Phosphorylated mammalian target of rapamycin is associated with an adverse outcome in oral squamous cell carcinoma

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Objectives. To evaluate the expression of phosphorylated mammalian target of rapamycin (p-mTOR) and phosphatase and tensin homolog deleted on chromosome TEN (PTEN) in oral squamous cell carcinomas (OSCCs) and relate them with clinicopathologic characteristics and outcome.

Study Design. We analyzed p-mTOR and PTEN protein expression by immunohistochemistry in 72 OSCCs. Multivariate analysis was conducted to examine their role in survival.

Results. p-mTOR expression was observed in 46 (63.9%) cancers and PTEN expression was absent in 22 (30.6%). An adverse independent prognostic value for high p-mTOR expression was found ($P = .043$) as well as for advanced tumor stage ($P = .010$) in patients' overall survival (OS). For disease-free survival (DFS), only advanced tumor stage ($P = .001$) presented an adverse independent prognostic value.

Conclusions. The effect of p-mTOR in OS of OSCC suggests that this marker may serve as a reliable biological marker to identify high-risk subgroups and as a guide to therapy. Furthermore, the high expression of p-mTOR suggests that this protein may be a promising therapeutic target in OSCC. (Oral Surg Oral Med Oral Pathol Oral Radiol 2013;115:638-645)

Oral cancer remains a significant cause of morbidity and mortality, with 263,020 new cases annually and 128,654 deaths worldwide in 2008.[1] Throughout the world, the survival rate for oral cancer has not changed significantly during last few decades.[2] However, recent US data show a statistically significant improvement in OS among patients treated for oral squamous cell carcinoma (OSCC) from 55% in 1984-1986 to 60% in the 1996-2003 timeframe.[3] In other regions, survival figures have remained at approximately 50%.[2]

Oral tumorigenesis is a multistep process caused by sequential accumulation of multiple gene alterations. Molecular analysis of oral cancers has so far failed to find any consistent changes associated with neoplastic progression or prognostic markers. These aberrations may occur in a number of cell pathways. One such key pathway is PI3K/AKT/mTOR. Molecular aberrations in this pathway may serve as prognostic markers or targets for molecular therapies.[4,5]

The mammalian target of rapamycin (mTOR) is a 290 kDa serine-threonine protein kinase and an important downstream target of the PI3K/AKT signaling pathway involved in the regulation of overall cellular anabolism, cell growth, proliferation, and survival. In response to multiple stimuli including nutrient, oxygen, insulin, growth factors, adenosine triphosphate, and tobacco metabolites mTOR is activated by phosphorylation of Ser2448 through the phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway.[6] This results in subsequent activation of 3 key modulators of protein biosynthesis: the eukaryotic translation factor 4E, the p70 ribosomal S6 kinase, and elongation factor 2.[7]

Statement of Clinical Relevance

p-mTOR is a promising therapeutic target for OSCC and an independent prognostic factor in the overall survival (OS), serving as a reliable biological marker to identify high-risk subgroups and as a guide to therapy.

This study was supported by a grant from the Centro de Investigação em Ciências da Saúde (01-GCD-CICS-09; 02-GCD-CICS-09).

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Received for publication Jan 7, 2013; returned for revision Jan 18, 2013; accepted for publication Jan 23, 2013.

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2212-4403/$ - see front matter

http://dx.doi.org/10.1016/j.oooo.2013.01.022

Previous studies have shown that activated mTOR is deregulated and could influence the prognosis of a number of cancers.[8-15] However, the prognostic significance of the active form of mTOR in OSCC remains uncertain. Furthermore, increasing interest has been shown with regard to this molecule as an important target for anticancer therapy with the advent of everolimus, an analog of rapamicin.[6,7]

PTEN gene (phosphatase and tensin homolog deleted on chromosome TEN) is a tumor suppressor gene, located at 10q23.3, that encodes a protein phosphatase with lipid and protein phosphatase activity. The major substrate for PTEN is a product of PI3K, phosphatidylinositol (3,4,5)-triphosphate (PIP-3).[16] Loss of PTEN function, reported in a number of tumors, may lead to increasing levels of PIP-3, resulting in a hyperactivation of AKT and, subsequently, unrestricted activity of mTOR.[17] The prognostic value of PTEN has been reported in several tumors.[18-20]

To date, few studies have examined both mTOR and PTEN protein expression in OSCC. The aim of the present study was to evaluate activated mTOR and PTEN expression in patients with OSCC and relate them to clinicopathologic characteristics and patient outcome.

