Ferreira et al. Applied Cancer Research (2017) 37:41
DOI 10.1186/s41241-017-0046-4

Applied Cancer Research

RESEARCH ARTICLE Open Access

Prognostic implications of the phosphatidylinositol 3-kinase/Akt signaling pathway in oral squamous cell carcinoma: overexpression of p-mTOR indicates an adverse prognosis

Darkle Modesto Ferreira[1], Tercia J. Neves[1*], Luiz Guilherme C. A. Lima[1], Fabio A. Alves[2] and Maria D. Begnami[1]

Abstract

Background: The development of oral cavity cancer is related to the accumulation of genetic alterations. The activation of AKT is associated with the proliferation and progression of many malignancies. It is thought that MAP kinases, together with the PI3K/AKT/mTOR signaling pathway, promote uncoordinated proliferation via inhibition of PTEN, thus increasing cell survival and mediating cancer progression. However, there are few studies regarding the expression of these proteins in oral squamous cell carcinoma (SCC).

Methods: The expression of PI3K, p-mTOR, p-AKT, p-MAPK, and PTEN in 125 oral SCCs, including gingival, palate hard, and alveolar ridge tumors, was examined by immunohistochemistry and correlated with clinicopathological data and survival rates.

Results: We observed PI3K, p-mTOR, p-MAPK, p-AKT, and PTEN positive staining in the cytoplasm of most SCC (92.4%, 88.2%, 88.3%, 94.2%, and 25%, respectively). Positive nuclear staining was observed for p-mTOR, PTEN, p-AKT, and p-MAPK (42.9%, 72%, 64.2%, and 58.2%, respectively). Only p-mTOR protein expression was observed on the cell membrane and was present in 44.5% of cases. A statistically significant correlation was found between p-MAPK expression and SCC clinicopathological stages III and IV ($p = 0.0042$). Lower rates of disease-free survival were found in patients with SCC III / IV ($p = 0.001$). Patients with positive nuclear staining of p-mTOR displayed a significant increase in disease-free survival rates.

Discussion: The identification of prognostic and predictive markers is clinically important because oral cancer is a group of heterogeneous diseases with various biological and clinical characteristics.

Conclusion: Our findings suggest that the PI3K/AKT pathway is activated in gingival, hard palate, and alveolar ridge SCCs. We have demonstrated that p-mTOR expression can function as a biomarker for survival in oral SCCs and could be a promising therapeutic target in oral SCC treatment.

Keywords: PI3K, AKT, mTOR, PTEN, Oral carcinoma, Squamous cell carcinoma, Immunohistochemistry, Molecular markers, Prognostic markers

* Correspondence: tercianeves@hotmail.com
[1]Departament of Pathology, A C Camargo Cancer Center, Rua Professor Antonio Prudente 211, Liberdade, São Paulo ZIP 01509-900, Brazil
Full list of author information is available at the end of the article

Background

Squamous cell carcinoma (SCC) of the oral cavity is a severe health problem in terms of morbidity and mortality. It is the sixth most common cancer worldwide and accounts for 4% of all malignancies and 2% of malignancies in women [1, 2]. These tumors primarily develop in individuals with a protracted history of tobacco and alcohol abuse. However, the specific cause of the disease remains unknown [3].

Despite the evolving model of multimodality management, which integrates surgical intervention, chemotherapy, and radiation therapy, overall survival remains poor with a five-year relative survival rate below 50% [4].

The majority of oral SCC patients presents with advanced disease and incur significant morbidity and mortality due to the limited availability of screening tools and markers for adjuvant therapy. Progress has been made regarding the identification and validation of biomarkers as targeted molecular therapies have advanced through clinical trials. To determine which patients will benefit from these treatments, personalized medical methods and analysis of factors specific to the individual and their tumor are required [5].

The phosphatidylinositol 3 kinase (PI3K)/protein kinase B (AKT) signaling pathway plays important roles in regulating tumor cell survival, apoptosis, and protein translation [6, 7]. PI3K is activated by receptor tyrosine kinases, leading to allosteric activation of the enzyme and tyrosine phosphorylation of its regulatory subunit. In response to extracellular stimuli, PI3K phosphorylates the 3′-hydroxyl group of phosphatidylinositol-4, 5-bi-phosphate (PIP2) to generate phosphatidylinositol-3, 4, 5-triphosphate (PIP3). AKT is activated by phosphorylation via PIP3 and mammalian target of rapamycin (mTOR) and is a component of the mTOR complex [8]. The phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a well-described negative regulator of the PI3K/AKT signaling pathway, which functions as a tumor suppressor gene by decreasing the levels of cyclin D1 and inducing G1 phase cell cycle arrest [9].

