to stromal expression.

Conclusions: BDNF appears to participate in the development of cystic, benign and malignant odontogenic epithelium in similar levels. Moreover, the acquisition of the invasive or malignant phenotype in odontogenic neoplasms is not associated with activation of BDNF/TrkB/Akt/RPS6 axis.

Key words: odontogenic lesions, growth factor, signaling pathway, mTOR, BDNF, TrkB.

1 INTRODUCTION

Odontogenic lesions (OL) are a heterogeneous group of disorders derived from tooth forming apparatus or its remnants. These entities present a wide spectrum of clinical and biological behavior.[1,2] OL may range from hamartomatous proliferations, cystic, benign neoplasms to malignant neoplasms with metastatic potential.[1,2,3] The vast majority of OL is intra-osseous and thus requires invasive and sometimes extensive surgeries, especially in OL with progressive and infiltrative growth.[4] Moreover, recurrences can occur in both cystic and neoplastic OL, leading to the necessity of new surgical procedures and further morbidity to the patients.[5,6]

Among OL, some stand out due to their higher prevalence as well as more aggressive clinical behavior. Odontogenic keratocyst (OK) and ameloblastoma (Am) are derived from odontogenic epithelial component and are among the most common OL.[1,5,6] OK is a peculiar odontogenic cyst with more aggressive growth compared to other odontogenic cysts and high recurrence rates after conservative.[6] These features have let to many discussions about the cystic or neoplastic nature of this entity. Currently, the World Health Organization (WHO) recognized these unique aspects but includes this lesion within odontogenic cysts.[1] Am is a benign odontogenic tumor with infiltrative growth leading to a significant local aggressiveness and the necessity of wide surgical resection to prevent tumor relapse.[5] The malignant counterpart of Am is called ameloblastic carcinoma (AmC) and shows an ameloblastic epithelium with malignant features. This tumor can arises *de novo* or secondary to a previous benign Am. Although AmC is a very rare lesion, it represents the most frequent malignant OL and presents extremely high recurrence and metastatic rates.[7] The etiology and pathogenesis of these OL remain poorly understood. Previous studies have identified some transcriptional alterations that may be responsible for their development and progression.[8,9] However, the mechanisms involved with the acquisition of the proliferative and invasive capacity of OL epithelial cells need to be better understood.

There is a vast literature reporting the role of growth factors and their receptors in the activation of intracellular signaling pathways involved in the regulation of cell differentiation, survival, metabolism, mobility and cell growth in different types of benign and malignant lesions. The deregulation of growth factors and their receptors can be responsible for the acquisition of the neoplastic phenotype, invasiveness and metastasis of malignant tumors.[10] Brain-derived neurotrophic factor (BDNF), a member of the

neurotrophin family, is originally known to play an important role in controlling cell survival, differentiation and death in the nervous system.[11] The major ligands for BDNF are the cell membrane receptors thrompiosycin kinase (Trk)[12], especially TrkB. BDNF binding to TrkB elicits its dimerization through conformational changes and auto-phosphorylation of tyrosine residues in the environment intracellular.[11,12] 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 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, due to its role in the processes of angiogenesis, invasion, metastasis and resistance to chemotherapy.[14,15,16,17]

During odontogenesis, neurotrophic factors may have multiple functions such as establishment of the dental innervation and modulating epithelial-mesenchymal interactions during tooth morphogenesis.[18,19,20] Nevertheless, the pattern of BDNF/TrkB signaling pathway activation in cystic and neoplastic OL still need to be elucidated. Thus, the aim of the present study was to evaluate the expression of BDNF/TrkB and two downstream targets of this pathway, Akt and ribosomal protein S6 (RPS6) in OK, Am and AmC.

2 METHODS

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

2.1 Study population

A manual 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 cases of OK, Am and AmC with adequate amount of material for the analysis of specimens were included in the study, resulting in a total of 60 cases of OL. The patients' records were manually evaluated and information concerning gender, age and site were collected.

2.2 Histopathologic analysis

Slides of the incisional biopsies were retrieved by two blinded pathologist and diagnosed according to the more recent Histological Classification of Head and Neck Tumors of the WHO.[3] The presence of intense inflammatory infiltrate was considered as exclusion criteria during histopathological evaluation. This is because; it is known that the inflammatory process can modify the immunoexpression of some proteins and also the epithelial behavior. Am were not sub-classified based on the histologic pattern once we evaluated incisional biopsies, which could lead to a misdiagnosis.

2.3 Immunohistochemistry

Immunohistochemical reactions were performed at Experimental Pathology Unit at the Hospital of Clinics at Porto Alegre (HCPA). 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 90ºC in a water bath. Endogenous peroxidase activity was blocked using 10% hydrogen peroxide in 5 baths during 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 (DAB; 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 brown citoplasmatic color regardless of the color intensity will be considered as positive marking.

2.4 Semi-quantitave analysis

All immunohistochemical analysis was performed in a blind manner. Two experienced pathologists and previously calibrated performed a semi-quantitative analysis. The final score was established by a consensus. For each case an immunoreactive score (IRS) at the epithelial and stromal tissue was designed. Only brown cytoplasmic staining was considered positive. The IRS was calculated by multiplying percentage of positive

cells (PP) (stained 0-4) by staining intensity (SI) (stained 0-3). 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% a 100% of stained cells. SI was scored as follows: 0 - no staining; 1 - weak staining; 2 - moderate staining; and 3 - strong staining.

The distribution 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.[21]

2.5 Statistical analysis

The immunohistochemical data were analyzed using SPSS software (IBM Corporation, Armonk, NY), version 20.0. Initially, a descriptive analysis of clinic-pathologic features was performed. Chi-square test was used to compare gender and site between diagnoses while differences in age were assessed by the Kruskal-Wallis test. Results from immunohistochemistry were compared using chi-square test (positive versus negative expression) and Kruskal-Wallis test followed by Dunn's post-hoc test adjusted for Bonferroni error correction (IRS score). 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.

3 RESULTS

3.1 Study population

Sixty cases of OL were included in the present study. The overall male:female ratio was 1:1.10 and the majority (77.2%) of cases were located at mandible. The mean age at diagnoses was 38.96, ranging from 11 to 86. The sample comprised 25 cases of OK (41.66%), 29 cases of Am (48.33%) and 6 cases of AmC (10%). The clinico-demographic features of each diagnosis are described in Table 1. No differences were observed concerning age and gender between the OL. Regarding the site, a significant difference was encountered. All lesions were predominantly in the mandible, If we compared OK were more prevalent at the maxilla to Am and AmC.

3.2 Immunohistochemical results

BDNF staining was observed in the cytoplasm of odontogenic epithelium in the majority of OK (83.3%), Am (82.1%) cases and all AmC cases (Figure 1A and Table 2). BDNF expression was similar in OK, Am and AmC, concerning both the percentage of positive cases as the IRS analysis (Figure 1B and Table 2). Aiming to evaluate if the activation of TrkB was more dependent of paracrine or autocrine mechanisms in epithelial cells, we evaluated the expression of BDNF in the stroma of OL. We observed no significant differences among the stromal IRS score between OK, Am and AmC (Figure 1C). Nevertheless, when comparing the epithelial and stromal expression within each diagnosis, Am exhibited a higher BNDF epithelial expression compared to stromal expression (Figure 1D). This difference was not observed in Ok and AmC, in which epithelial and stromal expressions had similar levels.

