# Molecular biology 1

## PD.64 Promoter hypermethylation, mRNA and protein expression of p16, DAPK, MGMT and GSTP1 genes in chewing-tobacco induced oral cancers

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**Introduction:** Hypermethylation in the promoter region of a gene is a critical mode of gene silencing in cancer, resulting in loss of mRNA transcripts and consequent loss of protein expression. With the aim of understanding the role of epigenetic alterations in development of oral cancer, p16, DAPK, MGMT and GSTP1 regulatory genes, were investigated for hypermethylation, percentage of CpGs methylation, and expression of the genes at the transcript/protein levels.

**Materials and Methods:** Hypermethylation status of the genes was investigated in 103 oral tumors, 76 corresponding tumor adjacent mucosa, 24 premalignant oral lesions, buccal scrapings from 67 long term tobacco users (LTTUs) and 50 healthy volunteers without tobacco habits, using methylation specific PCR. The density of methylated CpGs was assessed by bisulfite sequencing of the promoter regions of the genes. Gene transcripts were examined by quantitative real time PCR, and protein expression by immunohistochemistry.

**Results:** Hypermethylation of one or more genes – p16, DAPK and MGMT, was detected in 86% oral tumors, 75% tumor adjacent mucosa, 92% premalignant lesions and 70% LTTUs. GSTP1 was not hypermethylated in any of the samples. Hypermethylation was not observed in the buccal scrapings of healthy controls. The mean CpG density was higher in oral tumors as compared to premalignant lesions and LTTUs. We demonstrated a decrease in p16, DAPK and MGMT mRNA transcripts in the hypermethylated samples, as compared to the transcript levels in the buccal scrapings of normal individuals with no tobacco habits. A high concordance between hypermethylation and absence of protein expression was also observed in the oral cancers.

**Conclusion:** Our data shows that gene promoter hypermethylation is a frequent and early event in oral cancers, also observed in premalignant lesions and buccal scrapings of LTTUs, resulting in loss of mRNA transcripts and protein expression of p16, DAPK and MGMT genes, contributing to oral carcinogenesis.

## PD.65 Functional results of patients with total glossectomy without laryngectomy

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**Introduction:** Survival of patients with extensive squamous cell carcinoma of the entire tongue is usually very poor. Function and quality of life of patients submitted in total glossectomy with or without laryngectomy before the advent of tissue transfer was dismay. Free tissue transfer has in the recent years been successfully used in the improvement of these two important parameters.

**Materials and Methods:** From November 1999 to January 2003 five patients with advanced primary squamous cell carcinoma of the tongue and the floor of the mouth where submitted to total glossectomies without laryngectomies. Four patients were male and 1 female with ages ranging from 40 to 68 years, mean 55.2 years. All patients were submitted into bilateral neck dissection and en block resection of the lower oral tissues. Three patients were submitted simultaneously into horizontal hyperglottic laryngectomy with preservation of the upper laryngeal nerves. The mandible was preserved in all cases and in all patients peripheral marginal resection of the mandibular alveolus was simultaneously performed. The defects were reconstructed with one free rectus abdominis and 4 radial forearm flaps. Tracheostomy was maintained from 20 to 30 days in all patients. Oral feeding was achieved in all but 1 patient.

**Results:** All patients received both chemotherapy and radiotherapy as part of their oncological treatment. Three patients died during the post-operative period and 2 remain free of tumors 48 and 36 months postoperatively. The 3 patients who died from locoregional recurrence of disease had a median post-operative survival of 15 months.

**Conclusion:** Total glossectomy with or without laryngectomy remains an extremely severe surgical operation with poor functional and therapeutic results. This operation should be selectively undertaken as salvage surgery and only when other organ preservation treatment modalities have failed.

## PD.66 A non-invasive genetic screening test to detect oral preneoplastic lesions

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**Introduction:** Early diagnosis of oral squamous cell carcinoma (OSCC) may have a major impact on survival and quality of life. Recent studies have shown that the majority of OSCC are preceded by precursor lesions characterized by genetic alterations. The aim of this study was to develop and evaluate a non-invasive screening test for oral preneoplastic lesions, based on genetic alterations as marker.

**Materials and Methods:** Various methods to obtain a high yield of cells by brushing a small area of the oral mucosa were compared. An existing test for the measurement of numerical chromosomal alterations, multiplex ligation-dependent probe amplification (MLPA), was applied using 140 markers distributed over the genome. MLPA was performed on DNA of normal and dysplastic oral mucosa as well as of OSCC with the intention to select a specific probe set for accurate detection of precursor lesions in the oral cavity. The assay was correlated to loss of heterozygosity analysis using microsatellite markers.

**Results:** A non-invasive sampling method was developed with sufficient DNA yields. We could detect large differences with MLPA in the number of alterations between normal vs. dysplastic and dysplastic vs. tumor tissue with P-values lt; 0.0001. A significant correlation was found between the number of alterations as detected by MLPA and the analysis for allelic loss. The available data enabled the selection of a set of 42 MLPA probes, which had the power to optimally discriminate between normal and dysplastic tissue. It was shown that the assay enabled detection of precursor lesions on DNA of exfoliated cells.

**Conclusion:** Our data show that MLPA is a sensitive, reliable, high-throughput and easy-to-perform technique, enabling the detection of genetic alterations on small non-invasive samples and can be considered a promising method for early detection of preneoplastic lesions in the oral cavity