Title	p53, p21, Rb, MDM2 proteins in tongue carcinoma from patients <35 years
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2033 Identification of genes that correlate with the HPV-induced oral cancers.

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Oral carcinogenesis is a multistage process in which "high-risk" types of human papillomaviruses (HPVs) infection and cofactors, such as smoking and alcohol consumption, is frequently involved in the development of oral malignant cancer. However, the molecular mechanisms underlying these processes remain poorly understood. To identify the genetic changes that drive oral cancer progression, we used mRNA differential display method to analyze an *in vitro* model for neoplastic conversion of papillomavirus-immortalized human oral keratinocytes. Non-tumorigenic HPV 16-immortalized human oral keratinocytes (HOK-16B) were compared with their tumorigenic counterparts (HOK-16B-BaP-T), and 20 genes were identified which were either up or down regulated in different levels of gene expression. To determine the overall pattern and consistency of the differential expression of the identified genes, we have examined different cells, including normal oral keratinocytes, HPV-transfected non-tumorigenic immortalized oral keratinocytes, and oral cancer cell line. Two genes, #4 and #6, are particularly interesting, because their expression is upregulated in the tested oral cancer cells but not in the immortalized cell line. These two genes may be molecular markers for the progression to oral cancinogenesis and provide new avenues for early detection. This study is supported by NIDR DE00371 and DE11229.

2034 Stepwise loss of loci on 3p during oral carcinogenesis.

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Loss of heterozygosity (LOH) on chromosome 3p is one of the most frequent changes that occurs in oral cancers. This loss is complex, involving at least 3 different regions (3p14, 3p21, and 3p24-25), suggesting the involvement of 3 putative suppressor genes. Are these genes involved in the development of dysplasias or in later events, such as those needed for invasion? No previous studies have examined the relative frequency of loss at these 3 loci in premalignant lesions in the oral cavity. In this study we used 10 microsatellite markers, distributed among the 3 regions of loss, to determine the pattern of LOH at 3p in 38 early dysplasias (21 mild, 17 moderate), 17 late dysplasias (severe dysplasia and carcinoma in situ) and 21 squamous cell carcinomas (SCC). Overall, LOH on 3p was seen in 42% of early dysplasias, 53% of late dysplasias and 78% of SCCs. There was a tendency for the losses in early dysplasias to involve discrete regions of 3p whereas in SCC, the loss more often involves the entire region of 3p assayed. In early dysplasia, LOH was most frequently found in the centromeric region at 3p14 (32%), followed by 3p25 locus (24%), with only 4 cases of loss at 3p21 (11%). Among the severe dysplasia and CIS, LOH occurred in 9 cases (47%) at 3p14; 8 (42%) at 3p25; and 2 (11%) at 3p21. In contrast, in SCC, 20 cases showed LOH at 3p14 (74%); 16 cases at 3p25 (59%), and 16 cases at 3p21 (59%). These data suggest that at least two of the tumor suppressor genes (at 3p14 and 3p25) are involved in the initiation of carcinogenesis in head and neck region and that the 3p21 locus contains a gene that has a function more often associated with tumor invasion. (P=0.0017 two sided Fisher's exact test). Supported by a grant from NCI (Canada) with funds from the Canadian Cancer Society.

