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The Biological Effect Of Fenretinide In Nasopharyngeal Carcinoma Cells Y. XIA*, N. S. WONG, H. TIDEMAN (Dept. of OMFS, Faculty of Dentistry & Dept. of Biochemistry, Faculty of Medicine, University of Hong Kong, Hong Kong)

As a member of the retinoids family, fenretinide (*N*-(4-hydroxyphenyl)retinamide) is a promising chemopreventive agents under intensive investigation. It has demonstrated potent efficacy and reduced toxicity against a variety of tumors including some head and neck cancers. To study its biological activity on nasopharyngeal carcinoma (NPC), the CNE3 cells originated from a NPC were treated with fenretinide. The cell morphological changes were observed by fluorescent microscopy, and cellular DNA alterations were detected with biochemical approaches including gel electrophoresis and flow cytometry. The obvious cell shrinkage, chromatin condensation and nuclear fragmentation after treatment of fenretinide indicated the occurrence of apoptosis, which was supported by the characteristic DNA 'ladder' formation on agarose gel and the sub-G_1 peak presence on flow-cytometric histogram. Therefore, our findings suggest that fenretinide is an effective apoptosis inducer in NPC cells at clinically relevant doses. It is interesting and meaningful to explore further the cellular pathways activated by fenretinide which might give insight to the carcinogenesis of NPC and help with novel therapeutic strategies.

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The Association of Areca Quid Chewing and Oral Precancerous Lesions K LIN*, YH YANG, CH CHENG, CC LIN, and TY SHIEH (Graduate Institute of Oral Health Sciences, Kaohsiung Medical University, Kaohsiung, Taiwan)

The significant association between oral cancer and areca quid chewing has been reported in many southeast Asia countries. However, the relationship of chewing habit with oral precancerous lesions has not been conclusive. The purpose of this research is to investigate the association between oral precancerous lesions and different types of areca quid chewing (Lao-hwa quid and betel quid) in Taiwan. A matched case-control study was conducted in Kaohsiung Medical University Hospital. There were three groups of cases: 127 patients of oral cancer, 88 patients of oral submucous fibrosis and 129 patients of other oral precancerous lesions. The control group was matched by sex, age and occupation variables for each patient. The information of behavior of areca quid chewing, smoking and drinking was obtained by questionnaires. The odds ratio of chewing areca quid was 23.78 for having oral cancer, 164.96 for having oral submucous fibrosis and 23.68 for having other oral precancerous lesions, while adjusted by smoking and drinking. Among oral cancer patients, the percentage of chewing both Lao-hwa quid and betel quid was higher than chewing only betel quid (39.37% vs. 20.47%). The same feature was also seen among patients of oral submucous fibrosis (38.64% vs. 22.73%). Among patients of other oral precancerous lesions, the percentage of chewing only Lao-hwa quid was higher than chewing only betel quid (31.01% vs. 15.50%). We concluded that areca quid chewing was highly associated with oral precancerous lesions, and Lao-hwa quid might be more risky than betel quid. The risk of having oral cancer or precancerous lesions was also affected by longer chewing history and larger daily consumption.

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Growth Inhibition of an Oral Squamous Cell line by an Antisense Oligodeoxynucleotide Complementary to Human *CCND1* mRNA. CHEN, QIANMING*, LUO, GANG, LI, BINGQI, L. P. SAMARANAYAKE. (Oral BioSciences, Faculty of Dentistry, The University of Hong Kong, Hong Kong)

Background and Purpose: Cyclin D1, the product of proto-oncogene *CCND1*, has been identified as an important positive cell cycle regulator. Our recent studies have demonstrated that aberrant Cyclin D1 overexpression and *CCND1* amplification are frequently involved in oral carcinogenesis. In this study, we aimed to observe the growth inhibition of an oral squamous cell line by an anti-sense oligodeoxynucleotide to human *CCND1* mRNA. ***Material & Methods***: A human oral squamous cell carcinoma cell line, BcaCD885, was evaluated for the amplification of *CCND1* using a differential polymerase chain reaction (PCR; *CHEN, et al. Oral Oncology, 2000,36: 95 - 99*). A direct PCR- DNA sequencing method was used to confirm the accuracy of the PCR amplification. Then, an anti-sense oligodeoxynucleotides complementary to human *CCND1* mRNA was transducted into BcaCD885 cell line using leptofectin as a vector (*Zhou P, et al. Oncogene, 1995; 11(3): 571 – 80*). The yield of colony forming units of this cell line before and after the transduction were compared, as well as with an oligodeoxynucleotide-free, blank transduction. ***Results***: Based on the differential PCR results, BcaCD885 demonstrated the amplification of *CCND1*, whilst DNA sequencing confirmed the accuracy of the PCR products. The number of colony forming units from the squamous cell line in culture decreased significantly in the group treated with the anti- sense oligodeoxynucleotides, when compared with all the control groups. ***Conclusion:*** The results suggest that the antisense oligodeoxynucleotides complementary to human *CCND1* mRNA is able to inhibit the growth of the oral squamous cell line (with overexpression of *CCND1*). Hence, *CCND1* may be a target for gene therapy in oral cancers. (This work was supported in part by a grant from the University Research Committee Grants of the University of Hong Kong, Hong Kong, No.10203005 / 30713 / 08011 / 302 / 01).

