UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
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ANÁLISE IMUNOISTOQUÍMICA DO PAPEL DO TGF-β1 E SUA CORRELAÇÃO COM A PROLIFERAÇÃO CELULAR EM LEUCOPLASIAS E CARCINOMAS ESPINOCELULARES

Porto Alegre
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Orientador: Prof. Dr. Vinicuis Carrard

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RESUMO

RODRIGUES, Paula Cardoso. **O papel do TGF-β1 em leucoplasias e carcinomas espinocelulares orais**. 2014. 47f. Trabalho de Conclusão de Curso (Graduação) – Faculdade de Odontologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2014.

As alterações epiteliais observadas nas leucoplasias e carcinomas espinocelulares (CEC) de boca vem sendo estudadas por meio de diferentes marcadores, a fim de observar os fatores indicadores de transformação maligna e de comportamento dessas lesões. O objetivo do presente estudo foi analisar a imunomarcação do TGF- β1 e Ki-67 em leucoplasias e CEC de boca, bem como correlacioná-los com fatores de risco, graduação histológica e acompanhamento dos pacientes. Foram coletados dados sobre características demográficas, fatores de risco, aspectos clínicos, tratamento e evolução de 24 casos de leucoplasias e 87 casos de CEC de boca. Foram incluídos 10 casos de mucosa bucal normal, provenientes de biópsias diagnosticadas como mucoele. As lâminas de cada caso foram revisadas e classificadas de acordo com a OMS nas leucoplasias e pelo método de Bryne nos casos de CEC. Foram construídos microarranjos de tecido (TMAs) dos casos de CEC. Todos os casos foram submetidos à análise imunoistoquímica utilizando anticorpos anti-TGF-β1 e anti-Ki67. A existência de associação entre as variáveis independentes e os desfechos foi avaliada por meio do teste qui-quadrado e regressão de COX. Foram construídas as curvas de sobrevida pelo método de Kaplan-Meier. Em todos os testes foi utilizado o software SPSS 19 e o nível de significância estabelecido foi de 5%. A imunomarcação de TGF-β1 e Ki-67 foi significativamente maior nos casos de CEC quando comparados a leucoplasias e a mucosa bucal normal. Nenhum dos marcadores mostrou correlação com a graduação histológica e evolução das lesões. A graduação histológica e a forma de tratamento dos CEC mostram-se fatores preditivos de pior sobrevida. Concluímos que o TGF-β1 e o Ki-67 não representam bons marcadores prognósticos para leucoplasias e CEC de boca. Todavia, o TGF-β1 parece estar envolvido na carcinogenese do CEC de boca e pode ser um bom alvo terapêutico para essas lesões.

Palavras-chave: Neoplasias bucais. Leucoplasia. Carcinoma de células escamosas. Prognóstico. TGF-β1. Ki-67.

ABSTRACT

RODRIGUES, Paula Cardoso. **Role of TGF-β1 in leukoplakias and oral squamous cells carcinoma**. 47f. Final Paper (Graduation) – Faculdade de Odontologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2014.

Epithelium alterations found in leukoplakia and oral squamous cell carcinoma (OSCC) have been studied trough many markers in order to observe the indicators factors of malignant transformation and behavior of this lesions. The aim of this research was to analyze the immunostaning pattern of TGF- β1 and Ki-67 in leukoplakia and OSCC, correlating with risk factors, histological graduation and follow up of patients. Data about demographic characteristics, risk factors, clinical aspects, treatment and evolution of 24 cases of leukoplakia and 87 cases of OSCC were collected. Ten cases of normal oral mucosa were include, from biopsies diagnoses as mucocele. Each slide was reviewed and classified according to WHO in leukoplakias and according to Bryne´s method in OSCC. Tissue microarrays (TMAs) of OSCC cases were constructed. The cases were submitted to immunohistochemical analyzes with anti-TGF-β1 and anti-Ki67 antibodies. Associations between independent variables and outcomes were evaluated by chi-square test and COX regression. Survival curves were constructed trough Kaplan-Meier method. The software SPSS 19 was used in all cases and the significance level establish was 5%. The immunostaining of TGF-β1 and Ki-67 was significantly higher in OSCC when compare to leukoplakia and normal oral mucosa. None of the markers presented correlation with histological graduation and evolution of the lesions. Histological graduation and treatment of OSCC were predictive factors of worse survival rate. As conclusion, TGF-β1 and Ki-67 could not be considered prognostic markers to leukoplakia and OSCC. Nevertheless, TGF-β1 seems to be involved in OSCC carcinogenesis and might be a good therapeutic target in this lesions.

Keywords: Mouth neoplasms. Leukoplakia. Carcinoma, squamous cell. Prognosis. TGF-β1. Ki-67.

SUMÁRIO

1 ANTECEDENTES E JUSTIFICATIVAS

O câncer é uma das causas mais comuns de morbidade e mortalidade no mundo todo, tendo uma incidência anual de aproximadamente 10 milhões de casos novos (RICARDO et al., 2011). No Brasil, segundo o Instituto Nacional do Câncer, há uma estimativa de incidência dessa lesão em 11.180 homens e em 4.010 mulheres (INCA, 2014). Dentre os diferentes tipos de câncer, o carcinoma espinocelular (CEC), que se origina do epitélio de revestimento, corresponde à cerca de 95% dos casos na cavidade bucal (ZINI et al., 2010; PARKIN et al., 2005; JEMAL et al., 2007; JEMAL et al., 2002). Apesar dos esforços despendidos, apenas uma média de 50% dos pacientes com câncer de cabeça e pescoço apresentam sobrevida maior do que 5 anos (HASSONA et al., 2013; OHTA et al., 2013; HALL et al., 2013).

A carcinogênese é um processo que envolve várias etapas, dentre elas a iniciação, a promoção e a progressão, resultando em um acúmulo de alterações genéticas que levam à transformação maligna do tecido (CHEN et al., 2011) . A iniciação representa a fase na qual ocorre dano ao DNA por carcinógenos. Na promoção, as células iniciadas, após a ação de co-carcinógenos, são estimuladas a proliferar de forma descontrolada e a passar a falha genética às células filhas até que formem clones de células mutadas. Este processo é acompanhado de distúrbios de diferenciação e se consolida com a invasão dos tecidos adjacentes por parte das células mutadas, caracterizando as neoplasias malignas (CALIFANO, SIDRANSKI, 1999). A interação das células cancerígenas com o microambiente tumoral está intimamente relacionada com seu processo de crescimento e com o desenvolvimento de metástase (HWANG, PARK, CHUNG, 2014). No modelo aceito atualmente para descrever a carcinogênese em boca, o CEC propriamente dito pode ser precedido por lesões denominadas potencialmente malignas (EPSTEIN, 2002).

As fases iniciais da progressão tumoral compreendem alterações na arquitetura e nas células teciduais. Essas mudanças correspondem a um grupo

de desordens potencialmente malignas (SALVADORI et al., 2014). Dentre as lesões potencialmente malignas, a leucoplasia é a mais prevalente, estimada entre 0,4 e 5% (SCHEPMAN et al, 1996; SCHEIFELE, REICHART, DIETRICH, 2003; GAIO, 2012). Em relação à transformação maligna, os estudos referem taxas que variam entre 0% e 9% (PANDEY et al., 2001; SAITO et al., 2001; REIBEL, 2003; REDDI & SHAFER, 2006; EREA et al., 2013). As lesões localizadas em borda lateral e ventre de língua, bem como as de assoalho da boca são as que parecem apresentar maior risco de transformação maligna (HOLMSTRUP et al., 2005; VAN DER WAAL, 2006; NAPIER & SPEIGHT, 2008). O exame histopotológico pode apresentar hiperceratose, hiperplasia, acantose ou graus variados de displasia epitelial (WARNAKULASURIYA et al., 2008). Sabe-se que, à medida que ocorrem modificações gênicas, se observam alterações celulares e estruturais mais graves, as quais são acompanhadas por aumento progressivo na atividade proliferativa (TOMATIS, 1993).

