Evaluation of hTERC (3q26) gain by Fluorescence in situ hybridization (FISH) to predict the behaviour of oral epithelial precursor lesions

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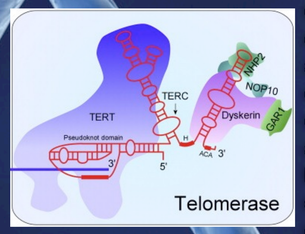
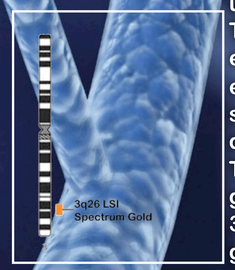
INTRODUCTION: Every year about 640.000 new cases (NIH, 2012) of head and neck squamous cell carcinoma (HNSCC) are diagnosed worldwide and two thirds occur in industrialized nations. HNSCC typically involves the oral cavity, oropharynx, hypopharynx and larynx and are characterized by substantial heterogenity, both at the clinical and molecular level. Squamous Cell Carcinoma (SCC) of the oral cavity and oropharynx are the most frequent malignant neoplasm, being found in more the 90% of patients. The prognosis of SSC is related to tumor size and 5-year survival drops from 90 % to zero across the various tumor stages. The squamous cell carcinoma progresses through a series of well defined clinical and histopathological grades in the form of precursor lesions with various degrees of dysplasia. Predication of malignancy in oral lesions can be achieved with the aid of new technologies but they are not able to completely assess epithelial alterations and biopsy is mandatory in the optimal management of the patient. At least the 50% (1) of these lesions reveal no dysplasia on biopsy and the patients undergo unnecessary surgical procedures; moreover, despite recent progress in the diagnosis and therapeutic modalities for OSSC, the 5-year survival rate has not improved in the last two decades. Therefore, an objective and noninvasive method is required for the detection of the oral lesions that may progress to malignancy. Molecular cytogenetic may help in the early diagnosis of OSSC considered that alteration in the number of chromosomes is reported to be the most consistent marker of malignancy and is the earliest and most distinctive preneoplastic genotype.(2) Aneuploidy is an early event in the development of OSSC and this is a multistep process since genetic and epigenetic events accumulate leading to cell cycle deregulation and chromosomal abnormalities (3). A consistent number of chromosomes present alteration in OSSC, both losses and gains of genes or chromosomes regions (4;5). Gain of 3q is a common feature of squamous cell carcinoma and in particular gain at 3q26 have been reported in carcinoma of different anatomic sites, including lung, cervix, esophagus and head and neck. The gene localized in this region is the human telomerase RNA component (hTERC) and encodes the RNA component of the human telomerase. Telomerase has been found to be reactivated in 90% of malignant neoplasm, including OSSC. Telomerase reactivation is a critical step in cellular immortalization and a crucial event in the multistep process of human carcinogenesis and it happens very early in the cancer progression. In the HPV-related cervical cancer the gain of hTERC, along with the integration of the oncogenic HPV in the genome, appear to be an important genetic event in the progression of cervical intraepithelial neoplasia to invasive cancer. Besides alcohol and tobacco abuse, which are considered the major risk factors for the development of OSSC, over the last decade there is overwhelming evidence that human papilloma virus plays an important role in the development of head and neck cancer(4). Based on the evidence that both integration of oncogenic HPV and gain of human telomerase RNA gene are common events in the development of preneoplastic cervical and oral squamous cell carcinoma and given that both the epithelia have similar morhphological features, we decided to evaluate the gain of hTERC in oral epithelial precursor lesion and to evaluate if this genetic alteration could predict the progression to OSSC.

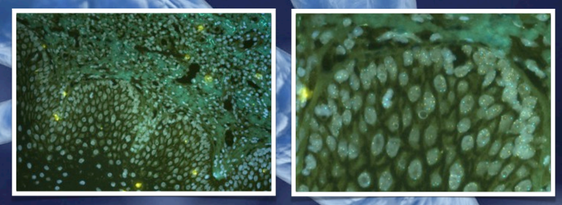


MATERIAL AND METHOD: We decided to perform a retrospective analysis on 40 oral samples with long term follow up (ranging from 5 to 10 years) with histological diagnosis of squamous cell hyperplasia (15 cases), mild dysplasia (10 cases), moderate dysplasia (8 cases) and severe dysplasia (3 cases); 2 samples are of poor quality and have been excluded from the study. All the hematoxylin & eosin slides were reviewed by two pathologists to confirm the diagnosis that were formulated using 2005 WHO classification. The formalin fixed paraffin embedded (FFPE) tissues from each case was cut into 3 µm sections and were assayed by dual-color interphase FISH. The two probes we used are: a gold locus specific (LSI) for the 3q26 region, to investigate the gain of hTERC, and an aqua centromeric probe (CEP) for the centromere of the chromosome 7 as control. The slides were analyzed with the aid of automated fluorescence microscope Ikoniskope® for counting the gold and aqua signals. Scoring was performed in the single non overlapping nuclei with clear intact nuclear membranes and signals of both probes present. The cell is considered abnormal if more than two hTERC signals were observed.



AUTOMATED TISSUE FLUORESCENCE SCANNING MICROSCOPY: The Ikoniscope platform is a robotic microscopy for whole slide digitization and analysis with true walk-away functionally and no dark room requirement. With ikonisoft explorer tissue software, areas of interest can be defined and mapped from H&E slide image acquired with the high resolution commercial slide scanner. Automated exposure setting and focusing allows localization of cells and precise signal acquisition and processing. The viewer software displays low-magnification (x10) images of all fields scanned to initially identify putative area of interest based on the algorithm that you select. High magnification (x40) images of cells are automatically chosen for analysis and presented for review. The system acquires the images and compiles a gallery for the operator to review and evaluate for significance and status.

RESULTS: We observed that 10 out of 38 samples (3 hyperplasia, 2 mild dysplasia, 3 moderate dysplasia and 2 severe dysplasia) showed tissue foci with an increase in the number of 3q26 signals. Looking at the follow up of the patients, 7 of these tissues belong to patient that developed OSSC. Only one patient progressed from mild to severe dysplasia at the time of the follow up (this doesn't exclude that he could progress to cancer in the future). The remaining 2 FISH-positive patients didn't progress to cancer or to severe dysplasia and this might be due to an effective treatment or to a radical surgery at the time of the diagnosis. Interestingly, we noticed that there were 5 patients that developed a carcinoma at the time of the follow up but our FISH results were negative and they didn't show an amplification of the hTERC gene. Probably, this might be for the fact that there are different mechanisms that leads to cancer development and progression and that not every kind of SSC in the oral cavity is related to hTERC amplification. Further experiments have to be done in order to elucidate this point. All the other tissues examined (23) didn't show an amplification of the hTERC gene and during the follow-up period they never developed cancer.



CONCLUSIONS: Our preliminary results are very promising since only those cases with the gain of hTERC developed SSC in the oral cavity. Since we have analyzed only a limited number of cases, we would like to increase the number of patients in order to evaluate if the results that we have obtained reflect the clinical outcome and the amplification of hTERC gene could be considered as an early predictive event in the development of Oral Squamous Cell Carcinoma. If this will be confirmed, the evaluation of hTERC gain by FISH could precisely predict the behaviour of oral epithelial precursor lesions and help the clinician in the early diagnosis of SSC of the oral cavity and address further clinical treatment.

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