particularly when taking BP plus another ONJ-related drug. It is our opinion that it is necessary to give attention for prevention protocols also to the patients in therapy with all drugs related to ONJ.

Overexpression of Nicotinamide n-Methyltransferase in HSC-2 OSCC cell line: effect on apoptosis and cell proliferation.

G. Giannatempo[1], M. Mascitti[2], O. Di Fede[3], R. Mauceri[3], A. Santarelli[2], R. Seta[2], D. Sartini[2], R. Campagna[2], M. Emanuelli[2], L. Lo Muzio[1]

[1]Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy; [2]Department of Clinical Specialistic and Dental Sciences, Polytechnic University of Marche, Ancona, Italy; [3]Department of Surgical, Oncology and Oral Sciences - Sector of Oral Medicine, Palermo University, Palermo, Italy

BACKGROUND: The oral squamous cell carcinoma (OSCC) is the most common head and neck malignancy, representing up to 90% of oral cavity cancers. In the last decades, despite progress in therapeutic strategies of OSCC, the 5-year survival rate showed no significant improvement, remaining slightly below 50%.

Nicotinamide N-Methyltransferase (NNMT) is a drug-metabolizing enzyme that is overexpressed in several tumors, including OSCC. In particular, NNMT overexpression in OSCC inversely correlates with lymph node metastasis, pT, pathological staging and histological grading. In addition to the potential role of NNMT as a prognostic factor, the measurement of salivary NNMT could serve as biomarker for early diagnosis of OSCC. In this study, in order to further explore the biological function of NNMT in OSCC cell metabolism, we investigated the effects of plasmid-mediated overexpression of NNMT in OSCC cell line.

METHODS: Human oral cancer cell line HSC-2 was transfected with the pcDNA3-NNMT plasmid. Control cells were transfected with the empty vector (pcDNA3) or treated with transfection reagent only (mock). Real-Time PCR, Western blot, and HPLC assay were used to evaluate NNMT expression, both at mRNA and protein levels. The assessment of cell proliferation was performed with MTT colorimetric assay. Furthermore, the effect of NNMT upregulation on β-catenin, survivin, and Ki-67 expression was also investigated. Data were analyzed using GraphPad Prism software. Differences between groups were determined using the Kruskal-Wallis test.

RESULTS: Compared with mock and pcDNA3-treated, cells transfected with pcDNA3-NNMT displayed significantly increased NNMT expression levels. Real-Time PCR showed a significant NNMT upregulation, that was confirmed at protein level by Western blot analysis. Furthermore, NNMT specific activity was significantly higher in transfected cells compared with controls. The results of MTT colorimetric assay showed that pcDNA3-NNMT plasmid was able to increase cell growth of HSC-2 cells compared with controls.

In order to explore the potential involvement of NNMT in cellular pathways, such as apoptosis, cell proliferation and cell signaling, we examined whether NNMT overexpression was able to affect the expression of β-catenin, survivin, and Ki-67. The results seem to indicate a statistically significant upregulation of survivin ΔEx3 isoform in pcDNA3-NNMT plasmid transfected cells, while the expression of 3B and 2α survivin isoforms was not detectable.

CONCLUSION: Our results show that NNMT overexpression in OSCC cell line significantly increases cell growth. The effect on the antiapoptotic survivin ΔEx3 isoform seems to suggest a possible involvement of NNMT in the proliferation and tumorigenic capacity of OSCC cells.

Prognostic value of mithocondrial DNA analysis in patients with secondary oral squamous cell carcinoma

D. B. Gissi, L. Sozzi, L. Luccarini, C. Amadasi, S. Kavaja, D. Servidio, L. Alessi, A. Gabusi

Department of Biomedical and Neuromotor Sciences, Section of Oral Sciences, University of Bologna, Bologna

BACKGROUND: In head and neck oncology a novel classification of the secondary tumors was recently proposed on the basis of the clonal analysis of the tumors and the genetically altered mucosal field. Second neoplastic lesions can be classified as: Second Primary Tumors (SPTs) independent from the index tumor at the molecular level, Local Recurrences (LRs) or metastases that are instead related to the primary tumor and ''second field tumors" (SFTs), derived from the same genetically altered mucosal field as the primary tumor. The distinction between LR, SPT and SFT is not a simply problem of classification but may influence prognosis and the choice of treatment. mtDNA (D-loop) sequence analysis was proposed in previous studies as a reliable method for establishing the clonal relationship between two neoplastic manifestations. In the present study mtDNA D-loop analysis was applied in a group of consecutive patients experiencing a second loco-regional neoplastic manifestation after surgical resection of a primary Oral Squamous Cell Carcinoma (OSCC). The purpose was to evaluate differences in terms of survival rate between LRs, SPTs and SFTs.

METHODS: The study population consisted of 24 patients who experienced a second neoplastic lesion after a surgical resection of a primary OSCC. 21/24 (87,5%) were limited to the oral cavity whereas 3/24 (12,5%) presented a neck nodal metastasis (LNM) as second event. mtDNA D-loop analysis was performed by deep sequencing and phylogenetic clusterization in all index OSCCs, in all secondary events and in respective normal mucosa. Disease-free survival endpoints was defined as the duration between appearance of second neoplastic lesion and dead of disease or last follow-up visit.

RESULTS: mtDNA analysis showed 7/24 second neoplastic events (31,1%) phylogenetically related to index OSCC, and 17/24 cases (68,9%) phylogenetically independent. The genetic distinction of secondary tumours in LR, SPT and SFT was acquired on the basis of the phylogenetic relationship between normal mucosa of index OSCC and normal mucosa of secondary OSCC. All 7 clonal paired tumours showed respective normal mucosa phylogenetically related, suggesting a genetic diagnosis of LR. Among non clonal patients 3 out of 17 presented respective normal mucosa phylogenetically related, suggesting a genetic diagnosis of SPT whereas in remaining 14 out of 17 non clonal paired lesions also the respective normal mucosa resulted phylogenetically distant entities suggesting the presence of an altered mucosal field and a genetic diagnosis of SFT. The presence of an altered mucosal field in non clonal patients resulted a variable significantly related with a better survival rate ($p<.05$), indeed 2/17 (11,8%) SFTs events failed as compared to 5/7 LR. (71,4%) and 3/3 SPTs (100%).