Os membros da família do fator de crescimento e transformação beta (TGF-β1, β2 e β3) foram descritos como indutores da homeostase, proliferação celular, cicatrização de feridas, imunossupressão e angiogênese. Essas ações se devem ao papel dessas proteínas em processos como proliferação, migração, diferenciação celular e apoptose (BIERIE, MOSES, 2006). Eventualmente, os CEC secretam uma maior quantidade de TGF- β e obtém como resposta uma elevada capacidade de invasão e metástase. Isso ocorre, porque a citocina reprime a atividade antitumoral das células T, células NK, neutrófilos, monócitos e macrófagos (CARNEIRO et al., 2012).

A superfamília dos TGF-β1 incluem mais de 100 diferentes proteínas e um número superior a 40 tem sido descrito em mamíferos (DERYNCK, ZHANG, 2003; GIEHL, IMAMICHI, MENKE, 2007). Quando o TGF-β1 estimula o crescimento de células mesenquimais, o mesmo age de forma a inibir o crescimento e a diferenciação de células epiteliais, podendo ainda induzir a desdiferenciação, assim como a transição epitélio-mesenquimal (WILKINS-PORT, HIGGINS, 2007; HE, BAZAN, 2008). Apesar do TGF-β1 agir inibindo a

proliferação de células epiteliais, paradoxalmente é encontrado em altas concentrações em células tumorais, contribuindo para a progressão do câncer e facilitando a migração e metástase em estágios mais tardios (AKHURST, DERYNCK, 2001; WAKEFIELD, ROBERTS, 2002; DUMONT, ARTEAGA, 2003; GIEHL, IMAMICHI, MENKE, 2007).

O TGF-β1 está envolvido em distintos estágios do câncer e é produzido por um complexo latente que requer um processamento para se tornar ativo (LUKAS et al., 2009). Ele foi relacionado com redução da resposta imunológica (BECK et al., 2001), estimulo da angiogênese (CHOI et al., 1997; DERYNCK et al., 2001; BERTOLINO et al., 2005), aumento da síntese de enzimas proteolíticas (SEOMUN et al., 2001; KIM et al., 2004) e estimulação da deposição de matriz extracelular (MEC) (CHENG, LOVETT, 2003) no microambiente tumoral.

O papel dessa citocina é complexo e parece variar de acordo com o estágio da carcinogênese (WANG et al., 2011). Nos estágios iniciais, quando ainda há uma resposta normal das células epiteliais ao TGF-β1, ele parece atuar como supressor da progressão tumoral (CHEN et al., 2011). Entretanto, nos estágios tardios, parece promover invasão celular e metástases, angiogênese e síntese de proteínas da matriz extracelular ou participar da regulação da resposta imune por meio de sinalização autócrina (CAMPISI, 2001; OHYAMA et al., 2013).

Estudos clínicos têm demonstrado haver associação positiva entre a expressão de TGF-β1 *in vivo* e o aumento da capacidade de invasão de tumores de mama (OSAMURA et al., 1990; GORCH et al., 1992) e próstata (STEINERAND, 1992; THOMPSON et al., 1992) e com redução da sobrevida em carcinomas pancreáticos (FRIESS et al., 1993). Foram evidenciadas altas concentrações deste fator de crescimento em câncer colorretal (HAWINKELS et al., 2009), carcinomas gástricos (MUTOH et al., 2010), carcinomas renais (KOMINSKY et al., 2007) e CEC de cabeça e pescoço (LOGULLO et al., 2003).

O antígeno Ki-67 vem sendo utilizado como marcador nuclear de células em proliferação, refletindo a fração de crescimento, ou seja, número de células em proliferação dentro de um tecido (SCHLÜTER et al., 1993; DUCHROW et al., 1995; GONZALEZ-MOLES et al., 2010). Essa relação se explica pela presença dessa proteína em células em proliferação nas fases G1, S G2, e durante a mitose (GERDES, 1990; WINKING et al., 2004), e pouca ou nenhuma imunomarcação em células quiescentes na fase G0 (GERDES, 1990; ENDL et al., 2001). A quantificação da imunomarcação de Ki-67 tem se mostrado útil como um indicador de padrão de crescimento tecidual (SAITO et al., 1999). O seu papel como marcador prognóstico em carcinoma de boca ainda não foi esclarecido por completo, sendo que, aparentemente, variações metodológicas podem explicar a observação de resultados discrepantes (SITTEL et al., 1999; GOZALES-MOLES et al.,1996; MONTEBUGNOLI et al., 2011; BENEVENUTO et al., 2012). Por outro lado, tem sido relatado aumento da marcação de Ki-67 em lesões potencialmente e as efetivamente malignas da mucosa bucal (SLOOTWEG et al.,1994; KUROKAWA et al., 2003; TABOR et al., 2003). Alguns estudos demonstram que a quantidade de células com marcação positiva para o Ki-67 aumenta gradativamente de casos de mucosa normal para displasia epitelial e carcinoma espinocelular de lábio; sugerindo que a progressão maligna se relaciona com o aumento da imunomarcação para o Ki-67 (SARTORI et al., 2014). Em estudo anterior realizado pelo nosso grupo foi observada relação entre a imunomarcação do Ki-67 e o TGF-β1 na carcinogênse labial (SALVADORI et al. 2014). Nos CEC de lábio foi observada uma correlação negativa entre esses marcadores indicando que quanto maior a marcação do Ki-67, menor a expressão do TGF-β.

Atualmente, muitas pesquisas utilizando marcadores celulares relacionados com a carcinogênese bucal vêm sendo desenvolvidos na busca de uma melhor compreensão das etapas envolvidas na transformação maligna e para esclarecer o comportamento biológico dessas lesões (GIROD et al., 1998; OHKURA et al., 2005; PIATTELLI et al., 2002; KUROKAWA et al., 2003; NASSER et al., 2011). Distintos autores indicam que as células tumorais apresentam uma síntese de TGF- β1 aumentada quando comparada com os seus homólogos normais (WALKER, DEARING, 1992). Em função disso, os

níveis elevados de TGF-β1 favoreceriam o crescimento do tumor e sua progressão (ROBSON, et al., 1996; PIVA et al., 2013). Nesse sentido, pode-se perceber que o papel do TGF-β1 na carcinogênese de boca ainda não foi totalmente verificado, bem como sua relação com marcadores de proliferação celular e dados prognósticos.

2 OBJETIVOS

O objetivo da presente pesquisa foi estudar a imunomarcação do TGF-β1 e Ki-67 em leucoplasias, CEC de boca e mucosa bucal normal, bem como correlacioná-los com fatores de risco, graduação histológica e acompanhamento dos pacientes. Amostras de mucosa bucal normal foram obtidas a partir de biópsias diagnosticadas como mucocele.

2.1 OBJETIVOS ESPECÍFICOS

Correlacionar a graduação histológica dos casos de leucoplasias, de CEC de boca e de mucosa bucal normal com a imunomarcação pelo TGF-β1 e Ki-67.

