



## 26\_PI3K/AKT/mTOR and ERK/MAPK pathways as key targets in oral cancer microenvironment

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**Background:** Head and neck cancers (HNC) represent the sixth most common cancer worldwide being diagnosis 664 000 new cases *per year* being oral cancer the most common. Over 95 % of oral cavity cancers are squamous cell carcinomas (OSCC). Despite the easy access of oral cavity, OSCC continues being diagnosed in a late stage, with an estimated survival rate for 5 years of 50%, being urgent the identification of diagnosis, prognosis and treatment targets. The oral cavity anatomy is particularly challenging since there are many different types of tissue located in this relatively small area. Considering these, the interplay between tumor cells and stroma structure has been receiving growing attention as it can contribute for malignant cell transformation. The phosphatidylinositol 3-kinase(PI3K)/protein kinase B(AKT)/mammalian target of rapamycin(mTOR) and the extracellular signal-regulated kinase(ERK)/mitogen-activated protein kinase(MAPK) pathways regulate cell survival, proliferation, and motility and can extensively converge to both positively and negatively regulate each other.

**Aim:** To understand the role of PI3K/AKT/mTOR and ERK/MAPK in oral cancer microenvironment, namely in the reciprocal interplay between malignant cells and stromal structure, in order to identify new diagnosis and therapeutic targets for OSCC.

**Material & Methods:** The human OSCC cell line, HSC-3, was cultured in order to establish mice models xenotransplants. For this, Balb/c nu/nu mice were inoculated with approximately 3, 15 or 30 million cells on tongue, jugal submucosa and dorsal side subcutaneously (back), respectively (4 mice *per location*) (Of. 3-CE-2011 FMUC, Portugal). During 3-7 weeks, the Balb/c nu/nu mice were supervised and after this period, they were killed by anesthetic overdose. Tumor nodules and involving tissue were excised for *ex vivo* studies. The histology was assessed using the hematoxylin-eosin double staining and the immunophenotype was evaluated by immunohistochemistry (IHC) (epithelial phenotype: low molecular weight cytokeratins AE1/AE3 and high molecular weight CK5.6.18/LP34; myoepithelial phenotype: P63; mesenchymal phenotype: Vimentin and  $\alpha$ -Actin; proliferative index: Ki67). The expression of phospho(p)-mTOR was also evaluated by IHC. For genetic studies the samples were Purezol<sup>®</sup> processed and the obtained RNA was converted to cDNA and a 48 array RT-PCR for PI3K/AKT/mTOR pathway specific for human was applied.

**Results:** We established and developed the three xenotransplant mice models with a success rate of 75% for tongue, jugal submucosa and back, according to histologic studies. Our results showed that depending on the inoculation/implantation site HSC-3 tumor cells present different gene expression and immunophenotype profiles as well as different



composition in keratinizing cells, large cells and basal/intermediate cells. Comparing to the other models, tongue localization showed increased IHC expression of AE1/AE3, Vimentin and pmTOR. The Ki67 index was higher in large and basal cells in all xenotransplant models. The gene expression profile analysis showed that the PI3K/AKT/mTOR pathway has a different transcription regulation according to the site of tumor implantation. The jugal submucosa xenotransplant model showed increased gene expression levels of *AKT3*, *PDK1*, *PDK2*, *PRKCA*, *IGF1R*, *MAPK3/ERK1*, *CTNNB1* (1.5x higher). We also verified a decrease in apoptotic *FASL* gene expression and in *NFKB1A* (0.5x higher). These results together with the ICH unstaining of pmTOR, suggest that in jugal submucosa location MAPK/ERK pathway has a critical role in carcinogenesis.