Whole exome sequencing analysis of *NOTCH* pathway gene mutations in salivary mucoepidermoid carcinomas

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BACKGROUND: Salivary mucoepidermoid carcinoma is the most common type of malignant salivary gland tumor and a subset of these carcinomas (ranging from 55 to 70%) have been frequently associated with chromosomal translocation t(11;19)(q21–22;p13). A consequence of this translocation is the creation of a new hybrid protein resulting from the fusion of the coding sequences of the genes *MAML2* and *CRTC1*.
HYPOTHESIS: As MAML proteins are important Notch co-activators and the Notch signaling pathway is involved in a number of key cellular processes, such as cell differentiation, growth and survival, we speculate that Notch signaling plays an important role in the origin of these tumors. The present study aimed to investigate the prognostic impact of mutations in Notch pathway genes in salivary mucoepidermoid carcinomas regardless of the fusion presence.
METHODS: Mutations were evaluated in DNA derived from a set of 11 samples, composed of 8 independent and three normal/tumor primary salivary mucoepidermoid carcinomas pairs. Samples were collected during surgery, before chemotherapy and/or radiotherapy. Whole Exome Sequencing was performed using the Ion Torrent TargetSeq kit. Single Nucleotide Variants (SNVs) as well as insertions or deletions of bases in DNA (InDels) were detected by GATK (variant database; dbSNP and 1000G) after mapping to UCSC Hg19. Genes involved in Notch signaling pathway, selected from KEGG pathway database, were investigated including those encoding proteins related to apoptosis, cell cycle, neurogenesis, transcription-regulation, as well as cell proliferation and differentiation. Genes from signaling pathways that crosstalk with Notch pathway were also included.
RESULTS: We generated an average of 70 million reads/sample, which led to at least 80% of the exome covered at least 50x. Whereas the most common alterations identified were missense mutations in genes such as *PTCRA, NOTCH1, NOTCH4, JAG2, PPARG, ERBB2*, splice-site mutations were only observed in the *NOTCH2* gene. Although less frequently altered, MAML1 and *MAML2* were also found to carry missense mutations. Frequent mutations in 3'-UTR were also found in other *NOTCH*-related genes such as *FZD4* and *HES5*. The full understanding of the role of Notch pathway in salivary mucoepidermoid carcinomas remains to be achieved. However, our whole exome data suggest its relevance in this tumor. The identification of MAML gene alterations also suggests that alterations in Notch pathway can be independent of *MAML2/CRTC1* fusion. Research supported by FAPESP 14/06186-1 and 14/07249-7.