The PI3K/AKT pathway is frequently overactive in various tumors, which triggers a cascade of responses that drive tumor progression, including aberrant cell growth, proliferation, increased cell survival, and increased cell motility [10]. Although a few reports have examined AKT, PTEN, and mTOR expression in head and neck SCC [11–13], there is no comprehensive study of these markers in rare groups of oral SCC that include gingival, palate hard, and alveolar ridge tumors.

In the present study, we have assessed the expression of PI3K/AKT pathway proteins in 125 oral SCCs using immunohistochemistry. Additionally, we examined the possible correlations between protein expression and clinicopathological parameters in this cohort of patients.

Methods

Patients

A total of 125 patients with oral SCC treated over a period from 1980 to 2006 were identified from files obtained from the Department of Pathology, A. C. Camargo Cancer Center (Sao Paulo, Brazil). The mean age of the patients was 65 years (range: 30–91 years), and samples were collected from 76 males and 49 females. The clinicopathological parameters available included age, gender, tumor location, clinical stage, and histological grade. Tissue slides were reviewed for histological classification according to WHO [14]. The series included 32 gingival, 31 hard palate, and 62 alveolar ridge tumors. Patient clinical outcome was followed from the date of diagnosis to a period of 0.6–108.6 months (mean of 28.3 months). Patients who were lost to follow-up or died from any cause other than oral SCC were excluded from the analysis of survival rates. No tumors associated with specific genetic mutations were included. The institutional board for ethical studies at the A. C. Camargo Cancer Center approved this study. All the clinical characteristics and pathological findings are summarized in Table 1.

Tissue microarray (TMA) construction

For TMA construction, paraffin blocks of formalin-fixed oral SCC were retrieved from the pathology archives. A slide containing a representative portion of the tumor was selected and an area of the tumor was circled on the slide. With the use of a tissue microarrayer (Beecher Instruments, Silver Spring, MD, EUA), the area of interest in the donor block was cored twice with a 1.0 mm diameter needle and was transferred to a recipient paraffin block. Cores were arrayed onto three separate blocks that contained two tumor cores from each case. An adequate case was defined as a tumor occupying >90% of the core area.

Immunohistochemistry

TMA sections were placed on adhesive coated slides for immunohistochemistry (IHC). A standard peroxidase-conjugated streptavidin-biotin method was used to detect the staining reaction (LSAB+, DAKO, Carpinteria, USA) according to the manufacturer's protocol. The antibodies, dilutions, sources and positive controls are detailed in Table 2. Four-micrometer thick sections were cut from the array block, deparaffinized with xylene, and dehydrated through a series of graded alcohols. Microwave antigen retrieval was used for all antibodies and was performed by placing the slides in 10 mM citrate buffer (pH 6.0) for 15 min. For negative controls, the primary antibodies were omitted and PBS was used as a substitute.

Table 1 Clinicopathological characteristics of the 125 oral carcinomas patients

Variable	Category	TUMOR SITE			Total N° (%)	*P* value
		Gingival N° (%)	Hard Palate N° (%)	Alveolar ridge N° (%)		
Age	30–59 years	17 (53.1)	11 (35.5)	15 (24.2)	43 (34.4)	0.062
	60–69 years	9 (28.1)	8 (25.8)	22 (35.5)	39 (31.2)	
	70–91 years	6 (18.8)	12 (38.7)	25 (40.3)	43 (34.4)	
Gender	Male	27 (84.4)	20 (64.5)	29 (46.8)	76 (60.8)	0.002
	Female	5 (15.6)	11 (35.5)	33 (53.2)	49 (39.2)	
Clinical stage	I + II	7 (21.9)	2 (6.5)	12 (19.4)	21 (16.8)	0.196
	III + IV	25 (78.1)	29 (93.5)	50 (80.6)	104 (83.2)	
Tumor histological grade	SCC I	25 (78.1)	23 (74.2)	51 (83.6)	99 (79.8)	0.546
	SCC II e III	7 (21.9)	8 (25.8)	10 (16.4)	25 (20.2)	

Evaluation of stained sections

The staining was evaluated by light microscopic examination and was interpreted by two independent pathologists who were blinded to the available clinical information. The proteins were observed in the nucleus, membrane, and cytoplasm of tumor cells. Protein analysis was performed for 10 randomly selected fields viewed at high-power. A total of 100 tumor cells were counted from each field for each case and for all antibodies analyzed. The percentage of positive cells was calculated by dividing the number of positive tumor cells by the total of tumor cells counted. Staining was considered to be positive when >10% of the cells were positive, this cut off was based in previous studies [15, 16].

