p16 and p63. Samples were grouped as follows: Dysplasia with history of OSCC at the same site (group 1), or history of OSCC at a different site (group 2), no history of OSCC (group 3), and group 4-OSCC. The labelling index (LI) was evaluated in 10 high power fields.

Results LI for Ki67 was 21% in group 3, significantly lower than groups 1 and 4 (31%, 39%) (ANOVA $P = 0.003$, Games Howell pairwise $P = 0.07$, <0.0001). p63 LI was 76% for group 3, significantly lower than groups 1 and 4 (82%, 88%, ANOVA $p = 0.001$, Games Howell pairwise $P = 0.06$, <0.0001). Groups 2 did not exhibit significant differences from any of the groups p53, p16 were expressed in all groups, without significant differences.

Discussion:LI for Ki67 and p63 increased from dysplasia only samples, through dysplasia with cancer history at the same site, to the highest levels in OSCC. Dysplastic samples in patients with OSCC at a different site did not show a characteristically different pattern.

Conclusion: High LI of Ki67 and p63 are possible indicators for changes in the oral mucosa associated with malignancy (before or after transformation) in a localized site.

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P3.60. Phenotypic and functional differences between oral carcinoma-associated fibroblasts and normal oral fibroblasts

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Introduction: A though it has become clear that initiation and progression of carcinomas depend not only on alterations in epithelial cells, but also on changes in their microenvironment, the specific changes of normal stromal microenvironment of oral carcinomas and their consequence on oral cancer cell behavior are not yet well defined.

Aim: To characterize at molecular and functional levels oral carcinoma-associated fibroblasts (CAFs) and their normal counterpart fibroblasts (NOFs).

Materials and methods: CAFs and NOFs ($n = 6$) were isolated from oral cancer and healthy volunteers respectively, after informed consent. Growth potential was evaluated by cell counts and the number of population doublings (PDs) was determined. Expression of various cell markers was assessed by immunohistochemistry and flow cytometry. Functional interaction with oral cancer cells was assessed in both monolayers and organotypic 3D co-culture models.

Results: CAFs had a slower growth rate and a restricted growth potential (PD = 28.59 ± 4.11) in routine fibroblast cell culture conditions when compared with NOFs (PD = 50.71 ± 1.26, $p = 0.007$). CAFs expressed more alpha-smooth muscle actin and fibroblast activated protein than NOFs. More CAFs than NOFs expressed CD146, a mesenchymal stem cell-related molecule, and its expression increased significantly after interaction with oral carcinoma-derived cells. CAFs also caused oral carcinoma-derived neoplastic cells to invade deeper than in presence of NOFs in organotypic 3D co-cultures.

Conclusions: CAFs displayed significant differences versus NOFs with respect to phenotype, cell proliferation, immunoreactions and interaction with oral carcinoma-derived neoplastic cells. These differences were supportive for specific tumor initiating effects of tumor associated fibroblasts on oral carcinoma cells.

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P3.61. A simple mouthwash method for genomic DNA isolation in molecular studies

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Background: Application of PCR techniques requiring only minute amounts of Genomic DNA. Thus, a less invasive, simpler to perform, and cheaper method to obtain DNA from exfoliated cells is desirable. We aim to develop a method that can obtain high quality of genomic DNA from one sample that allows for numerous application of PCR analysis.

Objective: This study describes a simple, inexpensive and non invasive protocol to isolate a high quality of genomic DNA from exfoliated cells by using swish method.

Methodology: Twenty two subjects vigorously swished 10 ml of normal saline in their mouth for 60 s and spitted into a collection tube. DNA extraction assay was performed by using saliva DNA isolation kit (Norgen, USA). The washed pellets were suspended in TE buffer and analyzed for the quality and purity of DNA content by using the NanoDrop Spectrophotometer. A ratio of A260/A280 was calculated. The extracted genomic DNA was amplified with primers of p53 intron 6 by using PCR machine. The presence of amplified DNA was then confirmed by electrophoreses analysis, which DNA bands were scanned by Typhoon 9410 variable imager.

Results: In this study, the extracted genomic DNA demonstrated an average value of 1.94 O.D. in DNA content purity and 42.9 μg/μl in DNA yields. The electrophoresis images of the DNA products showed visible and detectable bands of higher molecular weight DNA in all the samples.

Conclusion: The results showed that the extracted genomic DNA from the exfoliated cells by applying the swish method, that provides substantially larger amounts and higher molecular weight of DNA for down-stream DNA identification application. In addition, all samples were successfully genotyped by PCR-based assays for p53 gene intron 6 regions, which confirmed that the quality of isolated DNA was reliable in supporting the PCR amplification for the molecular studies.

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P3.62. Clinical significance of the dihydropyrimidine dehydrogenase and thmidylate synthase activity in oral cancer

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The rate-limiting enzyme to catabolize 5-fluorouracil (5-FU) is dihydropyrimidine dehydorogenase (DPD), which expression in cancerous tissue is reported to have a relation with anti-tumor effect for 5-FU, and thymididylate synthase (TS) is one of the essential nuclear enzymes for nucleic acid synthesis, and it is considered to participate in tumor growth.

In this study, we evaluated the immunohistochemical expression in oral cancer and who treated with 5-FU and TS-1, using immunostaining techniques with an anti-human DPD and TS polyclonal antibody. The possibility that DPD and TS might be a useful indicator of the anti-tumor effect was then assessed based on our results and the patients' clinicopathological factors and prognoses.

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