TrkB staining was observed in the membrane and/or cytoplasm of all OK and AmC and the majority of Am (80.8%) (Figure 2A and Table 2). The percentage of cases with positive TrkB expression was similar between all OL (Table 2). The IRS analysis, though, revealed that TrkB was significantly more expressed in epithelial cells of OK compared to AmC (Figure 2B). The protein pAkt was observed in the cytoplasm and/or nuclei of odontogenic epithelium, and for this marker all OK and AmC and the majority of Am (96%) had positive expression (Figure 2A and Table 2). The percentage of cases with positive pAkt expression was similar between OK, Am and AmC, however significant differences were observed in the IRS analysis. OK had significantly higher pAkt IRS compared to AmC (Figure 2C). All AmC and the majority of OK (90.9%) and Am (92%) presented cytoplasmic and/or nuclear expression of pRPS6 (Figure 2A and Table 2). The percentage of pRPS6 positive cases and the pRPS6 IRS did not differ between OK, Am and AmC (Table 2 and Figure 2D).

The different arrangements of these proteins within the epithelial tissue were evaluated by the pattern of expression (Table 3). In OK and AmC we observe a predominance of the full pattern of all markers. In Am, the full pattern was also more commonly observed, however TrkB appeared as an exception with a marked tendency of antistromal expression.

4 DISCUSSION

A varied spectrum of cysts and neoplasms might originate from the odontogenic apparatus or its remnants.[3] These lesions can arise from both epithelium and/or mesenchymal odontogenic cells, however epithelial OL are most commonly observed. Some lesions deserve a more deepen analysis due to their high prevalence and especially their peculiar clinical behavior, such as OK, Am and AmC.[3] Several studies have been conducted aiming to identify deregulated proteins and signaling pathways that could justify the behavior of this OL.[4,22,21] Growth factors are recognized as major regulators in determining the fate of cells in both normal and pathologic conditions. The signaling pathways triggered by them might influence important mechanisms such as cell proliferation, differentiation, invasion and migration.[10] In OL, some growth factors, such as epidermal growth factor, transforming growth factor (TGF)-α[24] and TGF-β[25], 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 pAkt. In other types of epithelial neoplasms, such as head and neck, lung and breast cancer, BDNF/TrkB/Akt axis has been associated with the acquisition of a malignant phenotype.[26,14,27]). We believe that the down regulation of TrkB and Akt in Am and AmC indicate that the invasive and malignant phenotypes are not associated with activation of BDNF/TrkB/Akt/RPS6 axis.

Our study was pioneer in evaluating the signaling pathway triggered by BDNF in OL. In the nervous system, BDNF is involved with the survival and differentiation of neurons and neurotransmission mechanisms[11,28], however several non-neural cells are able to produce and secrete BDNF and express its receptor TrkB. During odontogenesis, BDNF/TrkB appears to be involved in the sensory innervation of dental papilla.[18,19] Moreover, TrkB is expressed during the histo-morphogenesis and cytodifferentiation stages and therefore seems to be implicated in the epithelial-mesenchymal interactions that occur during these phases, which are necessary for the maturation of dental cells.[19,20] Despite these evidences supporting the involvement of BDNF/TrkB during odontogenesis, the expression of these proteins had never been evaluated in OL until now. We demonstrated that in the vast majority of OK, Am and AmC odontogenic epithelial cells produced BDNF. This result suggests that this pathway might be involved with the development of odontogenic cysts and tumors however, it is nondiscriminatory pertaining

to lesion behavior. The cell remnants that give rise to odontogenic cysts and tumors produce architectural patterns and structures that reproduce different stages of odontogenesis, suggesting that they maintain a genetic or epigenetic “memory”. This fact could explain the capacity of epithelial cells of OL to produce BDNF. Other studies have demonstrated a similar expression of other growth factors between different OL, such as TGF-β[25] and fibroblast growth factor (FGF) 8.[29]

Growth factors are short-range mediators that can be released by either parenchyma cells or stromal components.[10] In physiological processes, the paracrine mode of activation of growth factors usually dominates. In neoplasms, however, it is common of tumoral cells to have the capacity of self-stimulation, leading to autocrine loops and constitutive pathway activation.[10] Therefore, we believed it was important to evaluate if TrkB activation in odontogenic epithelial cells was more dependent of stromal- or self-stimulation. We detected that in OK and AmC, epithelial and stromal BDNF expression were similar, suggesting an equal paracrine and autocrine activation of TrkB in these lesions. In Am, by the other side, BDNF in epithelial cells was significantly higher compared to stromal BDNF, indicating a predominance in autrocrine mechanisms of TrkB activation. Previous studies have demonstrated presence of epithelial expression of other growth factors in Am, such as TGF-β[25], and FGF-8.[29] 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 need to be performed to confirm this hypothesis.

We unexpectedly observed a higher expression of TrkB and pAkt in OK compared to AmC. Moreover, despite no significant difference was observed, OK also presented elevated expression of these proteins compared to Am. OK is known for its increased growth capacity and higher chance of recurrence compared to others odontogenic cysts. Am and AmC, however, have true neoplastic nature hence they are associated with more aggressive phenotype, including an invasive mode of growth.[3] In Am, for example, epithelial cells have the capacity to degradate the extra-cellular matrix by MMP production.[30,31] OK, by the other side, growth as most cysts by differences in the osmotic pressure inside the cavity associated with bone resorption through compression.[32] As several studies have demonstrated the association of TrkB and Akt activation with an invasive and migratory phenotype.[13,28,33], we expected to observe an up regulation 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 activation of TrkB/Akt axis.

The activation of PI3K/Akt and its downstream proteins regulate many cellular functions, including cell growth, survival and metabolism, in both physiological and pathological conditions. Following PI3K phosphorylation, different targets can be activated, including mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2).[34] The RPS6, evaluated herein, is a downstream target of Akt/mTORC1. Previous studies evaluating these proteins in OL were identified. Chaisuparat et al. (2013)[35] evaluated the expression of Akt phosphorylated at serine 473 (Ser473) and at threonine 308 and pRPS6 in dentigerous cyst, OK and Am, and compared these expressions with dental follicle. Corroborating with our results, the majority of OK and Am cases were positive for both pAkt Ser473 and pRPS6. The authors did not evaluated differences between the diagnoses, which hampers the comparison with our results. Kumamoto and Ooya (2007)[36] identified a similar expression of pAkt in Am and AmC, which is in agreement with our findings. Scheper et al. (2008)[37] also demonstrated a higher percentage of Am cases with pAkt Ser473 and pRPS6. In this previous study, only Am were evaluated. In our study, Akt phosphorylation was significantly increased in OK compared to AmC. This finding had never been described in the literature until know. Akt is usually evaluated because its capacity to induce aggressive cell behaviors. Nevertheless, this protein can also stimulate keratinocyte differentiation.[38,39] The most accepted theory suggests that OK originates in remnants from the primordial odontogenic epithelium that maintains the potential of keratinization inherited from its parent tissue, the stomadeal.[40] Our hypothesis is that the greater activation of Akt observed in OK is associated with the need of differentiation of primordial odontogenic epithelial cells in keratin producing cells. More studies need to be performed to confirm this theory. Another important aspect is that different growth factors, oncogenes and cytokines can activate Akt.[41] Our results suggest that the up regulation of Akt in OK is triggered at least in part by TrkB activation. Further studies need to be conducted to evaluate whether others known regulators of Akt, such as EGF and HGF, have a role in stimulating Akt in OK. Concerning the downstream pathway of Akt, we observed no significant differences in pRPS6 between OK and AmC, as observed for Akt. This could imply that in this entity, Akt activation does not stimulates mTOR1 therefore, other downstream proteins need to be evaluated.