Statistical analysis

The survival curves were estimated using the Kaplan-Meier product-limit method, and the significant differences between the survival curves were determined using the log-rank test. The multivariate survival analysis was performed using the Cox proportional hazards model. Correlation coefficients between immunohistochemical expression and clinicopathological findings were estimated by Pearson correlation. Either the chi-square test or Fisher's exact test (two-sided) was performed to determine the correlation between protein expression and clinicopathological parameters. The results were considered statistically significant at $p < 0.05$. All statistical analyses were conducted using the SPSS 10.0 statistical software program (SPSS, Chicago, IL, USA).

Results

Expression of PI3K, p-AKT, p-mTOR, p-MAPK, and PTEN protein

The expression of p-AKT, p-mTOR, PTEN, PI3K, and Mitogen Activated Protein Kinases p-MAPK was observed in a considerable number of oral SCCs and these results are summarized in Table 3. For most of the antibodies tested, there were no statistically significant differences between immunostaining levels and tumor location in oral SCCs. Overall, 110 (92.4%) of the oral SCCs were positive for PI3K. We observed p-mTOR staining in the cytoplasm of the tumor cells in 105 (88.2%) cases, membrane staining in 53 cases (44.5%), and nuclear staining in 51 cases (42.9%). The majority of the cases (113 of 120) were positive for p-AKT in the cytoplasm of the tumor cells, and nuclear staining for this marker was observed in 77 (64.2%) of the cases. Cytoplasmic p-MAPK staining was found in 106 cases (88.3%), and 70 (58.3%) of the cases exhibited positive nuclear staining. PTEN expression was observed in the cytoplasm of the tumor cells in 30 cases (25%), and nuclear staining was observed in 35 (28%) cases. Figure 1 shows the positive oral SCC staining patterns for the markers studied by immunohistochemistry.

Association between p-AKT, p-mTOR, PTEN, PI3K, and p-MAPK expression and clinicopathological factors

The expression levels of all proteins studied were not significantly associated with clinicopathological features, and only the p-MAPK positive staining was

Table 2 Primary antibodies, clones, dilutions, sources, and positive controls used in immunohistochemistry

Antibodies	Clones	Dilution	Sources	Positive Controls
mTOR (phospho)	49F9	1:100	Cell-Signaling	Normal breast
PI3K	policlonal	Ready to use	Abcam	Placenta
MAPK p44/42 (phospho)	D13.14.45	1:50	Cell-Signaling	Normal breast
PTEN	6H2.1	1:500	Cascade	Normal thyroid
AKT (phospho)	587F11	1:25	Cell-Signaling	Colon Tumor

Table 3 Expression of the proteins studied by immunohistochemistry and association with tumor site of oral carcinomas

Variable	Category	TUMOR SITE			Total N° (%)	*P* value
		Gingival N° (%)	Hard Palate N° (%)	Alveolar ridge N° (%)		
PI3K	Negative	2 (6.7)	2 (6.9)	5 (8.3)	9 (7.6)	0.949
	Positive	28 (93.3)	27 (93.1)	55 (91.7)	110 (92.4)	
p-mTOR cytoplasm	Negative	2 (6.7)	5 (16.7)	7 (11.9)	14 (11.8)	0.485
	Positive	28 (93.3)	25 (83.3)	52 (88.1)	105 (88.2)	
p-mTOR membrane	Negative	15 (50.0)	22 (73.3)	29 (49.2)	66 (55.5)	0.075
	Positive	15 (50.0)	8 (26.7)	30 (50.8)	53 (44.5)	
p-mTOR nuclei	Negative	17 (56.7)	13 (43.3)	38 (64.4)	68 (57.1)	0.164
	Positive	13 (43.3)	17 (56.7)	21 (35.6)	51 (42.9)	
p-AKT cytoplasm	Negative	2 (6.7)	0 (0.0)	5 (8.3)	7 (5.8)	0.275
	Positive	28 (93.3)	30 (100.0)	55 (91.7)	113 (94.2)	
p-AKT nuclei	Negative	12 (40.0)	10 (33.3)	21 (35.0)	43 (35.8)	0.850
	Positive	18 (60.0)	20 (66.7)	39 (65.0)	77 (64.2)	
p-MAPK cytoplasm	Negative	2 (6.7)	3 (10.0)	9 (15.0)	14 (11.7)	0.483
	Positive	28 (93.3)	27 (90.0)	51 (85.0)	106 (88.3)	
p-MAPK nuclei	Negative	11 (36.7)	15 (50.0)	24 (40.0)	50 (41.7)	0.539
	Positive	19 (63.3)	15 (50.0)	36 (60.0)	70 (58.3)	

statistically associated with advanced tumors at clinical stages III and IV (P = 0.042).