2035 Decreased Cyclin E Expression in Oral Cancer after Transfer of the Wild-type p53 Gene.

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Background: Cyclins and wild-type p53 protein are prime cell cycle regulators and may be involved in tumorigenesis. Cyclin E is a late G1 cyclin and its abnormalities have been reported in several cancers, but not in oral squamous cell carcinoma. ***Objective***: to investigate the Cyclin E expression in oral carcinogenesis and establish its correlation with p53 gene. ***Methods***: The Tca8113 squamous cell line, where a genetic abnormality in p53 gene had been determined by polymerase chain reaction – single strand conformation polymorphism, was transferred by vectors i) carrying wild-type p53 gene, ii) mutant p53 gene, and iii) no additional gene (control) using electroporation techniques (Chen Q et al: *Natl J Med China*, 1996; 76(11): 876). The cell line with no vector transfer served as the blank control. Then, the cells were ex-planted into four groups of naked mice with four mice in each group. After tumor development, the mice were killed, the tumors collected and embedded using routine histopathological techniques. An immunohistochemistry assay, Envision™+system (Dako, Denmark), was used to study the expression of Cyclin E. ***Results***: The cyclin E was localized in the nuclei of the tumor cells and was highly expressed by this cell line. In the wild-type p53 gene group, the number of stained cells and the staining intensity of Cyclin E decreased significantly ($P<0.05$), as compared with the mutant p53 gene group or the no gene transfer group, which were similar to the blank controls (both, $P>0.05$). ***Conclusions:*** The data suggest that Cyclin E may contribute to the progression of oral cancer and its expression correlates with that of the p53 gene. This work was supported partially by a grant from the Committee on Research and Conference Grants, the University of Hong Kong. Grant No. 10201937/30713/08011/301/01

2036 p53, p21, Rb, MDM2 Proteins in Tongue Carcinoma from Patients <35 Years.

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Squamous cell carcinomas of the tongue are associated with a relatively poor prognosis, and exhibit a particularly aggressive course in young patients. Alterations in cell cycle proteins likely contribute to the biologic behavior of these neoplasms and may be age related. The purposes of this investigation were to evaluate expression of cell cycle proteins (p53, p21, Rb, MDM2) in tongue cancers from patients <35 years of age, and to compare results to staining in tongue cancers from patients >75 years. All available archived lateral tongue carcinomas from patients <35 years (N=36, 23 male & 13 females) were sectioned, immunohistochemically stained, and evaluated. Protein expression was scored as percent positive nuclei. An equal number of sequentially accessioned lateral tongue specimens from patients >75 years (23 male & 13 female) were stained and compared. Internal and external controls were utilized. Positive p53 staining was seen in 18/36 of the <35 group vs. 24/36 of the >75 group ($p=0.23$, Fisher's exact). Increased p21 staining (both percentage of positive cells & intensity) was evident in 30/33 of the <35 group vs. 25/32 of the >75 group ($p=0.185$). Both p53 positivity and increased p21 expression were present in 15/33 of the <35 group vs. 20/32 of the >75 group ($p=0.22$). Rb protein was increased in 18/29 of the <35 group vs. 16/24 of the >75 group ($p=0.78$). Six cases (2/30 vs. 4/24, $p=0.39$) showed positive MDM2 stain; 4/6 MDM2+ cases were also p53+. p53, p21, Rb, and MDM2 are over-expressed in lateral tongue cancers. Significant differences in expression of these proteins in tumors from patients <35 years vs >75 years were not detected. Supported in part by NIDR/NCI grant P50 DE/CA 11912-03

2037 c-Myc Oncoprotein Overexpression and DHFR Gene Amplification in Ameloblastoma.

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Our previous studies have shown that (i) in a variety of neoplastic cells, including those of squamous epithelia, premalignancy is associated with the deregulated expression of the c-Myc oncoprotein, (ii) c-Myc overexpression precedes the focus-specific amplification of the dihydrofolate reductase gene DHFR, and (iii) c-Myc deregulation is associated with enhanced formation of extrachromosomal elements as the neoplasm progresses. As the first step in examining the mechanisms of genetic instability in odontogenic tumors, we have employed combined protein/FISH (fluorescent *in situ* hybridization) analysis (CPFA) and quantitative image analysis, to determine the level of c-Myc expression and DHFR amplification in a series of samples including benign ameloblastoma, and desmoplastic ameloblastoma with borderline features of ameloblastic carcinoma; adjacent normal surface epithelium was used for comparison. The observations obtained by CPFA are in keeping with data of earlier studies in other neoplasms, indicating that 1) c-Myc overexpression and DHFR amplification (at least four-fold) can be detected in benign ameloblastoma but not in adjacent normal tissues, and 2) the changes are most pronounced in samples with features of ameloblastic carcinoma. Thus, it appears that in ameloblastoma, the features of genetic instability (c-myc-dependent, focus-specific amplification of a target gene, DHFR), are maintained and probably enhanced during the progression of neoplasia.

