

## SPECIAL ARTICLE

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# mTOR deregulation in oral cavity squamous cell carcinoma

Nicholas S. Mastronikolis<sup>1</sup>, Evangelos Tsiambas<sup>2</sup>, Theodoros A. Papadas<sup>1</sup>, Panagiotis P. Fotiades<sup>3</sup>, Athanasios T. Papadas<sup>4</sup>, Stylianos N. Mastronikolis<sup>5</sup>, Ioannis Kastanioudakis<sup>6</sup>, Vasileios Ragos<sup>7</sup>

<sup>1</sup>Department of Otorhinolaryngology, Head and Neck Surgery, Medical School, University of Patras, Patras, Greece;

<sup>2</sup>Department of Immunohistochemistry & Molecular Biology, 401 GAH, Athens, Greece; <sup>3</sup>Department of Surgery, Leicester General Hospital, Leicester, UK; <sup>4</sup>Department of Hematology and Oncology, University of Wisconsin-Madison School of Medicine, Madison, WI, USA; <sup>5</sup>Department of Medicine, School of Health Sciences, University of Crete, Heraklion, Greece;

<sup>6</sup>Department of Otolaryngology, Head and Neck Surgery, University Hospital of Ioannina, Ioannina, Greece; <sup>7</sup>Department of Maxillofacial and Department of Medicine, School of Health Sciences, University of Ioannina, Ioannina, Greece

## Summary

Signal transduction pathways consist of a variety of inter- and intra-cellular molecules. They act as supporting mechanisms for cell survival and homeostasis. Among them, the phosphatidylinositol 3-kinase (PI3K)/tumor suppressor phosphatase and tensin homologue deleted on chromosome ten (PTEN)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) pathway plays a crucial role in regulating normal cell growth based on growth factor receptors (GFRs) interaction, including epidermal GFR (type II-HER2) and insulin GFR (IGF). mTOR protein acts as a serine-threonine kinase that belongs to the PI3K-related kinase family. It mediates protein and lipid synthesis, mitochondrial metabolism, biogenesis, proliferation and also negatively regulates autophagy. Two distinct multiprotein complexes

have been mainly identified and cloned: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTOR is deregulated predominantly due to mutations, deletions, loss of heterozygosity (LOH) or abnormal phosphorylation of the upstream molecules inside the current pathway. Pure mTOR mutations are very rare. Development of specific inhibitors at the basis of targeted therapeutic strategies such as rapamycin (rapalogs) is an evolution in handling patients with mTOR abnormal overactivity.

In the current special article we explored the role of the gene deregulation leading to abnormal protein expression in oral cavity squamous cell carcinoma (SCC).

**Key words:** carcinoma, gene, mTOR, oral, signaling pathway

## Introduction

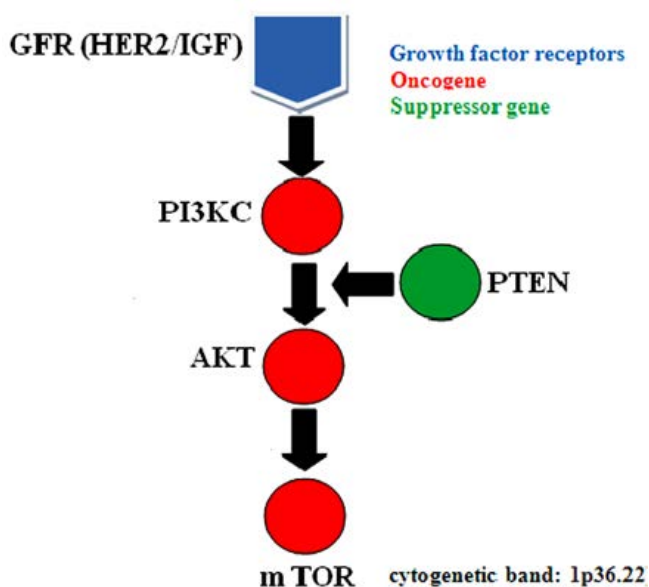
Understanding the exact role of signal transduction from the extracellular domain of the plasma membrane to the nucleus is a significant evolution in modern molecular biology. Based on extensive analyses, GFRs-dependent signaling pathways are critically involved in regulating the normal cell growth, proliferation and metabolism, securing tissue homeostasis [1]. These pathways demonstrate a chain-like cascade of molecules that act as inducers or inhibitors in the signal transduction process. Under specific abnormal

gene modifications - including point mutations, deletions, promoter abnormal methylation, and amplification or overphosphorylation - the corresponding protein products transform to oncogenes or non-functional suppressor genes [2]. Deregulation in one or more of them enhances and amplifies drastically the signal transduction to the nucleus desynchronizing the cell cycle, partially the normal apoptotic procedure and in combination with other genetic events lead the cell to a neoplastic and finally to a malignant (cancer-

ous) transformation.