The influence of overexpression of p-AKT, p-mTOR, PI3K, p-MAPK, and PTEN protein on survival in patients with oral SCC

Except for tumor histological grade (P = 0.001; Fig. 2), none of the clinicopathological features exhibited a significant influence on patient disease free survival analyses. In Cox's multiple regression analysis, patients with nuclear p-mTOR overexpression tumors had a significantly better rate of disease free survival than patients without nuclear p-mTOR overexpression (P = 0.037; Fig. 3). The results of univariate analysis of disease-free survival rate associated to clinicopathological and molecular parameters are summarized on Table 4.

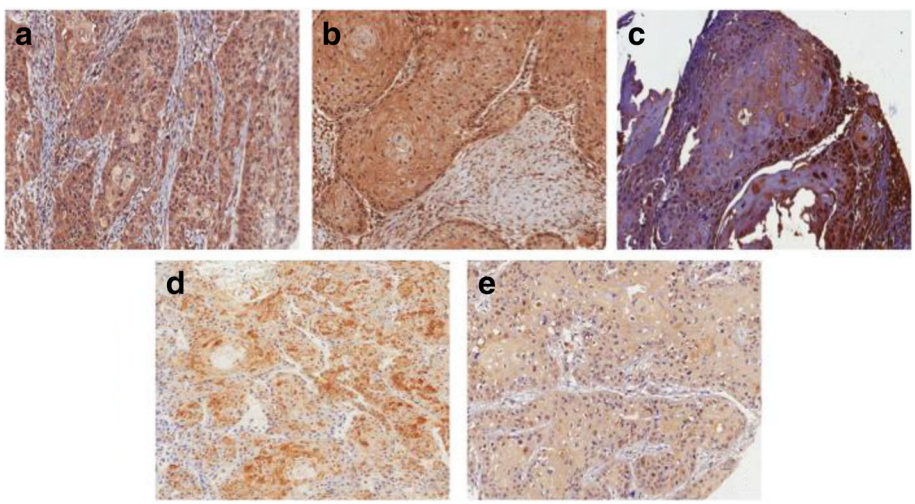

Fig. 1 Immunostaining of PI3K in oral SCC (**a**, original size 200×), pAKT overexpression (**b**, original 200×), p-MAPK (**c**, original size 200×), p-MTOR (**d**, original size 150×) and PTEN (**e**, original size 200×). The staining pattern is primarily in the membrane and/or cytoplasm of tumor cells (**a, b**, **d**), although the nuclear pattern can also be observed (**c**, **e**)