Correlacionar a evolução dos casos de de leucoplasias, de CEC de boca ede mucosa bucal normal com a imunomarcação pelo TGF-β1 e Ki67.

Correlacionar os dados de fatores de risco com a imunomarcação pelo TGF-β1 e Ki67.

3 ARTIGO CIENTÍFICO

TGF-β1 in Leukoplakia and Oral Squamous Cells Carcinoma

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Abstract

Aims: Analyze TGFβ-1 expression in cases of leukoplakia, oral squamous cell carcinoma (OSCC) and normal oral mucosa and further correlate with proliferative labeling index, clinico-pathological aspects and clinical outcomes.

Methods and results: Twenty four cases of leukoplakia, 87 cases of OSCC, 10 cases of normal oral mucosa and patient´s clinical data were retrieved from medical records. Cases of OSCC were organized in tissue microarray blocks and submitted to immunohistochemistry against to TGFβ-1 and Ki-67. A significant difference in TGFβ-1 and Ki-67 expression was observed among normal oral mucosa, leukoplakia and OSCC. Significant increase in TGFβ-1 and Ki-67 expression in OSCC compared to leukoplakia and normal mucosa. There was no association between TGFβ-1 with proliferative labeling index, clinico-pathological aspects and clinical outcomes in both lesions. High histopathologic graduation showed to be predictors for shorter survival rates in OSCC patients.

Conclusions: TGFβ-1 has a role in malignant transformation of oral epithelium participating of the malignant phenotype acquisition process since early stages of oral carcinogenesis process.

Key-words: Mouth neoplasms. Leukoplakia. Carcinoma, squamous cell. Prognosis. TGF-β1. Ki-67.

Introduction

Head and neck squamous cell carcinoma (HNSCC) represents the sixth most common cancer worldwide [1]. Despite the advances made in the last decades regarding the early detection and treatment of other types of cancer, oral squamous cell carcinoma (OSCC) prognosis has not demonstrated an absolute gain in survival rates [2] and is still responsible for more than 120,000 deaths per annum [1]. OSCC results from the accumulation of multiple genetic and epigenetic changes [3] and even though this type of cancer do not suit the criteria for a screenable disease, most cases are preceded by asymptomatic clinical lesions called oral potentially malignant disorders (OPMDs) [4]. The annual malignant transformation rate of leukoplakia, the most common OPMD, is approximately 2.6% [5].

The behavior of OPMDs as well as OSCC cannot be predicted using only conventional clinical and histopathological parameters. Investigate the mechanisms involved in cell signaling regulation, cell proliferation and invasive potential may lead to the identification of biological markers able to predict the malignant transformation of OPMDs or the prognosis of OSCC. The search of such markers has become a main goal once it might provide new insights into the development of new alternative treatments for OSCC and improves overall survival, as also help clinicians in stratifying OPMD´s patients into follow-up groups according to their relative risk [4, 6].

Transforming growth factor β1 (TGF-β1) is a member of a highly pleitotropic family of growth factors whose signaling pathway has multiple and paradoxical roles in epithelial cell types. Prior to initiation and in early progression TGF-β1 can exert tumor-suppressive effects. On the other hand, at later stages pathological forms of TGF-β1 signaling act promoting tumor growth and invasion. The signaling pathway triggered by TGF-β1 can promote cancer cells dissemination, immune system regulation and microenvironment modifications used by cancer cell to acquire competitive advantages [7, 8]. Previous studies with OSCC cells have demonstrated that TGF-β1 signaling enhances tumor growth and epithelial-mesenchymal transition (EMT). Also, TGF-β1 have been related to a more aggressive phenotype and treatment resistance [6, 9].

Few studies have analyzed the value of TGF-β1 immunohistochemical expression in predicting OPMD and OSCC outcomes, and some controversial results are found in literature [6, 10, 11]. Thus, the aim of the present study was to analyze TGFβ-1 expression in leukoplakia, OSCC and normal oral mucosa and further correlate our findings with proliferative labeling index, clinico-pathological aspects and clinical outcome.

Material and methods

Study population

Twenty four cases of leukoplakia and 87 cases of OSCC diagnosed between 1996 and 2010 were selected from the archives of the Pathology Laboratory of Clinics Hospital from Porto Alegre, Rio Grande do Sul, Brazil (Human Research Ethics Committee approval n.02810012.0.0000.5327). Patient´s clinical data concerning demographic characteristics, risk factors, clinical aspects, tumor localization, TNM system and follow-up information (clinical outcome and survival time) were retrieved from medical records. The follow-up period was defined as the date of diagnosis until the last visit to the hospital or date of death. Specimens retrospectively collected from OSCC cases were constructed into TMA (tissue microarray) blocks for immunohistochemical analysis. Were considerate inclusion factor have seventy percent or more information that should be collected on the medical record, presence of surgical sample of the case and the sample has the diagnoses of leukoplakia or OSCC.

Histopathological analysis

Slides of the surgical specimen stained with hematoxylin-eosin (H&E) were obtained for histological grading. In leukoplakia, the presence and degree of epithelial dysplasia was analyzed using the criteria proposed by the World Health Organization (2005). Cases of OSSC were graded according to the criteria described by Bryne et al. (1989). The specimens of normal oral mucosa were obtained from biopsies diagnosed as mucocele.

TMA construction

Tissue microarray (TMA) construction was done as previously described (Fonseca et al. 2014). Briefly, tumor areas were selected and marked on hematoxylin-eosin-stained sections using an objective marker (Nikon Corp, Tokyo, Japan). The slide was then overlaid on the original paraffin block to determine the corresponding area to be used. A manual tissue arrayer (Sakura Co, Japan) was used and 3 representative cylindrical cores from the invasive front of 2.0mm diameter were taken from each tissue block of OSCC and then arranged sequentially into a recipient ready-to-use paraffin block (Sakura Co, Japan). Three cores of normal mucosa were inserted in the left upper corner of each recipient block for orientation. A map specifying the exact position of each case was prepared to facilitate the interpretation of the immunohistochemical results.

Immunohistochemistry

For immunohistochemical staining, the samples were sectioned into 3-µm sections and placed on silanized slides. The slides were deparaffinized in xylene and hydrated in descending grades of ethanol. Antigen retrieval was performed prior to incubation of primary antibody. The primary antibodies, sources, antigen retrieval, dilutions and incubation times were as follows: Ki-67 (MIB-1, DAKO, low pH solution in a water bath at 90 °C for 18 h, 1:50, 1 h), TGF-β1 (Santa Cruz, sc-146, low pH solution in a water bath at 90 °C for 18 h, 1:100, 18h). The sections were then incubated with diaminobenzidine tetrahydrochloride (DAB, Novocastra, Newcastle, UK) and counterstained with Mayer's hematoxylin. Negative controls were obtained by replacing the primary antibodies with non-immune serum. The positive controls for Ki-67 and TGF-β1 were, respectively, human appendix and rat uterus.

