NOTCH1 Variants and Extracapsular Spread from Oral Squamous Cell Carcinoma Lymph Node Metastasis

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Background: The most adverse prognostic predictor for Oral Squamous Cell Carcinoma (OSCC) is Extracapsular Spread (ECS)) from lymph node metastasis, which needs further molecular characterization.

Methods: Next Generation Sequencing compared TP53 and NOTCH1 variants in OSCC primary tumour samples (n=50; 21 node negative [N-], 11 [N+ECS-], 18 [N+ECS-]). Sanger Sequencing independently validated candidate variants. Primary OSCC cell lines were chosen according to NOTCH1 variant status. Their cell growth and migration were compared following treatment with the γ-secretase inhibitor DAPT (N-[N-(3,5-difluorophenacetyl)- L-alanyl]-S-phenylglycine t-butyl ester), used for Notch inhibition.

Results: Data from 40/50 OSCC samples was included following quality control screening for average coverage. Known intronic and exonic variants (1000 Genomes) were found plus 14 NOTCH1 non-synonymous variants in 13 OSCC samples and 7 non-synonymous TP53 variants. 9/14 [N+ECS+] samples had NOTCH1 variants, compared to TP53 (2/14 of [N+ECS+] samples). Sanger Sequencing confirmed 57% of all variants with a rate of 7/40 (17.5%) for NOTCH1. 6/14 (43%) ECS+ samples had confirmed NOTCH1 variants compared to 1/26 (3.8%) ECS-, Fisher's exact test $p = 0.0044$. Variants clustered in the N-terminal, extracellular EGF-like repeat region.

In vitro, decreased cell growth and migration was observed after DAPT (25 μM) treatment for four weeks compared to controls. Western blot analysis for Notch proteins revealed absence of Notch1 and 3 after 3 weeks and Notch4 by week four.

Conclusion: Overall rates of NOTCH1 variants in our OSCC cohort were consistent with recent literature and NOTCH1 may be a key driver of ECS in OSCC.