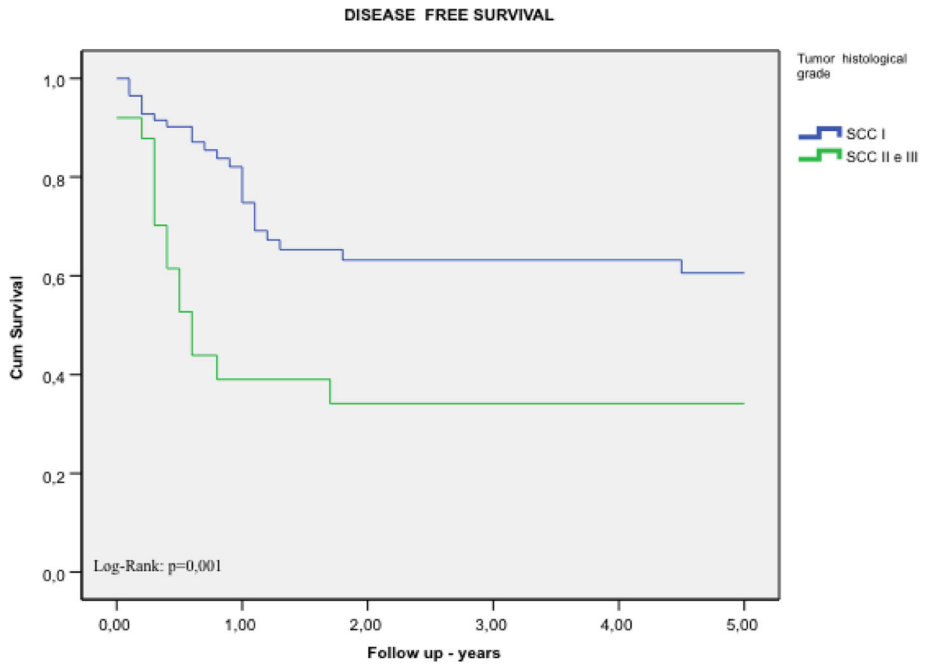

Fig. 2 Kaplan –Meier curve for the disease free survival rates in relation to tumor histological grade. The difference between the patients with SCC II e III (green line) and patients with oral SCC I (blue line) is statiscally significant (long-rank test, $p = 0.001$)

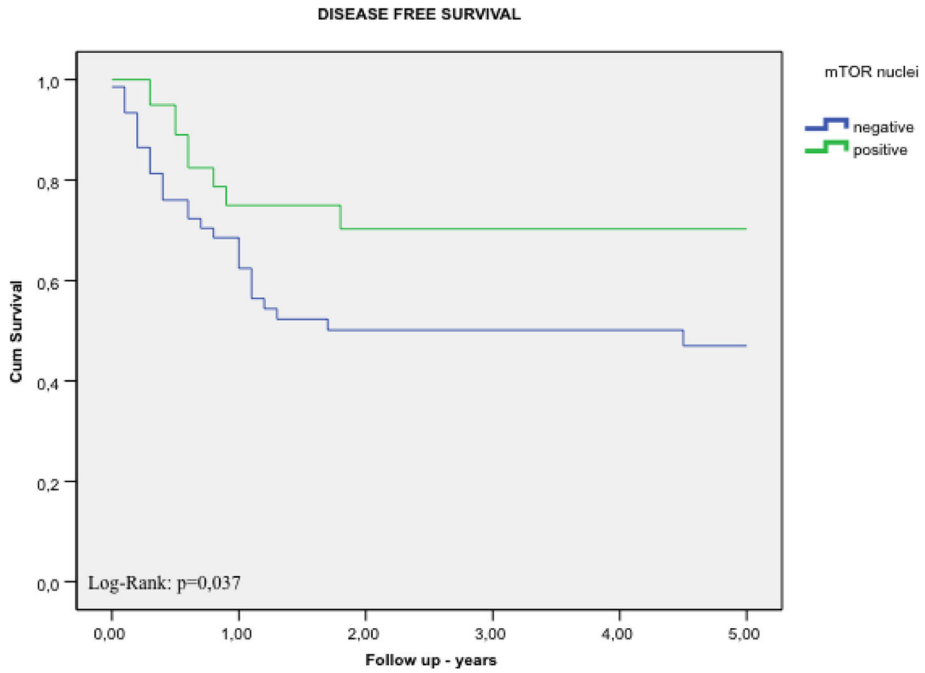

Fig. 3 Kaplan –Meier curve for the disease free survival rates in relation to p-MTOR nuclear expression. The difference between the patients with p-MTOR positive (green line) and patients with p-MTOR negative oral SCC (blue line) is statiscally significant (long-rank test, $p = 0.037$)

Table 4 Univariate analysis of disease-free survival rate according to clinicopathological findings and proteins expressions

Variable	Category	Probability of accumulated disease-free survival			*p** (log rank)
		1 year (%)	3 year (%)	5 years (%)	
Gender	Male	72.2	54.8	54.8	0.736
	Female	72.2	60.8	55.3	
Age	30–59 years	71.9	53.4	53.4	0.910
	60–69 years	76.8	58.5	58.5	
	70–91 years	67.4	61.7	49.4	
Tumor histological grade	SCC I	82.0	63.2	60.6	0.001
	SCC II e III	39.0	3.2	34.2	
Clinical Stage	I + II	70.4	70.4	70.4	0.457
	III + IV	72.5	53.6	51.0	
PI3K	Negative	83.3	83.3	83.3	0.157
	Positive	70.1	56.0	53.7	
p-mTOR cytoplasm	Negative	77.4	58.0	58.0	0.893
	Positive	70.5	57.4	54.9	
p-mTOR membrane	Negative	75.2	55.5	51.5	0.745
	Positive	67.3	60.7	60.7	
p-mTOR nuclei	Negative	68.5	50.1	47.0	0.037
	Positive	75.0	70.3	70.3	
p-MAPK cytoplasm	Negative	92.9	66.5	66.5	0.360
	Positive	68.1	56.3	53.9	
p-MAPK nuclei	Negative	73.6	60.6	53.9	0.837
	Positive	70.7	55.8	55.8	
p-AKT cytoplasm	Negative	85.7	68.6	68.6	0.568
	Positive	70.6	56.7	54.5	
p-AKT nuclei	Negative	67.9	59.9	59.9	0.923
	Positive	74.4	56.8	53.8	
PTEN cytoplasm	Negative	84.9	74.3	66.0	0.152
	Positive	66.6	51.0	51.0	
PTEN nuclei	Negative	84.4	70.3	62.5	0.228
	Positive	66.6	51.0	51.0	
TOTAL		72.3	57.0	54.9	