All immunohistochemical analysis was performed by blinded observers regarding case´s clinico-pathological aspects and outcome. Proliferative labeling index (PLI) was determined using Ki-67. The slides were quantitatively evaluated based on nuclear reactivity. Images of the selected fields were captured at a magnification of 400x using a conventional light microscope (CX41RF model, Olympus Latin America, Inc., Miami, Florida, USA) coupled to

a camera (QColor 5, RTV, Olympus Inc., BX51, Canada) and connected to a computer (Dimension 5150, Dell, Porto Alegre, RS, Brazil). The images were analyzed using the QCapture software program, version 2.81 (Quantitative Imaging Corporation, Inc., Surrey, DC, Canada). The number and percentage of positive cells were assessed in each case. In cases of leukoplakia, 1000 epithelial cells were counted in the entire epithelial length. In OSCC TMA sections, 500 cells were counted in each core (total of 1500 cells per case). The results were expressed as the percentage of positive cells (mean and standard deviation).

Slides stained with TGFβ1 were analyzed semi-quantitatively by 2 observers using scores based on the percentage of positive cells in both the epithelium (Salvadori et al., 2014). Each case was assigned a score as follows: 0 (0 to 10 % positive cells), 1 (10 to 50 %), or 2 (over 50 %).

Statistical analysis

All clinical and immunohistochemical data were analyzed with SPSS for Windows, version 18.0. Differences between groups were evaluated using Pearson's chi-squared test followed by Fisher's exact test and Student t test. Spearman's correlation coefficients were calculated to determine the correlation of TGFβ1 and Ki67 expression in leukoplakia and OSCC. Cox regression was performed to evaluate clinical, histopathological and immunohistochemical markers in determining death on cases of OSCC and Kaplan-Meier cumulative survival curves were constructed. For all tests, a p value <0.05 was considered to be statistically significant.

Results

Patient characteristics and TGF-β1 expression

Table 1 displays the demographic and clinical characteristics of the sample. Leukoplakia lesions were located at the tongue (22.7%), floor of the mouth (22.7%), labial mucosa (22.7%), palate (13.6%), gingival (13.3%) and buccal mucosa (4.2%). Regarding treatment of leukoplakia after incisional biopsy, 39.1% patients were submitted to further surgical excision while 60.9% of patients never underwent any type of treatment.

Tongue was the most common site for OSCC, representing 66.7% of cases, followed by the floor of the mouth (16.1%), palate (11.5%) and gingival (5.7%). Patients were diagnosed in TNM stage I in 8,6% of cases, stage II in 4.9% of cases, stage III in 16% of cases and in stage IV 70.5%. Nodal metastasis was present in 60.5% of cases and distant metastasis in 4.9%, all at the lungs. Regarding treatment modalities, surgery associated with radiotherapy was the most common treatment for patients with OSCC, accounting for 54.7% of the total sample. In 24.4% of cases, surgery was performed as the only treatment. Other treatment modalities included surgery associated with radiotherapy and chemotherapy (15.1%), surgery associated with chemotherapy (2.3%), only radiotherapy (2.3%) and radiotherapy associated with chemotherapy (1.2%).

No association was found between TGF-β1 expression and clinico-demographic characteristics in leukoplakia patients neither in OSCC patients.

Table 1. Demographic and clinical characteristics of patients with leukoplakia and OSCC.

Demographic/ clinical characteristics	Leukoplakia (n=24)	OSCC (n=87)	*p* value
Age			
Mean	56.61	59.15	0.35[§]
SD	14.92	10.16	
Gender			
Male	16 (66.7%)	78 (89.7%)	**0.01**[†]
Female	8 (33.3%)	9 (10.3%)	
Ethnicity			
Caucasian	23 (95.8%)	78 (90.7%)	0.68[†]
Black	1 (4.2%)	8 (9.3%)	
Residence			
Urban	15 (62.5%)	77 (90.6%)	**0.02**[†]
Rural	9 (37.5%)	4 (9.4%)	
Tobacco habits			
User/former user	14 (60.9%)	70 (97.2%)	**0.00**[†]
Non-user	9 (37.5%)	2 (2.8%)	
Alcohol consumption			
User/former user	11 (47.8%)	55 (78.6%)	**0.00**[†]
Non-user	12 (52.2%)	15 (24.4%)	
Site			
Tongue/ Floor of mouth	10 (45.5%)	72 (82.8%)	**0.00**[†]
Other locations	10 (45.5%)	15 (17.2%)	
Clinical aspects			
Ulcer	0 (0%)	68 (90.7%)	**0.00**[†]
Spot/Plaque/Nodule	22 (100%)	7 (9.3%)	
Pain			
Yes	8 (36.4%)	63 (91.3%)	**0.00**[†]
No	14 (63.6%)	6 (8.7%)	
TNM			
I/II		10 (12.5%)	
III/IV		70 (87.5%)	
Size			
<2 cm	18 (75%)		
≥2cm	6 (25%)		

[§] Student t test

[†]Pearson's chi-squared test

Histopathological analysis and TGF-β1 expression

Histopathologic analysis was performed in normal oral mucosa (Figure 1A), leukoplakia (Figure 1D) and OSCC (Figure 1G). The evaluation of the epithelial lining and degree of epithelial dysplasia in the 24 cases of leukoplakia revealed that 10 patients (41.4%) had no dysplasia, 8 (20.6%) had mild dysplasia, 3 (12.5%) had moderate dysplasia, and two (8.3%) had severe dysplasia. In OSCC sample, 6 patients (7.2%) exhibited a low degree of malignancy, 36 (43.4%) exhibited a moderate degree of malignancy, and 41 (49.4%) exhibited a high degree of malignancy.

Cases of normal mucosa were all negative to TGF-β1 expression and the mean PLI was 12.84±8.60 (Figure 1B and C). Table 2 compares TGF-β1 and Ki-67 labeling in leukoplakia and OSCC. While some cases of leukoplakia were negative to TGF-β1 protein or presented lower percentage of positive cells, other cases presented mild cytoplasmatic labeling in more than 50% of epithelial cells (Figure 1E). In OSCC, TGF-β1 was highly expressed, with strong cytoplasmatic labeling in almost 100% of neoplastic cells in the majority of cases (Figure 1H). Comparing to leukoplakia, OSCC presented a significant higher number of cases with more than 50% of positive cells ($p<0.00$). All cases of leukoplakia and OSCC were positive to Ki67 (Figure 1F and I). Patients with OSCC presented a higher mean of PLI, represented by Ki- 67 labeling, than patients with leukoplakia ($p<0.00$). In leukoplakia, Ki-67 was restricted to basal and suprabasal epithelial layers (Figure 1F).

Table 2. TGF-β1 and Ki- 67 labeling (mean and standard deviation) in leukoplakia and OSCC.

	Leukoplakia	**OSCC**	***p* value**
TGF-β1			
<50%	7 (30.4%)	5 (6.6%)	**<0.00**[†]
>50%	16 (69.6%)	7 (93.4%)	
Ki67			
Mean (SD)	36.20 (±18.09)	50.66 (±17.18)	**<0.00**[§]

[§] Student t test

[†]Pearson's chi-squared test

The TGF-β1 and PLI were not associated with histopathologic grading in leukoplakia and OSCC (Table 3). In leukoplakia cases, patients with tobacco habits presented a higher mean of Ki-67 expression (43.37±15.17) than patients without tobacco habits (27.10±18.46) (p=0.04). In patients with OSCC, lesions located in tongue and floor of the mouth presented a lower mean of Ki-67 expression (48.65±16.71) than patients with lesions in other sites (60.41±16.22) (p=0.02).

