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Title	Overexpression of CCND1 in oral carcinogenesis in a Chinese cohort
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For Office Use Only: Overexpression of CCND1 in Oral Carcinogenesis in a Chinese Cohort. CHEN, Qianming^{1,2*}, LUO, Gang ², L. P. Samaranayake ¹, LI, Bingqi ² (¹Oral Biosciences, Faculty of Dentistry, The University of Hong Kong, Hong Kong; ²College of Stomatology, West China University of Medical Sciences, Chengdu, P. R. China.)

Purposes and Methods: In order to investigate whether CCND1 exerts effects on oral carcinogenesis, a total of 55 formalin-fixed and paraffin-embedded samples of oral premalignant lesions (OPLs) and oral squamous cell carcinomas (OSCCs) were obtained from ethnic Chinese. The expression of Cyclin D1 protein in all the samples was investigated using a labeled streptavidin biotin (LSAB) immunohistochemistry (IHC) assay, and the gene amplification of CCND1 was evaluated using a differential PCR. A direct PCR-DNA sequencing method was performed to confirm the accuracy of the PCR product. In-situ hybridization (ISH) was also performed with 14 randomly selected samples to confirm the IHC and PCR data. In the second part, the identical differential polymerase chain reaction was used to evaluate the expression of CCND1 in human oral squamous cell carcinoma cell lines, BcaCD885 and Tca8113. Then, an anti-sense oligodeoxynucleotides (ODN) complementary to human CCND/ mRNA was transferred using leptofectin as a vector into BcaCD885 cell line. The growth patterns of this cell line before and after the transferation were compared with each other, as well as with an ODN-free blank transferation. Results: Immunohistochemically the Cyclin D1-positive staining was much stronger in OPLs and OSCCs groups than in normal controls and hyperkerotosis patients, and CCND1 amplification was also distinct in the former two groups based on the differential PCR results. ISH revealed positive hybridization signals in samples from three groups with hyperplasias and cancers, but not from the controls, corresponding well with the results of IHC and PCR. DNA sequencing confirmed the accuracy of the PCR products. Two tumor cell lines demonstrated the overexpression of CCND1, and decreased cell growth was noted in BcaCD885 cell line, treated with anti-sense ODN. Conclusions: These results suggest that the overexpression of CCND1 may contribute to the development and progression of oral carcinogenesis. (Supported by grants from the National Nature Science Foundation in China, Grant No. 30070814 & 30070815, and the CRCG of the University of Hong Kong)

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