Discussion

The identification of prognostic and predictive markers is clinically important because oral cancer is a group of heterogeneous diseases with various biological and clinical characteristics.

In recent years, the PI3K/AKT/mTOR signaling pathway has been shown to be a key regulator of cell cycle proliferation, growth survival, protein synthesis, and glucose metabolism [2, 6, 17]. The significance of this pathway in cancer is underscored by abundant evidence suggesting that multiple components of this pathway are frequently deregulated and serve as molecular targets in cancer development and progression [18].

Exploring the regulation of the PTEN/PI3K pathway in cancer will lead to insight into the malignant behavior of tumor cells. Such information will be useful for the optimization of rationally designed cancer therapies [5, 19].

Few studies exist regarding the expression of the signaling proteins of the PI3K/AKT/mTOR pathway in head and neck SCC. Several mutations were identified in this pathway, including PI3K (8–10%), mTOR (5–8%), and PTEN (23%) [20–22]. AKT is known to be activated in the majority of head and neck SCC tumor tissue and cell lines and its active form is detected in 50% of preneoplastic lesions [23, 24].

Our study is the first report to show the expression of PI3K/AKT proteins in gingival, hard palate, and alveolar ridge SCCs. We observed a high frequency of positive staining for PI3K, p-AKT, p-mTOR, and p-MAPK in oral SCCs, including gingival, hard palate, and alveolar ridge SCCs. However, the majority of our cases showed loss of PTEN expression, suggesting that activation of PI3K/AKT pathway occurred downstream of PTEN. A possible role for PTEN loss in the pathogenesis of head and neck SCC has been described by Califano et al. in a stepwise model of carcinogenesis [25]. The majority of PTEN gene dysfunction has been attributed to mutation, loss of heterozygosity (LOH), and epigenetic silencing [24]. As we presumed in our cases, these disorders lead to a decrease or loss of protein expression, thus providing a rationale for defective PTEN detection by immunohistochemistry.

The prognosis in oral carcinomas varies according to the tumor location. Local and regional lymph node involvement is the most important prognostic factor. An increased risk is observed with larger tumors, whereas poorly differentiated tumors metastasize more frequently than well-differentiated tumors [3]. Similarly, the only clinicopathological factor associated with patient prognosis in our study was tumor grade. We found that poor prognosis was associated with poorly differentiated oral SCC. Some molecular indicators of prognosis have been observed in oral SCCs. The overexpression of Epidermal growth factor receptor (EGFR) and Transforming growth factor alpha (TGF-α) is associated with a decrease in the disease-free and cancer specific survival rates [26]. Cyclin D1 overexpression and lack of p16 expression correlate with a worse prognosis, increased recurrence, and poor survival rates in oral cancer [27]. Overexpression of bcl-2 and loss of PTEN expression were correlated with poorly differentiated tumors, lymph node involvement, and late stages in oral SCC [28]. We found that PTEN protein expression was lost in more than 70% of the tumor cells. In a recent study, the authors found 56% of oral SCC lacked PTEN protein expression and associated this finding with pathogenesis and carcinogenesis of oral SCC [28].

Our study shows that p-mTOR nuclear expression was significantly related to overall survival. The nuclear import of mTOR has an important role in activating its cytoplasmic signaling. However, the mechanism of nuclear transportation of mTOR and the function of nuclear mTOR remain unclear. Rapamycin is a macrolide antibiotic and an immunosuppressive agent that inhibits mTOR. Its anti-proliferative effect is mediated through the formation of an active complex. Rapamycin also suppresses angiogenesis by decreasing the production of vascular endothelial growth factor [29]. In accordance with our results, a recent study has shown that adverse outcomes are associated with high expression of mTOR in a subset of 72 oral SCCs [30]. Indeed, mTOR inhibitors have shown promising efficacy rates in patients with renal cell and gastric carcinomas [31, 32].