In the present study we evaluated the pattern of expression of all markers in OK, Am and AmC. In this analysis, the TrkB pattern of expression in Am stood out. Clearly, this

protein was more expressed in the central cells of islands, cords and nests of Am and was less expressed in the peripheral cells. Jaafari-Ashkavandi et al. (2019)[42] 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 which express more TrkB. This finding reinforces that TrkB activation is not associated with increased proliferation in OL.

In conclusion, we have shown that the higher percentage of cases expressing BDNF suggests a participation of this growth factor in the development of OK, Am and AmC. The role of this protein in odontogenesis was previously known, and herein we demonstrated for the first time that BDNF is also present in odontogenic pathologic conditions. Surprisingly, OK exhibited higher levels of TrkB and Akt, indicating that the acquisition of the invasive or malignant phenotype in Am and AmC is not associated with activation of BDNF/TrkB/Akt/RPS6 axis.

Acknowledgments

The authors are grateful to Flavia Rejane Giusti for her technical support and the Biostatistics Unit of the Postgraduate Research Group of the Porto Alegre University Hospital for assisting the statistical analysis. The authors are also grateful to the São Paulo State Research Foundation (FAPESP 2016/21785-4) for student scholarship. This study was funded by the Postgraduate Research Group of the Hospital de Clínicas de Porto Alegre (GPPG/FIPE: 2018–0514). Manoela Domingues Martins and Pablo Agustin Vargas are research fellows funded by the Brazilian National Council for Scientific and Technological Development (CNPq).

Conflict of interest - The authors declare that they have no conflict of interest.

REFERENCES

1. Jaeger F, de Noronha MS, Silva ML, Amaral MB, Grossmann SM, Horta MC, de Souza PE, de Aguiar MC, Mesquita RA. Prevalence profile of odontogenic cysts and tumors on Brazilian sample after the reclassification of odontogenic keratocyst. J Craniomaxillofac Surg. 2017 Feb;45(2):267-270.
2. Wright JM, Vered M. Update from the 4th Edition of the World Health Organization Classification of Head and Neck Tumours: Odontogenic and Maxillofacial Bone Tumors. Head Neck Pathol. 2017 Mar;11(1):68-77.
3. El-Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg PJ. WHO Classification of Head and Neck Tumours, 4th Edition. IARC Press, Lyon, 2017.
4. Brito-Mendoza L, Bologna-Molina R, Irigoyen-Camacho ME, Martinez G, Sánchez-Romero C, Mosqueda-Taylor A. A Comparison of Ki67, Syndecan-1 (CD138), and Molecular RANK, RANKL, and OPG Triad Expression in Odontogenic Keratocyts, Unicystic Ameloblastoma, and Dentigerous Cysts. Dis Markers. 2018 Jul 29;2018:7048531.
5. Almeida Rde A, Andrade ES, Barbalho JC, Vajgel A, Vasconcelos BC.Recurrence rate following treatment for primary multicystic ameloblastoma: systematic review and meta-analysis. Int J Oral Maxillofac Surg. 2016 Mar;45(3):359-67.
6. Chrcanovic BR, Gomez RS. Recurrence probability for keratocystic odontogenic tumors: An analysis of 6427 cases. J Craniomaxillofac Surg. 2017 Feb;45(2):244-251.
7. Martínez Martínez M, Mosqueda-Taylor A, Carlos R, Delgado-Azañero W, de Almeida OP. Malignant odontogenic tumors: a multicentric Latin American study of 25 cases. Oral Dis. 2014 May;20(4):380-5.
8. Diniz MG, Gomes CC, de Sousa SF, Xavier GM, Gomez RS. Oncogenic signalling pathways in benign odontogenic cysts and tumours. Oral Oncol. 2017 Sep;72:165-173.
9. Amm HM, MacDougall M. Molecular Signaling in Benign Odontogenic Neoplasia Pathogenesis. Curr Oral Health Rep. 2016 Jun;3(2):82-92. Epub 2016 Mar 31.
10. Witsch E, Sela M, Yarden Y. Roles for growth factors in cancer progression. Physiology (Bethesda). 2010 Apr;25(2):85-101.

11. Arévalo JC, Wu SH. Neurotrophin signaling: many exciting surprises! Cell Mol Life Sci. 2006 Jul;63(13):1523-37.
12. Schecterson LC, Bothwell M. Neurotrophin receptors: Old friends with new partners. Dev Neurobiol. 2010 Apr;70(5):332-8.
13. Fujikawa H, Tanaka K, Toiyama Y, Saigusa S, Inoue Y, Uchida K, Kusunoki M. High TrkB expression levels are associated with poor prognosis and EMT induction in colorectal cancer cells. J Gastroenterol. 2012 Jul;47(7):775-84
14. Kupferman ME, Jiffar T, El-Naggar A, Yilmaz T, Zhou G, Xie T, Feng L, Wang J, Holsinger FC, Yu D, Myers JN. TrkB induces EMT and has a key role in invasion of head and neck squamous cell carcinoma.Oncogene. 2010 Apr 8;29(14):2047-59
15. Roesler R, de Farias CB, Abujamra AL, Brunetto AL, Schwartsmann G. BDNF/TrkB signaling as an anti-tumor target. Expert Rev Anticancer Ther. 2011 Oct;11(10):1473-5.
16. Okamura K, Harada T, Wang S, Ijichi K, Furuyama K, Koga T, Okamoto T, Takayama K, Yano T, Nakanishi Y. Expression of TrkB and BDNF is associated with poor prognosis in non-small cell lung cancer. Lung Cancer. 2012 Oct;78(1):100-6.
17. Sasahira T, Ueda N, Yamamoto K, Bhawal UK, Kurihara M, Kirita T, Kuniyasu H. Trks are novel oncogenes involved in the induction of neovascularization, tumor progression, and nodal metastasis in oral squamous cell carcinoma. Clin Exp Metastasis. 2013 Feb;30(2):165-76
18. Nosrat CA, Fried K, Lindskog S, Olson L. Cellular expression of neurotrophin mRNAs during tooth development.Cell Tissue Res. 1997 Dec;290(3):569-80.
19. Luukko K, Arumäe U, Karavanov A, Moshnyakov M, Sainio K, Sariola H, Saarma M, Thesleff I. Neurotrophin mRNA expression in the developing tooth suggests multiple roles in innervation and organogenesis.Dev Dyn. 1997 Oct;210(2):117-29.
20. Mitsiadis TA, Luukko K. Neurotrophins in odontogenesis. Int J Dev Biol. 1995 Feb;39(1):195-202.
21. Sánchez-Romero C, Bologna-Molina R, Mosqueda-Taylor A, Paes de Almeida O. Immunohistochemical Expression of GLUT-1 and HIF-1α in Tooth Germ, Ameloblastoma, and Ameloblastic Carcinoma. Int J Surg Pathol. 2016 Aug;24(5):410-8
22. Argyris PP, Malz C, Taleb R, Koutlas IG.Benign and malignant odontogenic neoplasms of the jaws show a concordant nondiscriminatory p63/p40 positive

immunophenotype. Oral Surg Oral Med Oral Pathol Oral Radiol. 2018 Sep 5. pii: S2212-4403(18)31162-3.