Table 3. TGF-β1 and Ki-67 labeling (absolute number and percentage) according to histopathological grading in leukoplakia (OMS, 2005) and OSCC (Bryne, 1992)

	Leukoplakia			**OSCC**		
	No dysplasia	**Dysplasia**	*p* value	**Low/Moderate**	**High**	*p* value
TGF-β1						
<50%	4 (40%)	5 (25%)	0.65	1 (2.7%)	4 (10.3%)	0.65[†]
>50%	6 (60%)	71 (75%)		36 (97.3%)	53 (98.7%)	
Ki67						
Mean (SD)	36.10 (±20.18)	36.44 (±17.93)	0.96	49.19 (±13.97)	52.58 (±18.04)	0.35[§]

[§] Student t test

[†]Pearson's chi-squared test

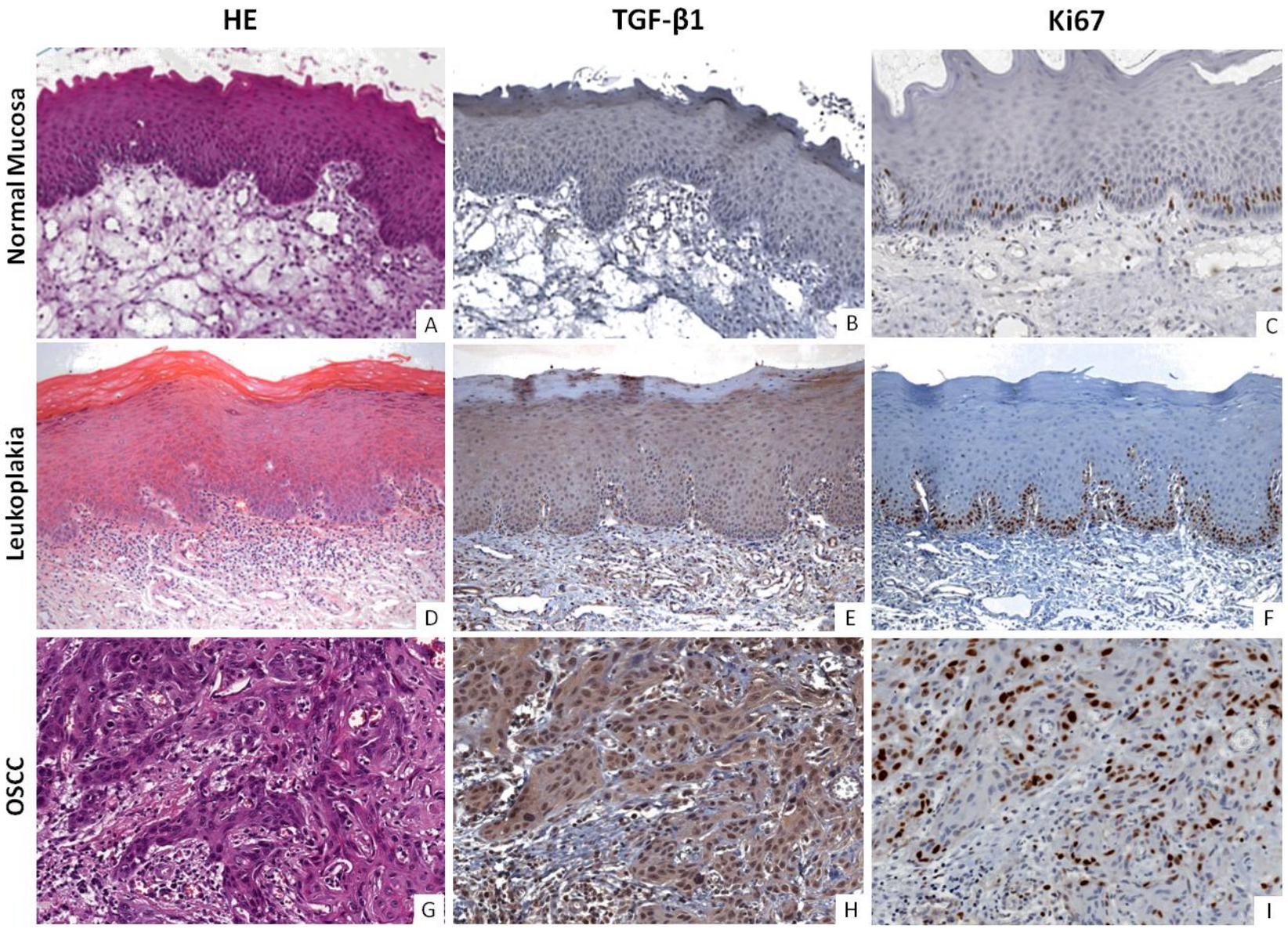

Figure 1. HE A,D and G) and immunohistochemical labeling of Ki-67 (B,E and H) and TGF-β1 (C,F and I) in normal mucosa (A, B and C- original magnification, ×100), leukoplakia and (D, E and F- original magnification, ×100) and OSCC (G, H and I - original magnification, ×200)

Correlation between TGF-β1 and Ki-67 in leukoplakia and OSCC

Spearman´s correlation coefficients were calculated to determine whether TGF-β1 could be implicated in changes in cell proliferation. No significant correlation was found in cases of leukoplakia neither in OSCC (respectively, p= 0.98 and p=0.72 data not shown).

Malignant transformation of leukoplakias and TGF-β1 expression

The mean follow-up time for patients with leukoplakia was 42,96 months. During this period, 4 cases suffered malignant transformation, leading to an annual malignant transformation rate of 1.11%. This lesions were situated in the floor of mouth (n=2), tongue (n=1) and palate (n=1). The initial biopsy showed no dysplasia in one case, mild dysplasia in one case, moderate dysplasia in one case and severe dysplasia in one case. All patients presented multiple lesions and in 3 cases the lesion that suffered malignant transformation measured 2cm or more. Only one case with malignant transformation presented a mild expression of TGF-β1, with less than 50% of positive epithelial cells. Other 3 cases presented cytoplasmatic expression in the entire epithelial length.

Evolution of OSCC and TGF-β1 expression

The mean follow-up time of patients with OSCC was 45.59 (±40.05) months. During the follow-up period 36 patients presented recurrence of the lesion, with a mean time for recurrence of 19.15 (±16.91) months. Thirty-one patients died because of the tumor, with a mean time for death of 22.83 (±21.82) months. The mean age of patients at time of death was 60.66 (±7.87) years. OSCC with higher histopathologic graduation and alternative treatments than surgery or surgery associated with radiotherapy showed to be predictors for death in the present study (Table 4). Patients with primary tumors graduated with high grade of malignancy presented 2.38-fold higher risk of dying, while patients treated with alternative treatments presented 4.32-fold higher risk of dying in the follow-up period analyzed. Kaplan-Meier cumulative survival curves were calculated and are displayed in Figure 2.

TGF-β1 expression was not associated with poor survival based in fact all OSCC analyzed reveled high expression of this protein.