Conclusion

We have evaluated p-AKT, p-mTOR, p-MAPK, PI3K, and PTEN expression by immunohistochemistry and observed that the PI3K/AKT pathway is activated in gingival, hard palate, and alveolar ridge SCCs. Our data show that p-mTOR expression can function as a biomarker of survival in oral SCCs and could be a promising therapeutic target in oral SCC.

Abbreviations

AKT: Protein kinase B; EGFR: Epidermal growth factor receptor; IHC: Immunohistochemistry; LOH: Loss of heterozygosity; MAPK: Mitogen Activated Protein Kinases; mTOR: Mammalian target of rapamycin; PI3K: Phosphatidylinositol 3 kinase; PIP2: Phosphatidylinositol-4, 5-bi-phosphate; PIP3: Phosphatidylinositol-3, 4, 5-triphosphate; PTEN: Phosphatase and tensin homolog deleted on chromosome 10; SCC: Squamous cell carcinoma; TGF-α: Transforming growth factor alpha; TMA: Tissue microarray

Acknowledgements

This work was funded by FAPESP, Grant 2012/03260-0.

Funding

This work was supported by Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (FAPESP, Grant 2012/03260–0).

Availability of data and materials

Not applicable.

Authors' contributions

DMF, TJN, LGCA, FAA, MDB contributed equally to the discussion, writing, and revision of the manuscript. TJN, MDB did the final revision and submission. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

I confirm that none of the authors have any competing interests in the manuscript.

Publisher's Note

Author details

[1]Departament of Pathology, A C Camargo Cancer Center, Rua Professor Antonio Prudente 211, Liberdade, São Paulo ZIP 01509-900, Brazil. [2]Department of stomatology, A C Camargo Cancer Center, Sao Paulo, Brazil.

Received: 12 July 2017 Accepted: 25 September 2017
Published online: 30 November 2017

References

1. Boyle P, Macfarlane GJ, Maisonneuve P, Zheng T, Scully C, Tedesco B. Epidemiology of mouth cancer in 1989: a review. J R Soc Med. 1990;83(11):724–30.