23. Sánchez-Romero C, Bologna-Molina R, Mosqueda-Taylor A, de Almeida OP. Immunohistochemical expression of podoplanin (D2-40), lymphangiogenesis, and neoangiogenesis in tooth germ, ameloblastomas, and ameloblastic carcinomas. J Oral Pathol Med. 2017 Sep;46(8):618-624.
24. Ribeiro AL, Nobre RM, Alves-Junior SM, Kataoka MS, Barroso RF, Jaeger RG, Pinheiro JJ.Matrix metalloproteinases, tissue inhibitors of metalloproteinases, and growth factors regulate the aggressiveness and proliferative activity of keratocystic odontogenic tumors. Oral Surg Oral Med Oral Pathol Oral Radiol. 2012 Oct;114(4):487-96.
25. Santos PPA, Nonaka CFW, Freitas RA, Pereira Pinto L, Souza LB.Immunohistochemical analysis of myofibroblasts, TGF-β1, and IFN-γ in epithelial odontogenic lesions. J Oral Pathol Med. 2017 May;46(5):365-370.
26. Kimura S, Harada T, Ijichi K, Tanaka K, Liu R, Shibahara D, Kawano Y, Otsubo K, Yoneshima Y, Iwama E, Nakanishi Y, Okamoto I.Expression of brain-derived neurotrophic factor and its receptor TrkB is associated with poor prognosis and a malignant phenotype in small cell lung cancer. Lung Cancer. 2018 Jun;120:98-107.
27. Tajbakhsh A, Mokhtari-Zaer A, Rezaee M, Afzaljavan F, Rivandi M, Hassanian SM, Ferns GA, Pasdar A, Avan A. Therapeutic Potentials of BDNF/TrkB in Breast Cancer; Current Status and Perspectives.J Cell Biochem. 2017 Sep;118(9):2502-2515
28. de Moraes JK, Wagner VP, Fonseca FP, Vargas PA, de Farias CB, Roesler R, Martins MD.Uncovering the role of brain-derived neurotrophic factor/tyrosine kinase receptor B signaling in head and neck malignancies. J Oral Pathol Med. 2018 Mar;47(3):221-227.
29. Swarup N, Nayak MT, Chowdhary Z, Mehendiratta M, Khatana S, Choi SJ, Sagolsem C. Evaluation and Immunolocalization of BMP4 and FGF8 in Odontogenic Cyst and Tumors. Anal Cell Pathol (Amst). 2018 Jul 12;2018:1204549
30. Siqueira AS, Carvalho MR, Monteiro AC, Freitas VM, Jaeger RG, Pinheiro JJ.Matrix metalloproteinases, TIMPs and growth factors regulating ameloblastoma behaviour. Histopathology. 2010 Jul;57(1):128-37.
31. da Silva AD, Nóbrega TG, Saudades AW, Otero MI, Danilevicz CK, Magnusson AS, Saraiva Homem de Carvalho AL, Rados PV, Carrard VC, Visioli F, Sant'Ana Filho

M. Ameloblastic neoplasia spectrum: a cross-sectional study of MMPS expression and proliferative activity. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016 Apr;121(4):396-401.e1.

32. Pogrel MA. Treatment of keratocysts: the case for decompression and marsupialization. J Oral Maxillofac Surg. 2005 Nov;63(11):1667-73.
33. Yuan Y, Ye HQ, Ren QC. Upregulation of the BDNF/TrKB pathway promotes epithelial-mesenchymal transition, as well as the migration and invasion of cervical cancer. Int J Oncol. 2018 Feb;52(2):461-472.
34. Yu JS, Cui W.Proliferation, survival and metabolism: the role of PI3K/AKT/mTOR signalling in pluripotency and cell fate determination. Development. 2016 Sep 1;143(17):3050-60.
35. Chaisuparat R, Yodsanga S, Montaner S, Jham BC.Activation of the Akt/mTOR pathway in dentigerous cysts, odontogenic keratocysts, and ameloblastomas. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013 Sep;116(3):336-42.
36. Kumamoto H, Ooya K.Immunohistochemical detection of phosphorylated Akt, PI3K, and PTEN in ameloblastic tumors. Oral Dis. 2007 Sep;13(5):461-7
37. Scheper MA, Chaisuparat R, Nikitakis NG, Sauk JJ.Expression and alterations of the PTEN / AKT / mTOR pathway in ameloblastomas.Oral Dis. 2008 Sep;14(6):561-8.
38. Calautti E, Li J, Saoncella S, Brissette JL, Goetinck PF. Phosphoinositide 3-kinase signaling to Akt promotes keratinocyte differentiation versus death. J Biol Chem. 2005 Sep 23;280(38):32856-65.
39. O'Shaughnessy RF, Welti JC, Cooke JC, Avilion AA, Monks B, Birnbaum MJ, Byrne C. AKT-dependent HspB1 (Hsp27) activity in epidermal differentiation. J Biol Chem. 2007 Jun 8;282(23):17297-305
40. Shear M, Speight P. Cysts of the Oral and Maxillofacial Regions, Fourth Edition. Oxford : Blackwell Munksgaard, 2007.
41. Castilho RM, Squarize CH, Gutkind JS. Exploiting PI3K/mTOR signaling to accelerate epithelial wound healing. Oral Dis. 2013 Sep;19(6):551-8.
42. Jaafari-Ashkavandi Z, Mehranmehr F, Roosta E.MCM3 and Ki67 proliferation markers in odontogenic cysts and ameloblastoma. J Oral Biol Craniofac Res. 2019 Jan-Mar;9(1):47-50.

FIGURES AND TABLES LEGENDS

Table 1. Clinico-demographic features of OL.
Table 2. Percentage of positive and negative cases.
Table 3. Pattern of BDNF,TrkB, pAKT and pRPS6 expression.

Figure 1. (A) Representative images of BDNF immunohistochemical expression in OK, Am and AmC (reference bar – 20µm). (B) Epithelial BNDF IRS (mean + SEM) according to each diagnostic entity. Similar lowercase letters denote absence of significant difference ($p>0.05$, Kruskal Wallis test). (C) Stromal BDNF IRS (mean + SEM) according to each diagnostic entity. Similar lowercase letters denote absence of significant difference ($p>0.05$, Kruskal Wallis test). (D) Comparison between epithelial and stromal BDNF IRS (mean + SEM) within each diagnostic entity (Wilcoxon test, **** denote a p value <0.0001). Abbreviations: ns – not significant. SEM – standard error deviation.

Figure 2. (A) Representative images of TrkB, pAkt and pRPS6 immunohistochemical expression in OK, Am and AmC (reference bar – 20µm). (B) Epithelial TrkB IRS (mean + SEM) according to each diagnostic entity. Different lowercase letters denote presence of significant difference ($p<0.05$, Kruskal Wallis test, followed by Dunn's post-hoc test adjusted for Bonferroni error correction). (C) Epithelial pAkt IRS (mean + SEM) according to each diagnostic entity. Different lowercase letters denote presence of significant difference ($p<0.05$, Kruskal Wallis test, followed by Dunn's post-hoc test adjusted for Bonferroni error correction). (D) Epithelial pRPS6 IRS (mean + SEM) according to each diagnostic entity. Similar lowercase letters denote absence of significant difference ($p>0.05$, Kruskal Wallis test).Abbreviations: SEM – standard error deviation.

Table 1. Clinico-demographic features of OL.

	OK (n=25)	Am (n=29)	AmC (n=6)
Gender (n, %)			
Male	11 (44%)	13 (44.8%)	4 (80%)
Female	14 (56%)	16 (55.2%)	1 (20%)
Missing	-	-	1
		p value 0.313*	
Age (mean, SD)	34.80 (±19.62)	38.03 (±17.44)	61.80 (±28.16)
		p value 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 value 0.048*	

*Chi-square test; #Kruskal-wallis test

Table 2. Percentage of positive and negative cases.

