Purpose: Scientific evidence exists that tongue squamous cell carcinoma has a higher local failure rate and poorer prognosis than other anatomical sites in the oral cavity.

Materials and methods: Tongue squamous cell carcinoma cell lines (SCC-4, SCC-15 and SCC-25) harbouring mutated/hypermethylated p53/p16 were used as models to study the influences exerted by p53 and p16 genes on the expression of micro RNAs (miRNAs). The study was performed on microarray chips harbouring 298 miRNA sequences, to obtain expression values normalized to healthy control of 298 miRNAs for each cell line.

Results: MiRNA 196b was found hyperexpressed in the three cell lines. MiRNAs 19b-1, 21, 27a, 30d, 134, 339, 379 and 465 were found altered in two out of three cell lines. miRNAs found altered in one cell line out of three were: 7b, 23a, 25, 30c, 30e-3p, 107, 125b, 124a, 214, 216, 325 and 384. A literature review for each miRNA found significant was performed. Some miRNAs have a well-known role in oral cancer; some have been put in correlation with other cancers/diseases; others are found significant for the first time.

Conclusion: After more than 10 years of research, a miRNA profile change in dysplastic cells, concurring to outline their “molecular fingerprint”, appears as a fact. These results on tongue cancer cell lines harbouring malfunctioning p16/p53 need further analyses to understand whether this variation of miRNA levels are directly influenced by the accumulation of genetic abnormalities and epigenetic changes, or if, vice versa, altered miRNA levels influence the biological function of p16 and p53. Moreover, discordant results among cancer cell lines of different part of the mouth, may confirm biological differences, which may lead to different diagnostic/therapeutic pathways.
aberrations without any alterations in the DNA sequence. Methylation profiling of tumour tissues has identified the epigenetic aberrations, especially silencing of promoter methylation has been reported as an early event in carcinogenesis.

Methods: Forty oral squamous cell carcinoma (OSCC) samples selected for methylation-specific polymerase chain (MSPCR) reaction and immunohistochemical staining (IHC). Chi Square and Fisher’s Exact tests were applied to determine the correlation between patient’s demographic and clinicopathological data with p16, DDAH2 and DUSP1 genes using SPSS version 17.0. A p value <0.05 was considered significant.

Results: In MSPCR, an overall analysis of tumours was made, where 100% (40/40) of the tumours revealed the hypermethylation of the promoter region was present in at least one of the genes analyzed. In 42.5% (17/40) of the tumours, the promoter hypermethylation was present in two of the genes analyzed and in 55% (22/40) hypermethylation was present in all of the three genes studied. Frequencies of methylation status of promoter hypermethylation of p16, DDAH2 and DUSP1 showed 78%, 80% and 88% positivity, respectively in our study. In IHC staining, DUSP1 revealed 42% of immunoreactivity. There was no statistically significant association between the presence of hypermethylation in the promoter regions of p16, DDAH2 and DUSP1 with the demographic data. However, a statistically significant association was found between p16 gene promoter region with tumour site of buccal, gum, tongue, and lip (p = 0.000).

Conclusions: Our findings reveal that the p16, DDAH2 and DUSP1 genes are aberrantly methylated in OSCC, which may serve as a diagnostic biomarker for OSCC patients’ prognosis in the future. In addition, aberrant methylation of DUSP1 genes more likely contribute to the loss of the protein expressions.

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OP008

Chromosome instability in tumor resection margins of oral cancers is a predictor of local recurrence

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Purpose: It is known that despite efforts of improving adequate ablation in head and neck cancer, the local recurrence rate has not decreased sufficiently over the last two decades. This is partly due to the presence of potentially malignant groups of cells in the resection margins (minimal residual disease). Chromosomal instability (CI) detection in these histologically diagnosed tumour-free margins might help predict patients at risk for recurrence.

Material and methods: We have included 25 patients with oral squamous cell carcinoma (OSCC) treated with surgery alone between 1994 and 2003. All resections were histopathologically radical. Follow-up was documented for at least 5 years. Fluorescence in situ hybridization (FISH) was used to examine all resection margins of all tumours for the presence of CI, which is indicated by the nuclear detection of chromosome imbalances and/or polypliodization for chromosomes 1 and 7. In addition, tissue sections were analysed for p53 expression by immunohistochemistry.

Results: Of the 25 patients, 11 developed a recurrence. FISH analysis showed that of the 11 recurrences, 8 exhibited CI in at least one resection margin. This relation was significant with a p-value of 0.011. There was a trend for p53 overexpression in the resection margins of tumours with recurrence, however, this trend was not statistically significant (p = 0.082).

Conclusion: CI in the resection margins of OSCCs, even though histologically tumour-free, can reliably identify patients at risk for developing a local or locoregional recurrence. A future goal will be to investigate how radiotherapy following surgery influences the prognosis of these patients.

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OP009

Genetic relationship between multiple squamous cell carcinomas arising in the oral cavity

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Purpose–objective: Histological and clinical criteria are generally utilized in routine practice to differentiate second primary tumors (SPTs) from local recurrences (LRs) in patients with oral squamous cell carcinoma (OSCC). The purpose of the present study was to apply mtDNA D-loop analysis in patients experiencing a second neoplastic lesion to differentiate SPT from LR and to validate the clinical classification.

Material/methods: The study population comprised 25 consecutive patients presenting multiple oral neoplastic lesions. mtDNA D-loop analysis was performed by direct sequencing and phylogenetic clusterization.

Results: Following the current clinical classification, 14 s neoplastic lesions were classified as SPT and included five cases showing a different OSCC histotype and seven cases showing an in situ carcinoma concomitant with the invasive OSCC. Agreement between mtDNA analysis and clinical classification was found in 16 cases. Discrepancies arose in three cases in which the clinical criteria based only on the spatial or temporal distance of the second lesion from the index tumor had led to a diagnosis of SPT (one case) or LR (two cases).

Conclusion: The present data highlight the value of mtDNA analysis in establishing the clonal relationship between the index tumor and the second neoplastic lesion. In routine practice, mtDNA analysis may be useful in association with clinical criteria to differentiate LR from SPT, especially in cases with uncertain clinical criteria.

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