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p21 $^{WAF1/CIP1}$ alterations in relation to the expression of p53 and Ki-67 in oral squamous cell carcinomas.

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To analyze relevant factors of neoplastic transformation in the oral cavity, the expression of p53, p21 $^{WAF1, CIP1}$ and Ki-67 was immunohistochemically investigated in oral squamous cell carcinomas (n=34). The positive rates of p53, p21 and Ki-67 expression were 59, 82 and 91%, respectively. Generally, p53 was mostly observed in basal layers while p21 was consistently elevated in the superficial, differentiated cells of the epithelium. The Ki-67 positive cells were located mainly in basal and suprabasal layers. Interestingly, 44% (15/34) of the cases showed p53 accumulation and overexpression of p21. Fifty-three percent (18/34) of the specimens expressed both p53 and Ki-67 proteins. No significant correlation could be found between p21 and p53 or proliferation index ($p>0.05$). In conclusion, p21 protein was overexpressed in oral squamous cell carcinomas. The overexpression was not related to cell proliferation and it may occur by both p53 dependent and independent mechanisms.

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Norcantharidin induced post G2/M arrest apoptosis is dependent with wild type p53

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Norcantharidin, a synthetic analogue of phosphatase type 2A (PP2A) inhibitors – cantharidin, has been reported to have effect on treatment of human and animal tumors. The tumor cell killing mechanisms by norcantharidin, however, remain unclear. In this experiment, the investigation was made to understand the mechanisms of norcantharidin mediated cytotoxicity. Effort was made to investigate if norcantharidin exerts its cytotoxicity through p53 dependent or independent mechanism. KB, CAL 27 (wt p53) and U251 (mutant p53) were used to expose to norcantharidin. Time course fluorescent-activated cell sorting (FACS) analysis showed that high doses of norcantharidin arrested the cells at the G2/M phase and post G2/M apoptosis in KB, CAL27 cell lines. There was, however, little apoptosis found in mutant p53 cell line exposed to norcantharidin. The results showed that norcantharidin kills tumor cells efficiently correspond to their p53 status. The results showedorcantharidin, at high dosage, kills tumor cells efficiently correspond to their p53 status. Western blot was used to further investigate whether p53 plays a role in the induction of G2/M arrest as well as apoptosis after expose to norcantharidin. Induction of p53 expression was found in wild type p53 cell line but not in mutnat p53 cell line exposed to norcantharidin using western blot analysis. p53 downstream genes were also detected afternorcanthardin exposure with western blot and immunohistochemistry at in vivo tumor model. The results showed that norcantharidin induced apoptosis through p53 and bax dependent mechanisms. The delivery of p53 into the mutant p53 cell lines would enhance norcantharidine act on cells. The results implied thatnorcantharidin induced apoptosis through p53 dependent mechanisms. Addition of p53 may increase the chemo-sensitization effect of Norcantharidin.

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Molecular Typing by Random Amplification of Polymorphic DNA (RAPD), and Biotyping of Sequential Oral Isolates of *Candida albicans* in HIV infection L. P. SAMARANAYAKE*, Y. H. SAMARANAYAKE, P. C. S. TSANG, S. ANIL Oral Bio-Sciences, Faculty of Dentistry, University of Hong Kong, SAR China

