PI3K/AKT/mTOR pathway activation in actinic cheilitis and lip squamous cell carcinomas.

Keywords: lip cancer, actinic cheilitis, potentially malignant disorders, biomarker, prognosis, immunohistochemistry
manuscript word count: 592
number of figures: 1

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Statement of all funding sources that supported the work: This study was funded by the Postgraduate Research Group of the Hospital de Clínicas de Porto Alegre (GPPG/FIPE: 2018–0514). Brazilian National Council for Scientific and Technological Development (CNPq student scholarship), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil (CAPES)-finance code 001 and São Paulo State Research Foundation (FAPESP 2016/21785-4) provided student scholarships. Manoela Domingues Martins and Pablo Agustin Vargas are research fellows funded by the Brazilian National Council for Scientific and Technological Development (CNPq).

Conflicts of interest: The authors declare that have no conflict of interest.

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Article type : Letter to Editor

Lower lip squamous cell carcinoma (LLSCC) is one of the most common cancers of the head and neck[1]. Actinic cheilitis (AC) is a potentially malignant disorder of the lip, and it is estimated that 3.07% of AC cases undergo malignant transformation[2]. Lip carcinogenesis is directly associated with exposure to ultraviolet (UV) radiation and tobacco.[1,2] The molecular circuitry involved in this process, however, remains poorly understood. The identification of deregulated pathways during the process of carcinogenesis may help in the identification of molecular markers capable of identifying early cellular transformation. The activation of the pathway comprised by phosphatidylinositol-3-kinase (PI3K), protein kinase B (Akt) and mammalian target of rapamycin (mTOR) is considered a key event in many types of tumors due to its ability to modulate important oncogenic outcomes.[3-5] Remarkably, this pathway had never been fully investigated in lip carcinogenesis until now. Herein, an immunohistochemical assessment of PI3K Kinase p110α, Akt^{Ser473} phosphorylation, and mTORC1 activation through analysis of ribosomal protein S6 kinase (RPS6) phosphorylation was performed in 38 cases of AC, 40 cases of LLSCC, and nine cases of normal lips (NL), as baseline controls. For each case, an immunoreactive score (IRS) was designated by multiplying the percentage of positive cells (0 – 0% to 10%; 1 – 11% to 50%; and 2 – 51% to 100%) by the staining intensity (0 – absent; 1 – weak; 2 – moderate; 3 – intense). Differences in the IRS were assessed through statistical analysis.

PI3K Kinase p110α showed a significant increase in immunoreactivity from NL to LLSCC ($p<0.01$) and from AC to LLSCC ($p<0.05$) (Fig. 1), suggesting that the activation of this protein is associated with the acquisition of the malignant phenotype. PI3K pathway alterations occur in 30% to 66% of head and neck squamous cell carcinomas and are also linked to advanced disease.[5] The oncogenic triggering of this pathway commonly occurs by activating mutations in the p110α isoform of PI3K or through the loss of the PTEN tumor suppressor.[5]

The $pAkt^{Ser473}$ marker was significantly higher in AC ($p<0.001$) and in LLSCC ($p<0.001$), both compared to NL (Fig. 1), suggesting that Akt modification occurs as an early event in lip carcinogenesis. The Akt pathway is considered an important element in cell survival and proliferation after UV exposure[6], which could justify why AC presents high levels of pAkt. Moreover, other groups have previously demonstrated an increase of $pAkt^{Thr303}$ in oral squamous cell carcinoma (OSCC) and oral epithelial dysplasia compared to non-dysplastic oral tissue[4], corroborating with the present findings. Several PI3K/Akt/mTOR target drugs are available, and their use as a topical treatment for AC deserves to be further investigated.

The activation of mTORC1 that results in phosphorylation of RPS6 was significantly decreased in LLSCC when compared to NL ($p<0.05$), contrasting with the previous proteins (Fig. 1). These findings agree with previous studies conducted with OSCC and leukoplakia.[4,7] Moreover, our group has previously studied the PI3K/PTEN/mTOR pathway in lip keratoacanthoma (KA),[8] a well-differentiated, well-keratinized tumor that may originate in the pilosebaceous unit. We observed a strong positivity for pRPS6 along with a lower expression of $pAkt^{Ser473}$. Our hypothesis is that mTORC1, but not mTORC2, is associated with KA development, and the absence of $pAkt^{Ser473}$ could be related to its low invasive capacity, whereas the increase of pRPS6 would be related to the tumor differentiation pattern.

Based on our results, it is possible to conclude that PI3K Kinase p110α and $pAkt^{Ser473}$ (mTOR2) enhanced activation are associated with more aggressive behavior during lip carcinogenesis by triggering AC and LLSC

progression. Contrastingly, pRPS6 (mTOR1) seems to prevent tumor development since its activity decreased during the progression of lip carcinogenesis.

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 Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES- finance code 001), Brazilian National Council for Scientific and Technological Development (CNPq) and the São Paulo State Research Foundation (FAPESP 2016/21785-4) for student scholarships. This study was funded by the Postgraduate Research Group of the Hospital de Clínicas de Porto Alegre (GPPG/FIPE: 2018–0047). Manoela Domingues Martins and Pablo Agustin Vargas are research fellows funded by the Brazilian National Council for Scientific and Technological Development (CNPq).

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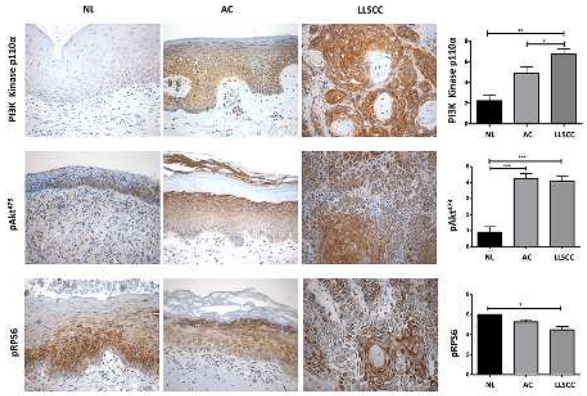
Figure Legends

Figure 1. Representative images of immunohistochemical labeling of PI3K Kinase p110α, pAkt^{Ser473,} and pRPS6 in NL, AC, and LLSCC (original magnification, x400). Mean expression of PI3K Kinase p110α, pAkt^{Ser473,} and pRPS6 in NL, AC, and LLSCC (* $p<0.05$; ** $p<0.01$; *** $p<0.001$ – Kruskal Wallis test followed by Dunn's posthoc test adjusted for Bonferroni error correction).



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