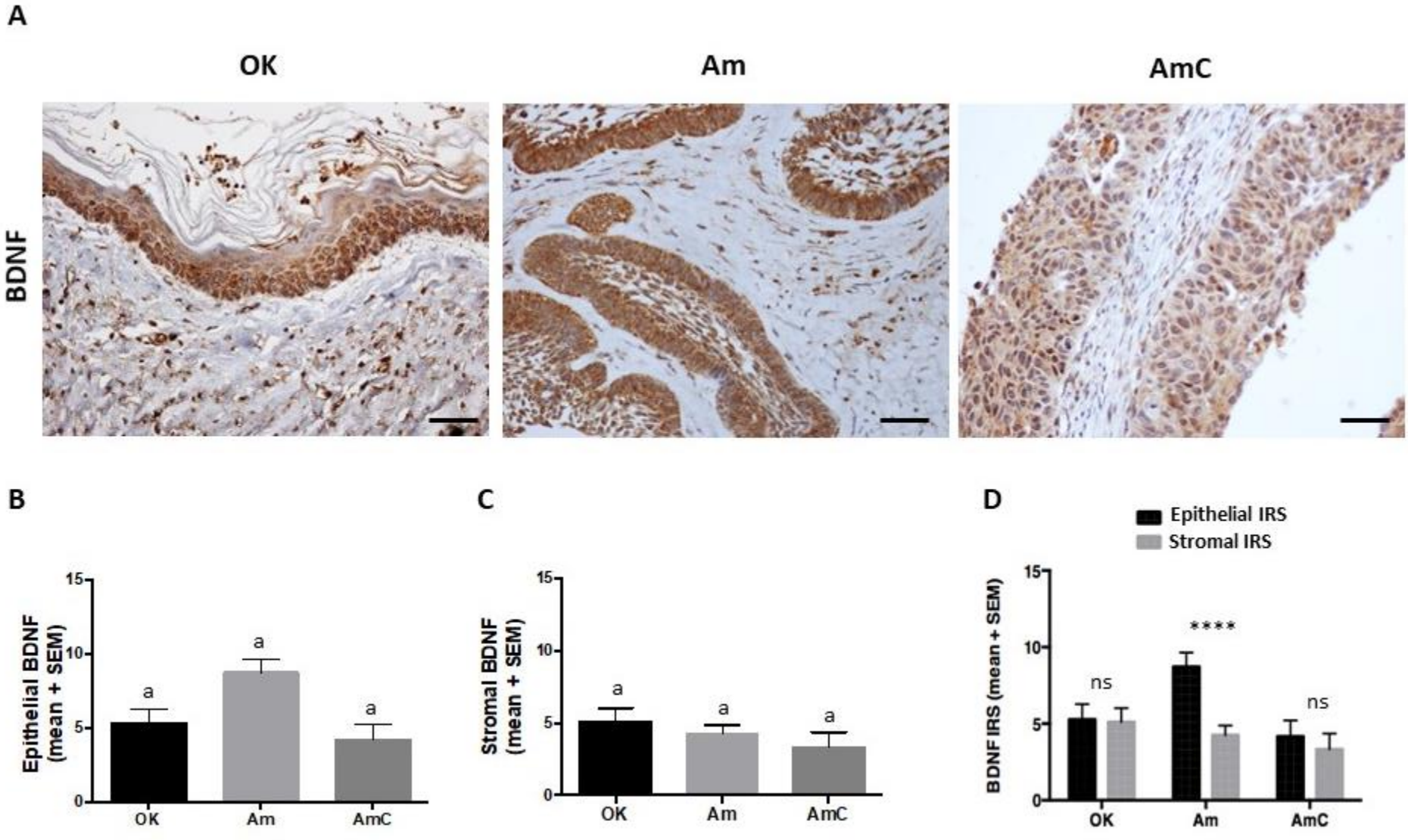
	OK (%)	Am (%)	AmC (%)	p value*
BDNF				
Positive expression	83.3%	82.1%	100%	0.537
Negative expression	16.7%	17.9%	0%	
TrkB				
Positive expression	100%	80.8%	100%	0.094
Negative expression	0%	19.2%	0%	
pAkt				
Positive expression	100%	96.0%	100%	0.625
Negative expression	0%	4.0%	0%	
pRPS6				
Positive expression	90.9%	92.0%	100%	0.751
Negative expression	9.1%	8.0%	0%	

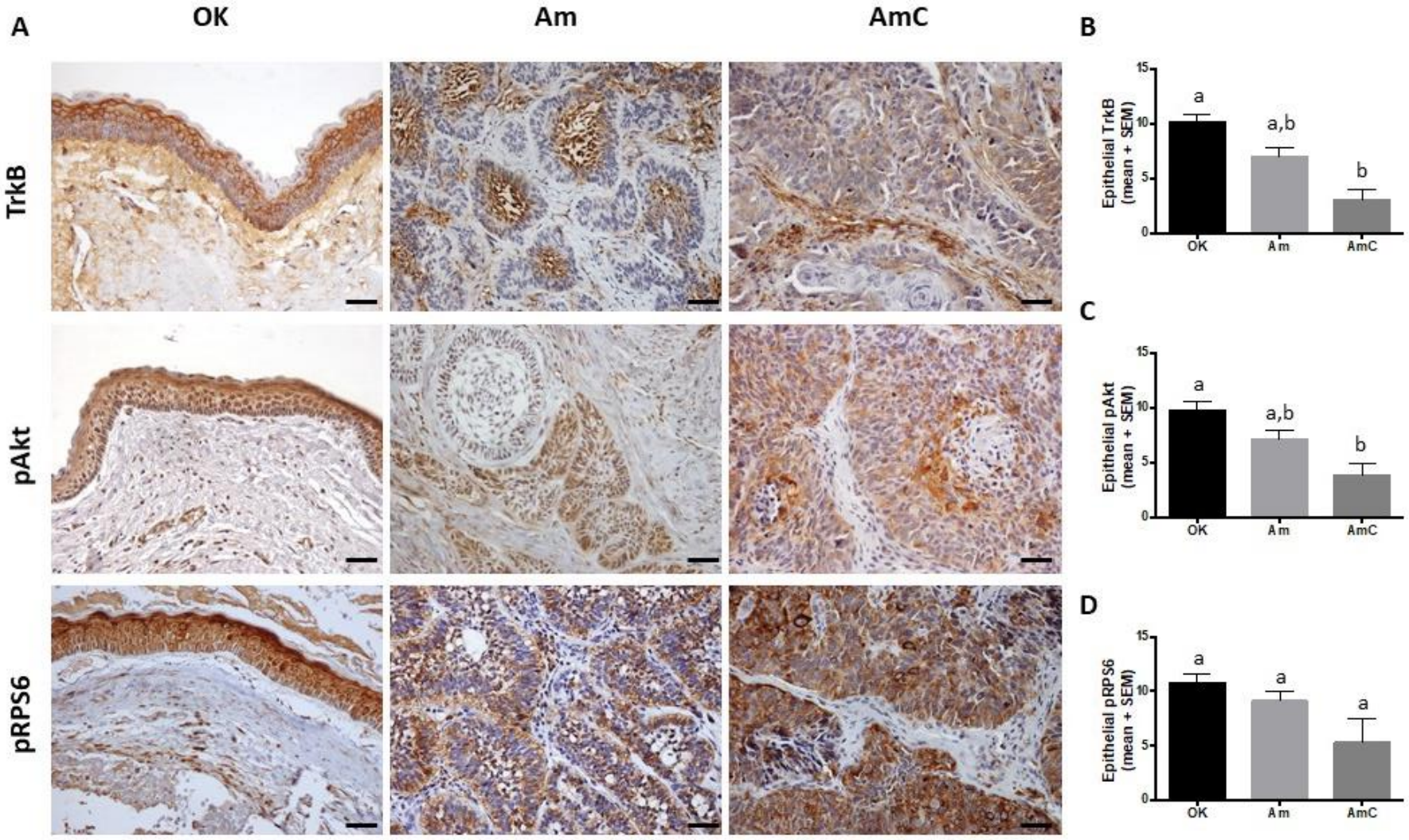
* Chi-square test

Table 3. Pattern of BDNF,TrkB, pAKT and pRPS6 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%
pAkt			
Prostromal	5.55%	4.34%	0%
Antistromal	0%	17.39%	0%
Full	94.44%	78.26%	100%
pRPS6			
Prostromal	0%	0%	0%
Antistromal	43.75%	42.85%	0%
Full	56.25%	57.14%	100%