Among those molecules, mTOR seems to be a very promising marker for handling patients with solid malignancies, especially at the basis of applying targeted therapeutic strategies. The protein is a main part of the PI3K/PTEN/Akt/mTOR pathway [3]. HER2/neu and also IGF receptors demonstrate, as downstream molecules, the previously referred proteins [4]. Interestingly, mTOR is involved also in the WNT and tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) pathways [5]. Deregulation at any level of PI3K/Akt/PTEN/mTOR route is a frequent genetic abnormality in a variety of solid malignancies (Figure 1). In particular, silence of PTEN suppressor gene combined with overactivation of the other oncogenes negatively influences the cell microenvironment, modifying the mitochondrial and nuclear activity.

In the current study we analyzed the crucial role of mTOR gene/protein in normal intracellular



**Figure 1.** The PI3K/Akt/PTEN/mTOR signaling transduction pathway.

functions, exploring also its deregulation's impact in oral cavity malignancies.

### Introducing the mTOR gene and protein

The mTOR gene that encodes for the corresponding protein (289 kDa) is located on chromosome 1 (cytogenetic band: 1p36.22). The molecule is a member of phosphoinositide 3-kinase related kinase family acting as a downstream serine-threonine kinase. It comprises mainly two distinct

multiprotein complexes: mTORC1 and mTORC2 [6]. In both of them, mTOR is the catalytic subunit of the complex. mTORC1 includes raptor and mammalian LST8 (mLST8) domains and also other two components: the proline-rich AKT substrate 40 (PRAS40) and DEP-domain-containing (DEPTOR). The clear function of those subunits is under investigation, whereas it seems that they represent positive and negative regulators in assembling the complex by recruiting substrates and binding them to the main catalytic subunit. mTORC1 is a significant mediator in a variety of intracellular functions affecting the communication inside a network that involves nucleus, mitochondria and production organelles. The complex induces main metabolic activities (anabolism) including lipid and protein biosynthesis. Concerning lipids, the molecule induces the levels and the activity of sterol regulatory element binding protein 1 (SREBP1) and also of peroxisome proliferator-activated receptor-g (PPARg) [7]. Both of these agents act as transcription factors in regulating lipid and cholesterol homeostasis. Similarly, protein synthesis is mediated by phosphorylating two critical factors: the eukaryotic initiation factor 4E (eIF4E)- binding protein 1 (4E-BP1) and the p70 ribosomal S6 kinase 1 (S6K1). Based on their increased activity, mTORC1 upregulates the mRNA and also the ribosomal RNA (r-RNA) one [8]. Additionally, the molecule regulates mitochondrial metabolism and biogenesis by enhancing its membrane potential. This modification leads to an elevated oxygen consumption promoting higher ATP levels. Furthermore, amplified mitochondrial DNA (increased copy number in specific genes), which leads to overexpression of specific proteins participating in oxidative metabolism, seems to correlate also with mTORC1 activation. Concerning autophagy, some studies have shown that the molecule is a negative regulator in this catabolic procedure. This activity is released by phosphorylating and thereby downregulating a protein complex consisting of composed of unc-51-like kinase 1 (ULK1), autophagy-related gene 13 (ATG13) and focal adhesion kinase family-interacting protein of 200 kDa (FIP200). Finally, mTORC1 is implicated in feedback loops based on energy deposits as AMP-activated protein kinase (AMPK) and also on oxygen levels. In fact, AMPK activation and hypoxia represses the molecule's expression [9]. Similarly, DNA damage which leads to genotoxic stress, activates p53 dependent AMPK, reducing indirectly mTORC1 expression. In contrast to this, inflammatory signals upregulate its expression as

a response to proinflammatory cytokines (TNF- $\alpha$ ) activation inducing also angiogenesis, especially in neoplasms and malignant tissue formations.

mTORC2 complex includes six different subunits sharing some of them with mTORC1. It consists of the basic mTOR protein, protein observed with Rictor-1 (Protor-1), mLST8, DEPTOR, rapamycin-insensitive companion of mTOR (Rictor) and mammalian stress-activated protein kinase interacting protein (mSIN1). mTORC2 is involved in the regulation of many intracellular activities, such as cytoskeletal organization due to inducing actin polymerization and PKCa – paxillin phosphorylation [10]. It is also implicated in cell proliferation, metabolism, ribosome interaction, and cell survival. Cell migration activity seems to be an alternative extracellular procedure that the protein positively affects.