Several investigators, using an array of genetic and phenotypic tools, have shown that HIV-infected patients are frequently infected with the identical sub-type of *C albicans* over multiple episodes of infection while others have found the opposite. **Aim:** Hence the purpose of this study was to evaluate the genetic and phenotypic diversity of oral *C. albicans* isolates on single and sequential visits in HIV-infected ethnic Chinese. **Materials:** A total of 16 ambulatory, community dwelling HIV-infected individuals were examined over a period of one year. At each visit the oral cavity was sampled using the oral rinse technique (Samaranayake *et al* J Oral Pathol. 1986; 15:251-4) and cultured on Sabourauds dextrose agar to obtain the yeast growth. Up to five colony forming units (CFU) of *C. albicans* were selected and identified at each visit, yielding a total of 441 isolates from 16 patients. To obtain the genotypic profile all isolates were typed by RAPD analysis using two custom synthesised primers (Gibco BR, Hong Kong; *Nucleic Acid Res* 1992; 20 : 5137-42). The organisms were biotyped using a combination of the API 20C, APIZym and boric acid resistance test (Xu and Samaranayake *Arch Oral Biol* 1995; 40: 577-579). **Conclusions:** 1) multiple clones of *C. albicans* exist intra-orally in HIV disease 2) hence, it is essential to select and evaluate more than a single CFU at each visit to decipher their complete pathogenic profile, 3) in general, most patients appear to carry the same strain throughout, 4) as there is little congruence between the RAPD genotypes and the biotypes it is imperative to use multiple typing tools to ascertain the 'true sequential carriage', 5) although the RAPD technique is simple, versatile and reproducible for evaluating sequential carriage of yeasts, judicial selection of primers and their combined use appear essential to obtain reliable data.
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Sequential isolates of *Candida albicans* in HIV infection exhibits progressive resistance to human lactoferrin. [1]Y. H. SAMARANAYAKE*, L. P. SAMARANAYAKE, V. T. BEENA and K. W. S. YEUNG , Oral Bio-Sciences, Faculty of Dentistry, The University of Hong Kong, Hong Kong, SAR.

A variety of innate defense factors in saliva such as lactoferrin contribute to mucosal protection, and changes in the salivary concentrations of lactoferrin (H_{LF}) have been observed in HIV-1 infection. **Aim**: The aim of this study was to determine the *in vitro* susceptibility of 59 genotyped, oral *C. albicans* isolates from six HIV-infected individuals during sequential visits, to HL_F using a blastospore viability assay. **Methods:** The organisms were genotyped using a RAPD assay according to Bostak *et al*. (J. Gen. Microbiol., 1993: 139: 2179-84). The H_{LF} viability assay was conducted according to the method of Soukka *et al*. (FEMS Microbiol Letts., 1992: 90: 223-28) by exposing a standard suspension of the organisms to 20 µg/ml human lactoferrin (Sigma) for 60 min. and estimating the yield of colony forming units on Sabourauds' dextrose agar. **Results:** Exposure to H_{LF} caused a rapid loss of viability among all isolates to varying degrees. None of the sequential, 59 *C. albicans* isolates demonstrated significant differences in sensitivity to H_{LF} from one visit to the next. The ten genotypes demonstrated no significant differences in susceptibility to H_{LF} and were uniformly sensitive to the enzyme. On regression analysis of a sequentially isolated genotype of one patient, a significant negative correlation ($r=0.78$) between the H_{LF} resistance and the HIV disease progression was seen. **Conclusion:** These results indicate that a minority of *C. albicans* that persist intra-orally in HIV disease may develop progressive resistance to human lactoferrin as an adaptive response, while the majority are unaffected.
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The Association of Areca Quid Chewing and Radiographic Alveolar Bone Loss., CN HSIAO*, GL HOU, YH YANG, CC LIN, and TY SHIEH (Graduate Institute of Oral Health Sciences, Kaohsiung Medical University, Kaohsiung, Taiwan)

Areca quid chewing is a common masticatory drug used in South East Asia, India, and the South Pacific. It is used daily by 600 million people worldwide, and is a public health problem. In the past fifteen years, many investigations about areca quid chewing have focused on exploring epidemiology, premalignant changes, genetic factors and components of areca quid, but investigations concerning periodontitis have been quite few. The purpose of this study is to evaluate the relationship between areca quid chewing and periodontitis. One hundred and seventy seven subjects were recruited in the sample with which 117 areca quid chewers and 60 subjects without habit. After taking panoramic films and using a questionnaire to survey individual characters, we measured radiographic alveolar bone loss (RAL) in the lower first molar. Areca quid chewer's annual radiographic alveolar bone loss was up to 2.88 times the no habit group. The odds ratio for association of chewers with severe alveolar bone loss was 22.07 for no habit samples. We concluded that a significant relationship does exist between areca quid chewing increase the loss of alveolar bone. These data support the concept that areca quid chewing are the important factors to radiographic alveolar bone loss.