Table 4. Clinical, histopathological and immunohistochemical markers determining death in cases of OSCC

	HR (95% IC)	p value
Age	0.97 (0.93-1.01)	0.22†
Gender		
Male	1	
Female	0.58 (0.13-2.44)	0.45†
Cigars/Day	1.00 (0.97-1.02)	0.90†
Alcohol consumption		
User/former user	1	
Non-user	1.52 (0.51-4.50)	0.44†
Pain		
Yes	1	
No	0.36 (0.04-2.70)	0.32†
Site		
Tongue/ Floor of mouth	1	
Other locations	0.87 (0.30-2.52)	0.80†
Nodal metastasis		
No	1	
Yes	0.99 (0.45-2.15)	0.98†
TNM		
I/II	1	
III/IV	5.71 (0.77-42.23)	0.08†
Recurrence		
Yes	1	
No	0.85 (0.40-1.78)	0.67†
Treatment		
Surgery	1	
Surgery + radiotherapy	2.81 (0.94-8.39)	0.06†
Others	4.32 (1.22-15.22)	**0.02**†
Histopathologic graduation		
Low/moderate	1	
High	2.38 (1.07-5.29)	**0.03**†
TGF- β1		
<50%	1	
>50%	0.46 (0.16-1.35)	0.16†
Ki67	1.01 (0.99-1.03)	0.13†

† Univariate Cox regression

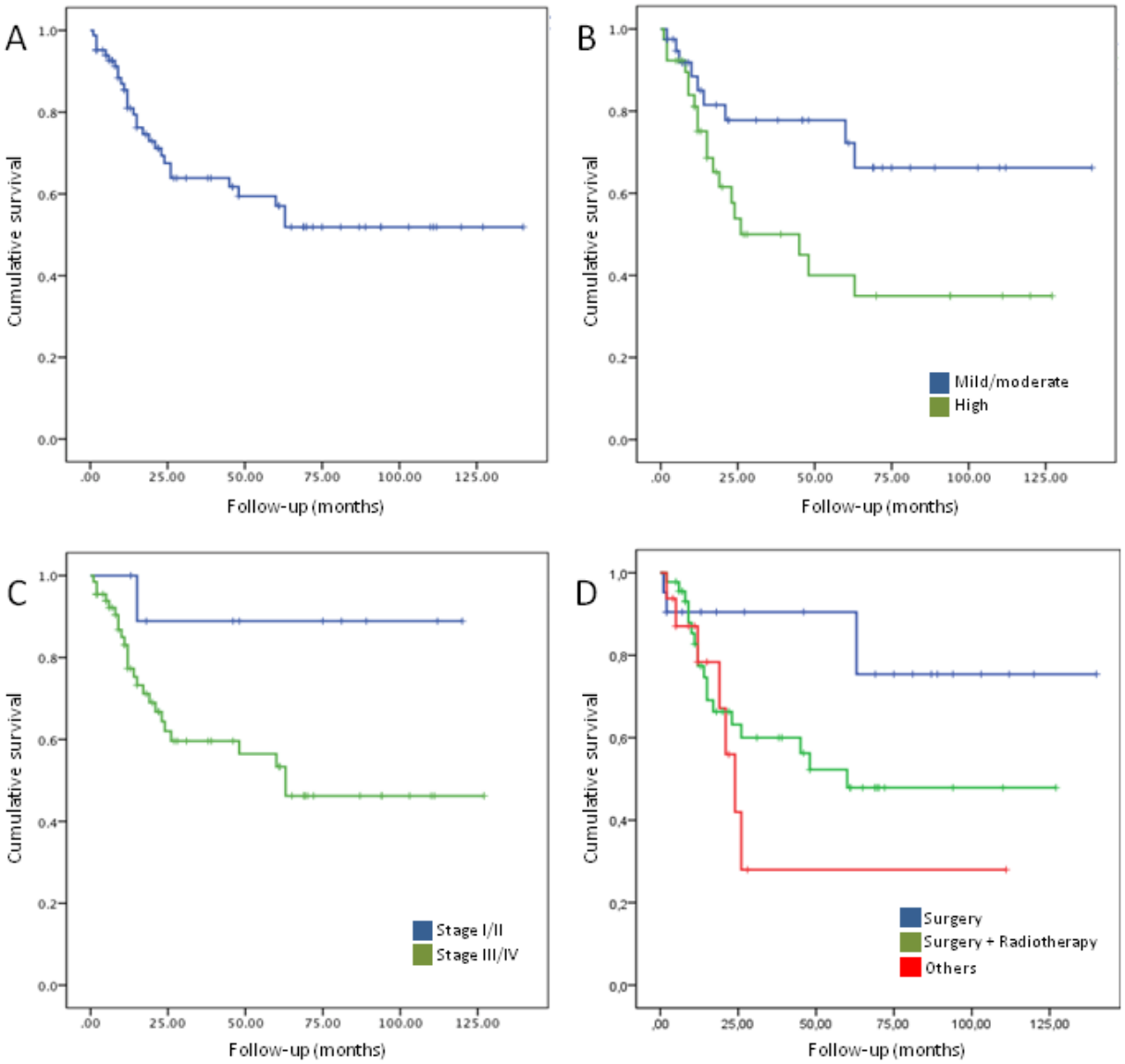

Figure 2. Kaplan-Meier analysis of overall OSCC survival (A) and according to Bryne´s graduation system (B); TNM (C) and treatment (C).

Discussion

Oral carcinogenesis is a multistage process comprising three main phases described as initiation, promotion, and progression, in which occur accumulated genetic changes responsible by the malignant transformation of normal mucosa [12]. Several molecular pathways have been recognized as being involved in OSCC development [3, 13] however, the role of TGF-β1 in different stages of oral tumorigenesis is complex and is not complete understand. In the

present study, TGF-β1 expression was analyzed in a spectrum ranging from normal through leukoplakia with and without dysplasia and OSCC. In addition, a correlation of TGF-β1 expression with proliferative labeling index, clinico-pathological aspects and clinical outcome was performed. Overall, the results indicate that epithelial cells in leukoplakia and OSCC lesions are positive for this protein. However, an increase in TGFβ-1, as well as, in PLI was observed in OSCC compared to leukoplakia and normal mucosa. Also, TGFβ-1 expression was not associated with clinico-pathological and outcome aspects.

TGF-β1 is a growth factors whose signaling pathway has multiple and paradoxical roles in different cell types. It is known that in normal conditions TGF-β1 is a potent growth inhibitor for normal epithelial, hematopoietic and immune cells, and plays an important function in normal tissue homeostasis [14]. However, many advanced tumors produce excessive amounts TGF-β1 indicating that in onco-genically activated cells use a complex set of cellular mechanisms that affect the carcinoma cell itself and its associated microenvironment [15, 16, 17, 18]. Besides that, some authors highlight dual role of TGF-β1 in tumors. Prior to initiation and in early progression, TGF-β1 can exert tumor-suppressive effects. But, at later stages pathological forms of TGF-β1 signaling contribute to tumor progression and invasiveness. These aspects underlines the complexity of TGF-β1 signaling and its apparently contrasting cellular effects that could be mediated by cross-talk with other growth factors and by activation of different signal transduction pathways [6, 7, 8, 14, 19, 20].

In the present study was demonstrated an increase in TGF-β1 in epithelial cell in leukoplakia and OSCC compared to oral normal mucosa. Also, OSCC revealed high expression of TGF-β1 than leukoplakia tissue. These results corroborated the literature indicating that TGF-β1 has a pro-tumorigenic role in several solid tumors as well in OSCC [21, 22, 23, 24, 25, 26, 27]. Briefly, TGF-β exerts its effect by binding to the TGF- type II receptor (TβRII) followed by recruiting TGF- βRI via multiple parallel signaling pathways, including the SMAD proteins [8, 28]. The inactivation of TGF-β pathway could occur by genetic mutation or epigenetic control but still not fully understood. TGF-β1 polymorphism was proved to be involved with increased susceptibility to OSCC,

demonstrating that individuals carrying this allele had an estimated 2.73-fold grated relative risk of developing cancer [29]. Evidence supports the concept that neoplastic transformation of oral cancers can result in loss of a growth inhibitory response to TGF-β by down regulation of their receptors or alterations in SMAD genes. The most commonly mutated TGF-β pathway genes are *TGFBR2, TGFBR1*, *SMAD4* and *SMAD2* [6, 13]. Also, in a majority of human cancers and cell lines, the expression of TβRI and TβRII is altered at the protein and/or mRNA levels [21, 28,] demonstrated that down regulation of TβRII and TβRIII, in human OSCC, was an early event that occurred initially during the leukoplakia stage. These alterations described in TGF-β receptors could explain the increased TGF-β1 expression during the carcinogenesis process that was observed in the present study.

