POSTER - RNA AND TRANSCRIPTOMICS

AN INTEGRATED IN SILICO - IN VITRO APPROACH TO CHARACTERIZE PRIMARY CILIUM AND HEDGEHOG SIGNALING IN ORAL SQUAMOUS CARCINOMA.

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Introduction. Oral squamous cell carcinoma (OSCC) is the most common oral malignancy, and the pathogenesis and cellular biology need to be better understood. Primary cilium (PC) are microtubule-based organelles that regulate signaling pathways in solid tumors, integrating signals from the microenvironment, including the Hedgehog pathway (Hh). The Hh is linked to cancer stem cell maintenance and regulation of gene expression related to the cell cycle and epithelial-mesenchymal transition. Evidence indicates that PC disruption can have varying effects on cancer progression, depending on the dysregulation of Hh signaling. However, research on PC in the context of OSCC is limited, despite indications of Hh pathway involvement. Goals. This work aims to predict and characterize PC and Hh pathway dynamics in OSCC using a combined in silico and in vitro approach. Methods. RNA-seq data was

downloaded from the TCGA database and validated with GEO data. The Limma R package was applied to screen DEGs, comparing normal and cancerous tissues. To identify gene modules with similar expression patterns, WGCNA was used. The obtained modules were correlated with the clinical feature (stage), and the main modules were selected for further analyses to investigate critical genes (hubs) related to the Hh and ciliogenesis pathways. Gene functional enrichment analysis was performed using Cytoscape. Kaplan–Meier curves were used to compare the clinical outcomes of the subgroups. For in vitro validation, 17 human OSCC samples were evaluated by immunohistochemistry and RT-qPCR. In addition, immunofluorescence and western-blot analyses were performed on the metastatic HSC3 cell line to determine cellular localization and expression of the investigated proteins. Results. We identified a total of 2179 DEGs, and using WGCNA, we selected the three modules most correlated with the disease, highlighting two containing highly related hub genes to the interest pathways. Highlight AURKA, KIF3A, BORA, GLI1, and IFT88 among these hub genes in the main modules. These genes demonstrated a significant association with patients' low survival rates, involvement in cytoskeleton organization, and regulation of microtubule and cell cycle. Immunohistochemical analysis divided the human OSCC samples into GLI1 positive and negative cases. Acetylated a-tubulin expression and the Ki-67 proliferation marker were detected in all OSCC tumor samples, regardless of GLI1-positive classification. Furthermore, RT-qPCR analysis noticed a high expression of the GLI1 gene in six OSCC samples. Higher PTCH1, SMO, and BORA transcript levels were observed in the OSCC group with GLI1 overexpression. Other investigated genes (AURKA, KIF3A, IFT88, and CCND1) showed similar mRNA expression levels regardless of GLI1 transcript levels. In addition, immunofluorescence assays demonstrated co-localization of acetylated a-tubulin protein with SMO and GLI1 in HSC-3 cells with assembled PC and without this assembled organelle. Western-blot also confirmed acetylated a-tubulin and GLI1 expression in HSC-3 cells. Conclusions. Altogether, these integrated analyses suggest that PC presence in tissues and oral cancer cells may not be essential for tumor cell proliferation and Hh pathway activation. These results provide important insights into PC and Hh mechanisms in tumor progression, impacting the survival of OSCC patients and revealing new therapeutic opportunities in this field.

Palavras-chave: bioinformatics; oral squamous cell carcinoma; hedgehog pathway and primary cilium.