PATIENTS AND METHODS

Patient population

This was a retrospective study of 72 patients newly diagnosed and treated for primary OSCC at the Hospital de Santo António (HSA), Porto, Portugal, between 2000 and 2006. The study was approved by the institutional review board of the hospital. Patients were excluded if they had undergone radiotherapy or chemotherapy prior to biopsy; if they lacked clinical and follow-up information or if their paraffin blocks lacked sufficient tumor tissue. From the patients' records, we obtained information with regard to age, gender, tumor location, tumor stages (I-IV), primary treatment, tumor grade, surgical margin status, and follow-up. Tumor stage was reclassified according to the seventh edition of the classification of malignant tumors of American Joint Committee on Cancer.[21]

For all tumors, 3 μm sections were cut and stained with hematoxylin-eosin (HE) to confirm the initial diagnosis. Tumor grade was reclassified following the World Health Organization classification (2005)—into well differentiated (G1), moderately differentiated (G2), and poorly differentiated (G3) OSCC.[22] The tumor stroma was inspected for the presence of lymphatic invasion and perineural permeation, reported as present or absent.

Tissue microarray construction

Representative tumor areas were selected on HE-stained sections and marked on paraffin blocks, avoiding necrosis and keratin areas. Three tissue cores (2 mm in diameter) were obtained from each selected specimen and deposited into a recipient paraffin block, using a microarray instrument (TMA Builder; Histopathology Ltd, Pécs, Hungary). The 12 tissue microarray (TMA) blocks were designed and constructed as previously described.[23]

Immunohistochemistry

The expression of p-mTOR and PTEN proteins was evaluated by immunohistochemistry on 3-μm TMA tissue sections, using the antibodies: anti-phospho-mTOR (Ser2448), recognizing the mTOR protein phosphorylated at Ser2448 (rabbit monoclonal antibody, clone 49F9; Cell Signaling Technology, Beverly, MA), and anti-PTEN, recognizing PTEN protein (rabbit polyclonal, clone PN37; Invitrogen Corporation, Carlsbad, CA). Tissue sections were deparaffinized followed by antigen-retrieval treatment (p-mTOR: citrate buffer 0.01 M pH 6.0; PTEN: EDTA buffer 0.01 M pH 9.0) at high temperature (water bath, 30 min at 98°C).

After blocking for nonspecific binding, primary antibody was added to the sections in a prestandardized dilution (p-mTOR 1/100; PTEN 1/50) and incubated for 60 min at room temperature. The primary antibodies were detected using a standard peroxidase-labeled dextran polymer for visualization with diaminobenzidine as chromogen (NovoLink Polymer Detection System; Novocastra, Leica Biosystems Newcastle Ltd), according to the manufacturer's instructions. TMA tissue sections were lightly counterstained with Mayer hematoxylin and cover-slipped. Negative and positive controls were used in each staining run.

Evaluation of immunohistochemical expression

All samples were evaluated independently by 2 investigators blinded to clinicopathologic characteristics. The discordant cases were reviewed under a multihead microscope to achieve a consensus. We used the higher score out of at least 2 of the 3 cores examined per case.

p-mTOR and PTEN staining were evaluated on the basis of extent and intensity immunolabeling of tumor cell cytoplasm. The intensity of staining was scored as 0 (absent), 1 (weak), 2 (moderate), and 3 (strong). The extent of staining was scored as 0 (0%-9%), 1 (10%-24%), 2 (25%-49%), 3 (50%-74%), and 4 (75%-100%), according to the percentage of cells stained positive for each protein. The sum of the intensity and extent scores was used as the final score (0-7). Tissues having a final score of 0-1 were considered negatives. Final scores of 2-3, 4-5, and 6-7 were considered 1+, 2+, and 3+, respectively. For data analysis, the score of these

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