Other important aspect that have been associated with increase of TGF-β1 expression in cancer cells is the decrease of E-cadherin expression and an increase in vimentin, hallmarks of EMT, a key event during invasion [30]. Under the influence of the transgenic TGF-β1, the benign skin tumor cells underwent EMT, thus forming invasive spindle carcinoma cells *in vivo*, which expressed high levels of TGF-β. In this mouse skin model, TGF-β acted directly on the carcinoma cells that secreted the cytokine, leading to EMT, which was sufficient for the acceleration of malignancy *in vivo* [31]. Based on these previous reports, it could be expected high levels of TGF-β1 in OSCC when compared to leukoplakia because EMT is a critical differentiation switch that allows epithelial cells to migrate, invade their local tissue environment and even advantage the metastasis process.

TGF-β1 was overexpressed in the vast majority of OSCC cases, suggesting that this pathway is highly active in this tumor and might be considered as a good therapeutic target. Several studies have demonstrated the positive association between high expression of TGF-β1 and invasive characteristics of mammary tumors [22, 23] and prostate [32], reduction in overall survival in pancreas carcinoma [33]. Also, high expression TGF-β1 was observed in differet cancers like colorectal [24], gastric [34], renal [25]and head and neck squamous cell carcinoma [26].

Despite all challenges involved on the development of TGF-β1 inhibitors for cancer therapy, several evaluated drugs proved to be safe and there are now at least four different drugs that have progressed further in clinical development. These drugs were tested in phase II clinical trials for hepatocellular and pancreatic carcinomas; and in phase III clinical trials for glioblastoma and non-small-cell lung carcinoma [35.] To the best of our knowledge there are no clinical trials evaluating TGF-β1 pathway as a therapeutic target in OSCC.

In conclusion, the results obtained in the present study suggest that TGF-β1 may participate in the malignant phenotype acquisition process of OSCC since early stages of oral carcinogenesis process. Therefore, this protein might be valid targets for therapeutic approaches in patients with OSCC and potentially malignant oral lesions; however, further studies with other validating methods are necessary to add strength to the results obtained in the current study.

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4 CONSIDERAÇÕES FINAIS

Neste estudo buscamos avaliar a associação da expressão imunoistoquímica de TGF-β1 com o índice de proliferação e variáveis clínico-patológicas do CEC e das leucoplasias. Apesar de não haver associação entre este marcador e os desfechos analisados, obtivemos restuldados importantes em relação a sua expressão precoce durante a carcinogênese oral, assim como uma super expressão na quase totalidade dos casos de CEC, quando comparados à mucosa normal que foi negativa para esta proteína. Neste sentido, é de extrema importância o desenvolvimento de novos estudos *in vitro* e *in vivo* que busquem novas alternativas para o tratamento do CEC de boca utilizando o TGF-β1. Analisando nossos resultados, em conjunto com demais estudos da literatura que mostram a relevância das vias de sinalização ativadas pelo TGF-β1 na progessão tumoral, concluimos que esta via pode ser um bom alvo terapêutico no tratamento do câncer oral.

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ANEXO A- CARTA DE APROVAÇÃO DO COMITÊ DE ÉTICA

Plataforma Brasil - Ministério da Saúde

Hospital de Clínicas de Porto Alegre - HCPA / UFRGS

PROJETO DE PESQUISA

Título: ANÁLISE IMUNOISTOQUÍMICA DO PAPEL DO TGF-B1 E SUA CORRELAÇÃO COM A PROLIFERAÇÃO CELULAR EM LEUCOPLASIAS E CARCINOMAS ESPINOCELULARES DE LÍNGUA

Área Temática:

Pesquisador: Luise Meurer **Versão:** 3

Instituição: Hospital de Clínicas de Porto Alegre - HCPA / UFRGS **CAAE:** 02810012.0.0000.5327

PARECER CONSUBSTANCIADO DO CEP

Número do Parecer: 65350

Data da Relatoria: 01/08/2012

Apresentação do Projeto:

Projeto a ser realizado em material biológico e pesquisa em prontuários, visa correlacionar dados clínicos com os resultados laboratoriais. O objetivo geral é verificar se a expressão de TGF-B1 tem papel na carcinogênese da língua.

Objetivo da Pesquisa:

Verificar se a expressão de TGF-B1 tem papel na carcinogênese de língua. Correlacionar a expressão de TGFB1 com proliferação celular, graduação das lesões, evolução clínica e exposição a fatores de risco para câncer de língua.

Avaliação dos Riscos e Benefícios:

Aparentemente não há riscos nem benefícios diretos para os pacientes. Esclarecer.
Existe a possibilidade de o paciente se beneficiar desta revisão? Em caso afirmativo os pacientes deverão ser informados sobre o estudo e autorizar a revisão do material. Caso não exista possibilidade de benefício direto os autores devem apresentar termo de compromisso para uso de material biológico.
Os pesquisadores esclarecem que 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 da doença estudada. Os pesquisadores assinarão o termo de compromisso na utilização de dados e o termo de compromisso na utilização de material biológico. Apenas os pesquisadores envolvidos no estudo terão acesso à identidade dos pacientes a qual será substituída por códigos no momento em que for criado o banco de dados. Foi adicionado no sistema WEBGPPG o termo de compromisso para uso de material biológico.

Comentários e Considerações sobre a Pesquisa:

Seria importante incluir uma estimativa do número de casos que foram atendidos no HCPA no período eleito para inclusão. É muito provável que ocorram muitas perdas (exclusões), considerando-se que casos só serão incluídos quando 70% das informações estiverem disponíveis e que não haverá outro método de busca de informações.
Pelas informações apresentadas, a correlação a ser pesquisa com a expressão de KI-67 é intuitiva. Os resultados de sua expressão não tem uma definida correlação com o prognóstico, como mencionado as informações da literatura são controversas. O que poderia melhor embasar a pesquisa e análise deste marcador, seria comprovar por outros métodos ou apresentar dados de literatura, mostrando que, também na leucoplasia e no CEC de língua há uma correlação deste marcador com a proliferação celular. Há ambiguidade na apresentação das variáveis que serão levantadas.
Os pesquisadores esclarecem que foi realizada consulta ao banco de dados do Serviço de Patologia do HCPA e encontramos 140 diagnósticos de carcinoma espinocelular em língua no período previsto para o estudo. Acreditamos que pelo menos 30% dos casos irão atender os critérios de inclusão da

pesquisa e portanto, há casos suficientes disponíveis para a realização do estudo.
Adicionalmente esclarecem que foi realizada uma nova versão da revisão de literatura do Ki-67 para deixar claro no projeto que existe na literatura científica informações consistentes para suportar a relação do Ki-67 como um importante marcador de proliferação, sendo um dos mais utilizados para este fim. Alguns estudos foram realizados com lesões potencialmente malignas de boca e carcinoma espinocelular, porém, observa-se que os estudos variam do ponto de vista metodológico no que tange a associação entre Ki-67 com variáveis clínicas (TNM e comportamento) e histopatológicas (graus de displasia e de diferenciação tumoral). A nossa proposta é utilizar o Ki-67 como um referencial da atividade proliferativa de cada lesão para poder compreender em paralelo o papel do TGF em lesões mais ou menos proliferativas bem como, associar estes dados com a evolução clínica de cada caso.