2038 Cyclin D1 expression is correlated with poor prognosis in patients with areca quid chewing-related oral squamous cell carcinomas in Taiwan.

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Abnormal expression of cell cycle regulatory proteins, particularly cyclin D1, has been implicated in pathogenesis of several types of cancer. We have examined the expression of cyclin D1 in oral squamous cell carcinomas (SCCs) using anti-cyclin D1 antibodies with an immunoperoxidase technique. Cyclin D1 nuclear staining was observed in 73 of 88 (83%) cases of oral SCC. In 54 of 73 (74%) cases, positive cyclin D1 staining was also found in the normal appearing epithelium immediately adjacent to the cyclin D1-positive SCCs. No significant correlation was found between the expression of cyclin D1 and the patients' age, sex, oral habit, cancer location and STNM status. The Kaplan-Meier analysis showed patients with tumors containing more than 10% cyclin D1-positive cells had significantly shorter overall survival than those with tumors containing less than 10% cyclin D1-positive cells or cyclin D1-negative tumors ($P<0.05$). Patients with positive lymph node status also had significantly shorter overall survival ($P<0.01$). These results indicate that cyclin D1 may play an important role in the carcinogenesis of oral SCC and may serve as an adjuvant marker of worse prognosis in patients with oral SCCs in Taiwan. This study was supported by National Science Council grant NSC 85-2331-B002-304-M14, Taiwan.

2039 Activity of Human Papillomavirus Type 16 P97 Promoter in Immortal and Tumorigenic Human Oral Keratinocytes.

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We previously immortalized normal human oral keratinocytes (NHOK) by transfection with cloned human papillomavirus type 16 (HPV-16) genome and converted these immortalized cells to tumorigenic cells with chemical carcinogens. Since the tumorigenic cells expressed higher level of HPV-16 E6/E7 transcripts, we predicted that enhanced E6/E7 expression was induced by mutations at the long control region (LCR) of the viral genome integrated into cellular chromosome. To test this possibility, we sequenced the entire HPV-16 LCR from immortalized and tumorigenic cells, but no difference in the sequences in all of the tested cells was observed. However, it is possible that such differences in the expression of E6/E7 could have originated from different activities of cellular transcription factors in the different cells. To examine this prospect, we subcloned entire LCR into a reporter gene and determined the promoter activity of LCR in immortalized and tumorigenic cells. We found that the LCR promoter activity was significantly higher in tumorigenic cells when comparing to immortalized cells. We also observed that at least 477 nucleotides at the upstream of E6 open reading frame are needed for the maximum LCR promoter activity in tumorigenic cells. This study was supported by the academic research fund of Ministry of Education, Republic of Korea (1995).

2040 Study of P53 Gene Mutation in Oral Leukoplakia.

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Previous studies have shown that p53 gene mutations are most frequently found in various human cancers. The purpose of the present study was to investigate the relationships between p53 gene mutation and the genesis of well differentiated squamous cell carcinoma from oral leukoplakia by PCR and single strand conformation polymorphism (SSCP) method. The 33 specimens were pathological diagnosed and leukoplakia samples were classified to with or without epithelial displasia. The template DNA was extracted from paraffin-embedded tissue. The each genom of exon 5 , 6 , 7 and 8 were amplified by PCR and gene mutation was analyzed by SSCP on a gradient (5 to 20%) polyacrylamide slab gel followed by silver staining. The following results were obtained: The p53 gene mutation was showed in 9.1% (1/11) of leukoplakia without epithelial displasia, 27.3% (3/11) of leukoplakia with epithelial displasia and 54.5% (6/11) of well differentiated squamous cell carcinoma. Among the samples which have shown mutation, the frequency of gene mutations in exon 5, 6, 7 and 8 was 20, 50, 10 and 30%, respectively. In addition, one case showed mutations in both exon 6 and 8. From the result presented here, we conclude that p53 gene mutation may have relation to epithelial displasia in oral leukoplakia.