It is well established that the mTOR whole protein complex regulates energy balance, adipogenesis, oxidative metabolism, muscle mass, insulin secretion, and glucose homeostasis in different tissues. Its deregulation influences negatively the progress of metabolic diseases, including type II diabetes (as a result of insulin resistance) [11]. Another potentially significant role of the protein is referred to aging process regulation. Some experimental studies have shown that inhibition of mTOR activity induces life extension in yeasts, worms, flies, even in mice. Although the exact mechanism is under investigation, it is considered that the molecule plays a crucial role in modifying cellular responses to nutrient availability [12].

### **mTOR deregulation in oral SCC: targeting the abnormal protein**

GFRs/PI3K/AKT/PTEN/mTOR pathway is frequently destabilized in many carcinomas of different histogenetic origin. In fact, mTORC2 complex is directly involved in the current pathway, whereas mTORC1 complex interacts with RAS/RAF/MEK/ERK ones. Overexpression of mTOR protein results mainly as a coincidence of upstream oncogene overactivation combined with suppressor gene downregulation, whereas its pure mutations are very rare. Concerning oral cavity SCC, there are limited data in investigating the complete mechanism of its alterations. Based on semi-quantitative RT-PCR analysis, a study group suggested that LOH and promoter methylation are two important mechanisms for downregulation of suppressor PTEN and TSC genes, leading to an indirect abnormal activation of the mole-

cule [13]. Similarly, amplifications and mutations of the PIK3CA oncogene in oral cancer combined with suppressor gene silencing provide the suitable substrate for mTOR overexpression [14]. Another important observation is the pattern of mTOR expression detected by immunohistochemistry (IHC). A study showed that patients with nuclear phosphorylated (p)-mTOR expression in extranodal tumors had significantly worse regional and distant disease control. Multivariate analysis also confirmed that nuclear p-mTOR expression in extranodal tumors was a significant independent adverse factor. It seems that p-mTOR expression can be used as a prognostic indicator predictive of 5-year disease-free survival (DFS) and 5-year overall survival (OS) in advanced oral SCC patients with extranodal extension (ENE) [15]. Interestingly, the recently cloned neuron-restrictive silencer factor/repressor element 1-silencing transcription factor (NRSF/REST) which acts as a transcriptional repressor of neuronal genes in non-neuronal cells seems to correlate with mTOR expression in oral SCC. A study group concluded that downregulation of NRSF/REST by siRNAs disrupted the mTOR signaling pathway in a series of oral SCC KB cell lines affecting negatively their survival [16].

Rapamycin - a macrolide produced by *Streptomyces Hygroscopius* bacteria (sirolimus) - is the most renowned inhibitor of mTOR altered expression. Other analogues (rapalogues) include everolimus and also temsirolimus. Although suppressing the current pathway, loss of PTEN function combined or not with the presence of numerous negative feedback loops in the mTOR pathway influence critically their efficacy [17]. Additionally, there is a skepticism regarding the resistance of carcinomas to first-generation rapalogues and also to second-generation mTOR-kinase inhibitors (TORKi) due to pure activating mTOR mutations. According to another study, development and implementation of the third-generation mTOR inhibitors could allow inhibition of these resistant mutants [18]. Furthermore, a significant result in exploring the role of combined targeted therapeutic strategies in oral SCC was observed regarding mTOR and PD-L1 inhibition. A study group showed that PD-L1 monoclonal antibody (mAb) enhanced durable primary tumor control and survival when combined with rapamycin in syngeneic models of oral carcinomas [19]. In the same study, rapamycin increased also IFN $\gamma$  production capacity in peripheral and tumor-infiltrating CD8 T cells in contrast to com-

mon idea that it mainly acts as an immunosuppressant factor. Based on this modern aspect of rapamycin multiple functionality, another study group investigated the influence of a newly developed small molecular ATP-competitive inhibitor of mTORC1 and mTORC2 kinase (AZD2014). They observed that AZD2014 synergized with radiation, increased the apoptotic level of cancer cells and also induced tumor cell cycle arrest at the G1 and G2/M phases, leading to disruption of cyclin D1-CDK4 and cyclin B1-CDC2 complexes. For this reasons, AZD2014 is considered as a very promising agent that should play a crucial role in inhibiting AKT/mTOR axis in oral SCC [20].

In conclusion, PI3K/AKT/PTEN/mTOR pathway deregulates frequently in oral carcinomas. mTOR molecule plays a significant role by its overactivation in the corresponding cancer cells by amplifying the signal transduction process to the nucleus. Novel targeted therapeutic regimens are based on a combination of current or next-generation rapalogues and new agents such as PD-L1 mabs and AZD2014 that disrupt the cascade of reactions inside the pathway in different levels.

### Conflict of interests

The authors declare no conflict of interests.

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