Considerações sobre os Termos de apresentação obrigatória:

Apresentado Termo de Compromisso para Uso de Material Biológico com a assinatura de todos os participantes.

Recomendações:

Sem outras recomendações.

Conclusões ou Pendências e Lista de Inadequações:

1. Informar estimativa de número de casos no período.
Foi realizada estimativa de casos diagnosticados no Serviço de Patologia do HCPA e acreditamos que o número encontrado de 140 casos no período proposto seja suficiente.
Pendência atendida.

2. Acrescentar informações sobre Ki-67 nas lesões estadadas ou acrescentar estudo de proliferação celular.
Novas informações foram acrescidas na introdução como forma de revisão de literatura do Ki-67 para suportar o papel do Ki-67 como um importante marcador de proliferação.
Pendência atendida.

3. Unificar a apresentação das variáveis do estudo no item Materiais e Métodos.
Pendência atendida.

4. Corrigir o primeiro objetivo (carcinogênese labial).
Pendência atendida.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

A versão do projeto aprovada corresponde ao documento submetido em 27/07/2012.

PORTO ALEGRE, 01 de Agosto de 2012

Assinado por:
José Roberto Goldim

ANEXO B- CARTA DE APROVAÇÃO DA COMPESQ

UFRGS

Sistema Pesquisa - Pesquisador: Manoela Domingues Martins

Linhas de Pesquisa
Projetos de Pesquisa
Áreas de Atuação
Bolsas de Pesquisa
Programa de Iniciação Científica Voluntário
Programa de Fomento à Pesquisa(auxílio)
Pós-doutorado

Retornar

Dados Gerais:

Projeto Nº:	22720	**Título:**	ANALISE IMUNOISTOQUIMICA DO PAPEL DO TGF-β1 E SUA CORRELACAO COM A PROLIFERACAO CELULAR EM LEUCOPLASIAS E CARCINOMAS ESPINOCELULARES DE LINGUA
Área de conhecimento:	Odontologia	**Início:**	01/04/2012 **Previsão de conclusão:** 31/08/2015
Situação:	Projeto em Andamento		
	Não possui projeto pai		**Não possui subprojetos**
Origem:	Faculdade de Odontologia Programa de Pós-Graduação em Odontologia		**Projeto da linha de pesquisa:** CÂNCER BUCAL
Local de Realização:	não informado		**Projeto sem finalidade adicional** **Projeto não envolve aspecos éticos**

Não apresenta relação com Patrimônio Genético ou Conhecimento Tradicional Associado.

Objetivo:

O objetivo do presente estudo será verificar a imunomarcação do TGF-β1 em leucoplasias e CEC de língua com intuito de verificar um possível papel desta proteína na carcinogênese labial.

Palavras Chave:

Linhas de Pesquisa
Projetos de Pesquisa
Áreas de Atuação
Bolsas de Pesquisa
Programa de Iniciação Científica Voluntário
Programa de Fomento à Pesquisa(auxílio)
Pós-doutorado

Avaliações:

Comissão de Pesquisa de Odontologia - Aprovado em 14/05/2012 Clique aqui para visualizar o parecer

Apoio Externo:

Instituição: HCPA - Hospital de Clínicas de Porto Alegre

Anexos:

Projeto Completo	**Data de Envio:** 30/03/2012
Instrumento de Coleta de Dados	**Data de Envio:** 30/03/2012
Concordância de Instituição	**Data de Envio:** 08/05/2012
Formulário de Encaminhamento do Protocolo de Pesquisa com Animais	**Data de Envio:** 08/05/2012
Documento de Aprovação	**Data de Envio:** 04/08/2012
Documento de Aprovação	**Data de Envio:** 04/08/2012
Relatório de Andamento	**Data de Envio:** 05/05/2014

Período: 01/04/2012 a 05/05/2014

Bolsas:

Projeto associado à bolsa PIBIC CNPq-UFRGS **No Período:** 01/08/2013 **a** 31/07/2014
Bolsista: PAULA CARDOSO RODRIGUES **no período de** 01/08/2013 **a** 31/07/2014
Projeto associado à bolsa BIC UFRGS **No Período:** 01/08/2014 **a** 31/07/2015
Bolsista: LISLEY VACARI ORTIZ **no período de** 01/08/2014 **a** 31/07/2015

ANEXO C- FICHA DE LEVANTAMENTO DE DADOS

Ficha de Levantamento de dados- Leucoplasias e CEC de língua

Prontuário:_____ Nº AP_____

Nome do paciente:_____

Idade: _____

Sexo: 1 ( ) masculino 2 ( ) feminino

Ocupação: _____

Cor: 1 ( ) branca 2 ( ) preta 3 ( ) amarela 4 ( ) outra

Residência: 1 ( ) urbana 2 ( ) rural

Fumo: 1 ( ) sim 2 ( ) não

6.1- Tipo: ( ) cigarro ( )charuto ( ) cachimbo ( ) palheiro ( ) outros.

6.2- Quantidade: _____

6.3- Periodo de uso: _____

6.4- Ex-fumante há quanto tempo:_____

Alcool: 1( ) sim 2 ( ) não

7.1- Tipo: ( )cerveja ( )cachaça ( )whisky ( )vinho ( )outros

7.2- Quantidade .....

7.3- Periodo de uso: .....

7.4- Há quanto tempo parou o uso:_____

Dor: 1 ( ) sim 2 ( ) não

Lesão:

Sitio: 1 ( ) Anterior da língua 2 ( ) Posterior da língua 3 ( ) base da língua 4 ( ) Bordo da língua 5 ( )outras _____

Aspecto clínico: ( )mancha branca ( )mancha vermelha ( )placa ( )nódulo ( ) úlcera ( )úlcero-vegetante ( )úlcero-infiltrativo

Tamanho: _____mm

Tamanho: ( )Tx ( )T0 ( )carcinoma “in situ” ( ) T1 ( )T2 ( )T3 ( )T4

Metástase regional: ( )NX ( )N0 ( )N1 ( )N2 ( )N2a ( )N2b ( )N2c ( ) N3

Metástase à distância: ( )MX ( )M0 ( )M1

TNM: ( )Estádio 0 ( )Estádio I ( )Estádio II ( )Estádio III ( )Estádio IVa ( )Estádio IVb ( )Estádio IVc

Tratamento:

Leucoplasias: ( )remoção parcial, ( )remoção total, ( ) acompanhamento clinico ( ) outros _____

CEC: 1 ( ) cirugia 2 ( ) radioterapia3 ( ) quimioterapia 2 ( ) cirugia e radioterapia 4 ( ) cirugia e quimioterapia 5 ( ) radio e quimioterapia 6 ( ) cirugia, radio e quimioterapia

Evolução:

Leucoplasia: 1( ) sem lesão, 2 ( ) com lesão, 3 ( ) evoluiu para CEC

CEC: 1 ( ) vivo 2 ( ) falecido pelo tumor 3 ( ) falecido por outra causa

Há quantos anos:_____

Recidiva: 1 ( ) sim 2 ( ) não