* Percentage of cases among positive ones
Only positive cases were included in this analysis


A
OK
Am
AmC
BDNF
B
Epithelial BDNF (mean + SEM)
15
10
5
0
a
a
a
OK
Am
AmC
C
Stromal BDNF (mean + SEM)
15
10
5
0
a
a
a
OK
Am
AmC
D
Epithelial IRS
Stromal IRS
BDNF IRS (mean + SEM)
15
10
5
0
ns
****
ns
OK
Am
AmC


A
OK
Am
AmC
TrkB
pAkt
pRPS6
B
Epithelial TrkB (mean + SEM)
a
a,b
b
C
Epithelial pAkt (mean + SEM)
a
a,b
b
D
Epithelial pRPS6 (mean + SEM)
a
a
a
0
5
10
15
OK
Am
AmC

4. CONSIDERAÇÔES FINAIS

Em diferentes tipo de câncer, a estimulação da via de sinalização do BDNF/TrkB promove a ativação de moléculas de sinalização como Akt , STAT3, Src, ERK e MAPK resultando no aumento da proliferação celular, resistência a apoptose, invasão, metástase e resistência a quimioterapia. Desta forma, o aumento da expressão de BDNF/TrkB/Akt tem sido associado com comportamento mais agressivo de diferentes tumores malignos e pior prognóstico. Todavia, esta via de sinalização nunca havia sido estudada em lesões odontogênicas as quais compreendem um importante grupo de patologias orais e maxilofaciais com comportamentos distintos. Desta forma, nosso estudo foi pioneiro em avaliar o papel a via de sinalização BDNF/TrkB/Akt em lesões benignas e malignas odontogênicas epiteliais. Nossos resultados mostram um alto percentual de casos expressando BDNF de forma semelhante entre as lesões estudadas indicando que este fatore de crescimento parece participar tanto do desenvolvimento de lesões císticas como de neoplasias benignas e malignas. O papel desta proteína na odontogênese era conhecido anteriormente, e aqui demonstramos pela primeira vez que o BDNF também está presente em condições patológicas odontogênicas.

No que diz respeito ao TrkB e Akt, surpreendentemente, o CO exibiu níveis mais elevados, indicando que a aquisição do fenótipo invasivo ou maligno em Am e Cam não está associada à ativação do eixo BDNF/ TrkB / Akt / RPS6. A Akt é geralmente avaliada por sua capacidade de induzir comportamentos agressivos de células. No entanto, essa proteína também pode estimular a diferenciação dos queratinócitos. Desta forma, acreditamos que esse seja o principal papel destas proteínas nas lesões odontogênicas epiteliais. Nossa hipótese é que a maior ativação da Akt observada em ceratocistos odontogênicos está associada à necessidade de diferenciação de células epiteliais odontogênicas primordiais em células produtoras de queratina. Além disso, nossos resultados sugerem que a regulação positiva de Akt em CO é desencadeada pelo menos em parte pela ativação de TrkB. Mais estudos precisam ser realizados para avaliar se outros reguladores conhecidos da Akt, como EGFR e C-MET, têm um papel em estimular a Akt em OK. Novo estudos com outros fatores de crescimentos e vias de sinalização se fazem necessários para a compreensão do papel destas proteínas em lesões odontogências com diferentes comportamentos biológicos.

REFERÊNCIAS

Akil H, Perraud A, Jauberteau MO, Mathonnet M.Tropomyosin-related kinase B/brain derived-neurotrophic factor signaling pathway as a potential therapeutic target for colorectal cancer. World J Gastroenterol. 2016 Jan 14;22(2):490-500.

Al-Rawi NH, Awad M, Al-Zuebi IE, Hariri RA, Salah EW. Prevalence of odontogenic cysts and tumors among UAE population. Journal of Orofacial Sciences. 2013 Jul; 5(2):95-99.

AlSheddi MA, AlSenani MA, AlDosarib, AW. Odontogenic tumors: analysis of 188 cases from Saudi Arabia. Ann Saudi Med. 2015; 35(2):146-150.

Alyasiri NS, Mehdi SJ, Alam MS, Ali A, Mandal AK, Gupta S, Singh I, Rizvi MM. PTEN-mediated AKT activation contributes to the reduced apoptosis among Indian oral squamous cell carcinoma patients. J Cancer Res ClinOncol. 2012 Jan;138(1):103-9.

Arce K, Streff CS, Ettinger KS. Pediatric Odontogenic Cysts of the Jaws. Oral Maxilofacial Surg Clin N Am. 2016; 28: 21-30.

Baghaei F, Zargaran M, Najmi H, Moghimbeigi A. A Clinicopathological Study of Odontogenic Cysts ans Tumors in Hamadan, Iran. J. Dent. Univ. Med. Sci. 2014 Dec; 15(4): 167-172.

Barnes L, Eveson JW, Reichart P, Sidransky, D. World Health Organization Classification of tumours. Pathology and genetics of head and neck tumours. Lyon: IARC PRESS, 2005.

Costa DOP, Maurício AS, Faria PAS, Silva LE, Taylor AM, Lourenço SQC. Odontogenic tumors: A retrospective study of four Brazilian diagnostic pathology centers. Med Oral Patol Oral Cir Bucal. 2012 May; 17(3):389-94.

de Farias CB, Heinen TE, dos Santos RP, Abujamra AL, Schwartsmann G, Roesler R. BDNF/TrkB signaling protects HT-29 human colon cancer cells from EGFR inhibition. Biochem Biophys Res Commun. 2012 Aug 24;425(2):328-32.

de Moraes JK, Wagner VP, Fonseca FP, Vargas PA, de Farias CB, Roesler R, Martins MD. Uncovering the role of brain-derived neurotrophic factor/tyrosine kinase receptor B signaling in head and neck malignancies.J Oral Pathol Med. 2018 Mar;47(3):221-227.

Deepthi P, Beena V, Padmakumar S, Rajeev R, Sivakumar R. A study of 1177 odontogenic lesions in a South Kerala population. Journal of Oral and Maxillofacial Pathology. 2016 May-August; 20(2):202.

Ferreres JB, Torres AS, Escoda CG. Malignant changes developing from odontogenic cysts: A systematic review. J Clin Exp Dent. 2016 Dec; 8(5): 622–628.

Fujikawa H, Tanaka K, Toiyama Y, Saigusa S, Inoue Y, Uchida K, Kusunoki M. High TrkB expression levels are associated with poor prognosis and EMT induction in colorectal cancer cells. J Gastroenterol. 2012 Jul;47(7):775-84.

Goteti SHL. Odontogenic Tumors: A Review of 675 Cases in Eastern Libya. Niger J Surg. 2016 Jan-Jun; 22(1): 37–40.

Grossmann SM, Machado VC, Xavier GM, Moura MD, Gomez RS, Aguiar MCF, Mesquita RA. Demographic profile of odontogenic and selected nonodontogenic cysts in a Brazilian population. OOOOE. 2014 Dec; 104(6):35-41.