2. Simpson D, Mell L, Cohen E. Targeting the PI3K/AKT/mTOR pathway in squamous cell carcinoma of the head and neck. Oral Oncol. 2014;51(4):291–8.
3. Das BR, Nagpal JK. Understanding the biology of oral cancer. Med Sci Monit. 2002;8(11):RA258–67.
4. Li Q, Wu D, Liu WW, Li H, Liao WG, Zhang XR, et al. Survival impact of cervical metastasis in squamous cell carcinoma of hard palate. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013;116(1):23–7.
5. Williams MD. Integration of biomarkers including molecular targeted therapies in head and neck cancer. Head Neck Pathol. 2010;4(1):62–9.
6. Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nat Rev Cancer. 2002;2(7):489–501.
7. Smolensky D, Rathore K, Bourn J, Cekanova M. Inhibition of the PI3K/AKT Pathway Sensitizes Oral Squamous Cell Carcinoma Cells to Anthracycline-Based Chemotherapy In Vitro. J Cell Biochem 2016; doi:10.1002.
8. Hay N, Sonenberg N. Upstream and downstream of mTOR. Genes Dev. 2004;18(16):1926–45.
9. Radu A, Neubauer V, Akagi T, Hanafusa H, Georgescu MMPTEN. induces cell cycle arrest by decreasing the level and nuclear localization of cyclin D1. Mol Cell Biol. 2003;23(17):6139–49.
10. Bussink J, van der Kogel AJ, Kaanders JH. Activation of the PI3-K/AKT pathway and implications for radioresistance mechanisms in head and neck cancer. The lancet oncology. 2008;9(3):288–96.
11. Abrahao AC, Giudice FS, Sperandio FF, Pinto Junior DD. Effects of celecoxib treatment over the AKT pathway in head and neck squamous cell carcinoma. Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology. 2013;42(10):793-8.
12. Squarize CH, Castilho RM, Abrahao AC, Molinolo A, Lingen MW, Gutkind JSPTEN. deficiency contributes to the development and progression of head and neck cancer. Neoplasia. 2013;15(5):461–71.
13. Holsinger FC, Piha-Paul SA, Janku F, Hong DS, Atkins JT, Tsimberidou AM, et al. Biomarker-directed therapy of squamous carcinomas of the head and neck: targeting PI3K/PTEN/mTOR pathway. J Clin Oncol. 2013;31(9):e137–40.
14. El-Naggar A, Chan J, Grandis J, et al. WHO Classification of Head and Neck Tumors. 4.ed. Lyon: International Agency for Research on Cancer. 2017;109.
15. Lee HS, Cho SB, Lee HE, et al. Protein expression profiling and molecular classification of gastric cancer by the tissue array method. Clin Cancer Res. 2007;13(14):4154–63.
16. Begnami MD, Fregnani JH, Brentani H, et al. Identification of protein expression signatures in gastric carcinoma using clustering analysis. J Gastroenterol Hepatol. 2012;27(2):378–84.
17. Horn D, Hess J, Freier K, et al. Targeting EGFR-PI3K-AKT-mTOR signaling enhances radiosensitivity in head and neck squamous cell carcinoma. Expert Opin Ther Targets. 2015;19(6):795–805.
18. Bellacosa A, Kumar CC, Di Cristofano A, Testa JR. Activation of AKT kinases in cancer: implications for therapeutic targeting. Adv Cancer Res. 2005;94:29–86.
19. Parsons R. Human cancer, PTEN and the PI-3 kinase pathway. Semin Cell Dev Biol. 2004;15(2):171–6.
20. Stransky N, Egloff AM, Tward AD, Kostic AD, Cibulskis K, Sivachenko A, et al. The mutational landscape of head and neck squamous cell carcinoma. Science. 2011;333(6046):1157–60.
21. Poetsch M, Lorenz G, Kleist B. Detection of new PTEN/MMAC1 mutations in head and neck squamous cell carcinomas with loss of chromosome 10. Cancer Genet Cytogenet. 2002;132(1):20–4.
22. Shao X, Tandon R, Samara G, Kanki H, Yano H, Close LG, et al. Mutational analysis of the PTEN gene in head and neck squamous cell carcinoma. Int J Cancer. 1998;77(5):684–8.
23. Molinolo AA, Hewitt SM, Amornphimoltham P, Keelawat S, Rangdaeng S, Meneses Garcia A, et al. Dissecting the Akt/mammalian target of rapamycin signaling network: emerging results from the head and neck cancer tissue array initiative. Clin Cancer Res. 2007;13(17):4964–73.
24. Amornphimoltham P, Sriuranpong V, Patel V, Benavides F, Conti CJ, Sauk J, et al. Persistent activation of the Akt pathway in head and neck squamous cell carcinoma: a potential target for UCN-01. Clin Cancer Res. 2004; 10(12 Pt 1):4029–37.
25. Califano J, van der Riet P, Westra W, Nawroz H, Clayman G, Piantadosi S, et al. Genetic progression model for head and neck cancer: implications for field cancerization. Cancer Res. 1996;56(11):2488–92.
26. Grandis JR, Tweardy DJ. Elevated levels of transforming growth factor alpha and epidermal growth factor receptor messenger RNA are early markers of carcinogenesis in head and neck cancer. Cancer Res. 1993;53(15):3579–84.
27. Bova RJ, Quinn DI, Nankervis JS, Cole IE, Sheridan BF, Jensen MJ, et al. Cyclin D1 and p16INK4A expression predict reduced survival in carcinoma of the anterior tongue. Clin Cancer Res. 1999;5(10):2810–9.
28. Rahmani A, Alzohairy M, Babiker AY, Rizvi MA, Elkarimahmad HG. Clinicopathological significance of PTEN and bcl2 expressions in oral squamous cell carcinoma. Int J Clin Exp Pathol. 2012;5(9):965–71.
29. Guba M, von Breitenbuch P, Steinbauer M, Koehl G, Flegel S, Hornung M, et al. Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. Nat Med. 2002;8(2):128–35.
30. Monteiro LS, Delgado ML, Ricardo S, Garcez F, do Amaral B, Warnakulasuriya S, et al. Phosphorylated mammalian target of rapamycin is associated with an adverse outcome in oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013;115(5):638–45.
31. Motzer RJ, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, et al. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. Lancet. 2008;372(9637):449–56.
32. Shitara K, Muro K, Ura T, Takahari D, Yokota T, Sawaki A, et al. Chemotherapy for gastric cancer that recurs after adjuvant chemotherapy with S-1. Jpn J Clin Oncol. 2008;38(11):786–9.