Guo D, Hou X, Zhang H, Sun W, Zhu L, Liang J, Jiang X. More expressions of BDNF and TrkB in multiple hepatocellular carcinoma and anti-BDNF or K252a induced apoptosis, supressed invasion of HepG2 and HCCLM3 cells. J Exp Clin Cancer Res. 2011 Oct 14;30:97.

Imran A, Jayanthi P, Gobu SC. Classification of odontogenic cysts and tumors – Antecedents. J. Oral Maxilofac Pathol. 2016 May-August; 20(2):269-71.

Iyogun CA, Omitola OG, Ukegheson GE. Odontogenic tumors in Port Harcourt: South–South geopolitical zone of Nigeria. J Oral Maxillofac Pathol. 2016 May-Aug; 20(2):190–193.

Jia S, Wang W, Hu Z, Shan C, Wang L, Wu B, Yang Z, Yang X, Lei D. BDNF mediated TrkB activation contributes to the EMT progression and the poor prognosis in human salivary adenoid cystic carcinoma. Oral Oncol. 2015 Jan;51(1):64-70.

Jones AV, Craig GT, Franklin CD. Range and demographics of odontogenic cysts diagnosed in a UK population over a 30-year period. J Oral Pathol Med. 2006; 35:500-507.

Kim MS, Lee WS, Jeong J, Kim SJ, Jin W. Induction of metastatic potential by TrkB via activation of IL6/JAK2/STAT3 and PI3K/AKT signaling in breast cancer. Oncotarget. 2015 Nov 24;6(37):40158-71

Kupferman ME, Jiffar T, El-Naggar A, Yilmaz T, Zhou G, Xie T, Feng L, Wang J, Holsinger FC, Yu D, Myers JN. TrkB induces EMT and has a key role in invasion of head and neck squamous cell carcinoma. Oncogene. 2010 Apr 8;29(14):2047-59

Lai PC, Chiu TH, Huang YT. Overexpression of BDNF and TrkB in human bladder cancer specimens. Oncol Rep. 2010 Nov;24(5):1265-70.

Lee J, Jiffar T, Kupferman ME. A novel role for BDNF-TrkB in the regulation of chemotherapy resistance in head and neck squamous cell carcinoma. PLoS One. 2012;7(1):e30246.

Lee RJ, Tong EL, Patel R, Go LA, Christensen RE. Epidemiology, prognostic factors, and management of malignant odontogenic tumors: an analysis of 295 cases. Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology.2015; 120(5):616 - 621.

Liu C, Chan CB, Ye K. 7,8-dihydroxyflavone, a small molecular TrkB agonist, is useful for treating various BDNF-implicated human disorders. Transl Neurodegener. 2016 Jan 6;5:2.

Meningaud JP, Oprean N, Pitak-Arnnop P, Bertrand JC. Odontogenic cysts: a clinical study of 695 cases. J Oral Sci. 48:59-62.

Mosqueda AT, Camacho MEI, Franco MAD, Tejero MAT. Quistes odontogénicos. Análisis de 856 casos. Medicina Oral. 2002 Mar-Abr; 7(2):89-96.

Ochsenius G, Escobar E, Godoy L, Peñafiel C. Odontogenic Cysts: Analysis of 2944 cases in Chile. Med Oral Patol Oral Cir Bucal. 2007; 12:85-91.

Okamura K, Harada T, Wang S, Ijichi K, Furuyama K, Koga T, Okamoto T, Takayama K, Yano T, Nakanishi Y. Expression of TrkB and BDNF is associated with poor prognosis in non-small cell lung cancer. Lung Cancer. 2012 Oct;78(1):100-6.

Osterne RLV, Brito RGM, Alves APNN, Cavalcante RB, Sousa FB. Odontogenic tumors: a 5-year retrospective study in a Brazilian population and analysis of 3406 cases reported in the literature. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011; 111:474-481.

Pereira JV, Figueirêdo DU, Souza EA, Holmes TSV, Gomes DQC, Cavalcanti AL. Prevalência de cistos e tumores odontogênicos em pacientes atendidos na Fundação Assistencial da Paraíba: estudo retrospectivo. Arquivos em Odontologia. 2010 Abr-Jun; 49(02):75-81.

Roesler R, de Farias CB, Abujamra AL, Brunetto AL, Schwartsmann G. BDNF/TrkB signaling as an anti-tumor target. Expert Rev Anticancer Ther. 2011 Oct;11(10):1473-5.

Sasahira T, Ueda N, Yamamoto K, Bhawal UK, Kurihara M, Kirita T, Kuniyasu H. Trks are novel oncogenes involved in the induction of neovascularization, tumor progression, and nodal metastasis in oral squamous cell carcinoma. Clin Exp Metastasis. 2013 Feb;30(2):165-76.

Schecterson LC, Bothwell M. Neurotrophin receptors: Old friends with new partners. Dev Neurobiol. 2010 Apr;70(5):332-8.

Sekerci AE, Nazlım S, Etoz M, Denız K, Yasa Y. Odontogenic tumors: A collaborative study of 218 cases diagnosed over 12 years and comprehensive review of the literature. Med Oral Patol Oral Cir Bucal. 2015 Jan; 20(1):34-44.

Sharifian MJ, Khalili M. Odontogenic cysts: a retrospective study of 1227 cases in an Iranian population from 1987 to 2007. Journal of Oral Science. 2011; 53(3):361-367.

Souza LB, Núñez MAG, Nonaka CFW, Medeiros MC, Torres TF, Emiliano GBG. Odontogenic cysts: Demographic profile in a Brazilian population over a 38-year period. Med Oral Patol Oral Cir Bucal. 2010 Jul; 15(4):83-90.

Thomaz A, Jaeger M, Buendia M, Bambini-Junior V, Gregianin LJ, Brunetto AL, Brunetto AT, de Farias CB, Roesler R BDNF/TrkB Signaling as a Potential Novel Target in Pediatric Brain Tumors: Anticancer Activity of Selective TrkB Inhibition in Medulloblastoma Cells. J Mol Neurosci. 2015 Nov 27

Tortorici S, Amodio E, Massenti MF, Buzzanca ML, Burruano F, Vitale F. Prevalence and distribution of odontogenic cysts in Sicily: 1986-2005. J Oral Sci. 2008; 50:15-18.

Urrutia SN, Figueiredo R, Escoda CG. Retrospective clinicopathological study of 418 odontogenic cysts. Med Oral Patol Cir Bucal. 2010 Set; 15(5):767-73.

Wright JM, Vered M. Update from the 4th Edition of the World Health Organization Classification of Head and Neck Tumours: Odontogenic and Maxillofacial Bone Tumors. Head and Neck Pathol. 2017; 11:68–77.

Yilmaz T, Jiffar T, de la Garza G, Lin H, Milas Z, Takahashi Y, Hanna E, MacIntyre T, Brown JL, Myers JN, Kupferman ME. Theraputic targeting of Trk supresses tumor proliferation and enhances cisplatin activity in HNSCC. Cancer Biol Ther. 2010 Sep 15;10(6):644-53.

Yin B, Ma ZY, Zhou ZW, Gao WC, Du ZG, Zhao ZH, Li QQ. The TrkB+ cancer stem cells contribute to post-chemotherapy recurrence of triple-negative breast cancers in an orthotopic mouse model. Oncogene. 2015 Feb 5;34(6):761-70.

Zhu L, Werner JA, Mandic R. Implications of tropomyosin-related kinase B (TrkB) in head and neck cancer. Anticancer Res. 2007 Sep-Oct;27(5A):3121-6

ANEXO-Parecer do CEP

UFRGS - HOSPITAL DE CLÍNICAS DE PORTO ALEGRE DA UNIVERSIDADE FEDERAL

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: ESTUDO DA VIA DE SINALIZAÇÃO BDNF/TrkB EM LESÕES ODONTOGÊNICAS
Pesquisador: Manoela Domingues Martins
Área Temática:
Versão: 1
CAAE: 95687218.6.0000.5327
Instituição Proponente: Hospital de Clínicas de Porto Alegre
Patrocinador Principal: Hospital de Clínicas de Porto Alegre

DADOS DO PARECER

Número do Parecer: 2.881.948

Apresentação do Projeto:
A via do BDNF/TrkB está envolvida em proliferação, invasão e sobrevivência de células neoplásicas malignas. Neste projeto, os autores pretendem avaliar a expressão de marcadores da via BDNF/TrkB por imunohistoquímica em biópsias de cistos e tumores odontogênicos. Serão utilizadas amostras de blocos de parafina já disponíveis no Laboratório de Patologia Bucal da Faculdade de Odontologia da UFRGS (centro onde são realizados diagnósticos específicos de neoplasias bucais). As análises de IHC serão realizadas na UPE do CPE. Trata-se de projeto de mestrado do PPG de odontologia. O projeto está claro, objetivo e é relevante. Apresenta TCDU, termo de autorização de acesso às amostras do Laboratório de Patologia Bucal, cálculo amostral e metodologia adequados. Projeto em condições de aprovação.

Objetivo da Pesquisa:
Objetivo Geral
-Estudar a via de sinalização BDNF/TrkB em lesões odontogênicas.

Objetivos Específicos
-Correlacionar os dados clínicos das lesões odontogênicas com a marcação de BNDF, TrkB, pS6

Endereço: Rua Ramiro Barcelos 2.350 sala 2229
Bairro: Santa Cecília **CEP:** 90.035-903
UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3359-7640 **Fax:** (51)3359-7640 **E-mail:** cep@hcpa.edu.br

(mTORC1), p-Akt 473 (mTORC2)

-Correlacionar os tipos histológicos de lesões odontogênicas com imunomarcação de BNDF, TrkB, pS6 (mTORC1), p-Akt 473 (mTORC2)

Avaliação dos Riscos e Benefícios:

Segundo os pesquisadores,

Riscos

Não são conhecidos ou previstos riscos diretos a integridade física, psíquica, moral, intelectual, social, cultural ou espiritual dos pacientes participantes do estudo, uma vez que serão trabalhados apenas dados de prontuários e material biológico armazenado em blocos de parafina. Um potencial risco envolve a quebra de confidencialidade das informações relativas aos pacientes. Contudo, os pesquisadores se comprometem a cumprir todos os requisitos relativos à proteção das informações dos pacientes. Para tanto, os pesquisadores assinam a declaração de compromisso para utilização de dados de material biológico. Apenas os pesquisadores envolvidos no estudo, e que assinaram as declarações terão acesso à identidade dos pacientes, que, por sua vez, será substituída por códigos no momento em que for criado o banco de dados do estudo.

Benefícios

A realização deste estudo não trará benefício direto aos pacientes, pois os pacientes já foram tratados. O possível benefício indireto poderá ser uma melhor compreensão a respeito das doenças estudadas. Caso na revisão de lâminas houver alguma modificação (reclassificação) do diagnóstico será informado ao responsável pelo Serviço de Patologia para que tome as providências cabíveis no que diz respeito a comunicação com o clínico responsável pelo paciente.

Comentários e Considerações sobre a Pesquisa:

Trata-se de projeto de mestrado do PPGOD onde será investigada a expressão de marcadores da via BDNF/TrkB por imuno-histoquímica em diferentes espécimes de tumores bucais já disponíveis no laboratório de patologia bucal da faculdade de odontologia da UFRGS. As análises por IHC serão realizadas na UPE do CPE/HCPA. Projeto bem escrito, objetivo e relevante.

UFRGS - HOSPITAL DE CLÍNICAS DE PORTO ALEGRE DA UNIVERSIDADE FEDERAL

Considerações sobre os Termos de apresentação obrigatória:

Os autores pedem dispensa do TCLE.

Recomendações:

Nada a recomendar

Conclusões ou Pendências e Lista de Inadequações:

Projeto não apresenta pendências e está em condições de aprovação.

Considerações Finais a critério do CEP:

Lembramos que a presente aprovação (versão projeto 11/08/2018 e demais documentos que atendem às solicitações do CEP) refere-se apenas aos aspectos éticos e metodológicos do projeto.

Os pesquisadores devem atentar ao cumprimento dos seguintes itens:

a) Este projeto está aprovado para inclusão de 126 participantes, de acordo com as informações do projeto. Qualquer alteração deste número deverá ser comunicada ao CEP e ao Serviço de Gestão em Pesquisa para autorizações e atualizações cabíveis.

b) O projeto deverá ser cadastrado no sistema AGHUse Pesquisa para fins de avaliação logística e financeira e somente poderá ser iniciado após aprovação final do Grupo de Pesquisa e Pós-Graduação.

c) Qualquer alteração nestes documentos deverá ser encaminhada para avaliação do CEP. Informamos que obrigatoriamente a versão do TCLE a ser utilizada deverá corresponder na íntegra à versão vigente aprovada.

d) Deverão ser encaminhados ao CEP relatórios semestrais e um relatório final do projeto.

e) A comunicação de eventos adversos classificados como sérios e inesperados, ocorridos com pacientes incluídos no centro HCPA, assim como os desvios de protocolo quando envolver diretamente estes pacientes, deverá ser realizada através do Sistema GEO (Gestão Estratégica Operacional) disponível na intranet do HCPA.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1127738.pdf	11/08/2018 09:27:47		Aceito
TCLE / Termos de	TCLEdispensa.docx	11/08/2018	Manoela	Aceito

Endereço: Rua Ramiro Barcelos 2.350 sala 2229
Bairro: Santa Cecília **CEP:** 90.035-903
UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3359-7640 **Fax:** (51)3359-7640 **E-mail:** cep@hcpa.edu.br

UFRGS - HOSPITAL DE CLÍNICAS DE PORTO ALEGRE DA UNIVERSIDADE FEDERAL

Continuação do Parecer: 2.881.948

Assentimento / Justificativa de Ausência	TCLEdispensa.docx	09:26:49	Domingues Martins	Aceito
Declaração de Pesquisadores	delegacaopesquisaFlor.doc	11/08/2018 09:19:52	Manoela Domingues Martins	Aceito
Outros	autorizacaousomateriais.pdf	11/08/2018 09:18:33	Manoela Domingues Martins	Aceito
Orçamento	orcamentoFlor.pdf	11/08/2018 09:17:48	Manoela Domingues Martins	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	termoutilizacaodedadosemateriais.docx	11/08/2018 09:17:10	Manoela Domingues Martins	Aceito
Cronograma	cronograma.pdf	11/08/2018 09:15:14	Manoela Domingues Martins	Aceito
Projeto Detalhado / Brochura Investigador	ProjetoFlorenciaeticafim.docx	11/08/2018 09:14:26	Manoela Domingues Martins	Aceito
Folha de Rosto	FlorenciafolhaDeRosto.pdf	11/08/2018 09:12:20	Manoela Domingues Martins	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

PORTO ALEGRE, 10 de Setembro de 2018

Assinado por:
Marcia Mocellin Raymundo
(Coordenador)

Endereço: Rua Ramiro Barcelos 2.350 sala 2229
Bairro: Santa Cecília **CEP:** 90.035-903
UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3359-7640 **Fax:** (51)3359-7640 **E-mail:** cep@hcpa.edu.br