MARKERS OF AGGRESSIVE BEHAVIOUR IN HEAD AND NECK TUMORS

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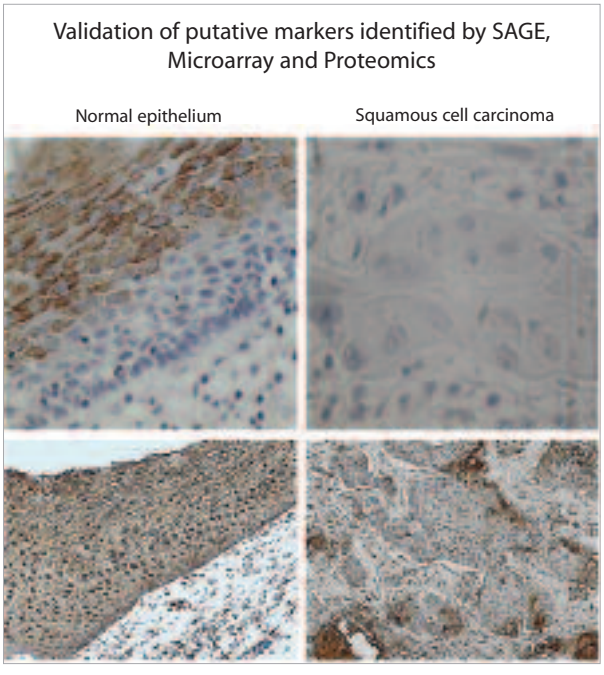

Above, KRT4 low expression in oral cell carcinomas. Below, ANXA1 low expression in larygeal squamous cell carcinomas

Head and neck squamous cell carcinoma is one of the most frequent neoplasias in Brazil. Patients in early stages frequently exhibit few symptoms, resulting in diagnosis delay and decrease in survival. Also, clinically and histologically similar lesions can follow significantly different clinical courses and show different responses to therapy. Unfortunately, despite intense research and improvements in early detection and therapeutic strategies, the prediction of tumor behavior in this group of neoplasias is still limited and the 5-year survival rate remains low.

The present study aims to investigate molecular markers of tumor aggressiveness in head and neck carcinomas that may be relevant for prognosis and therapeutic strategies. Specific aims include: (1) combining genomic and proteomic techniques, to investigate differences in gene and protein expression profiles from head and neck carcinomas and apparently normal counterparts (2) and their relations to clinical and laboratory parameters (disease stages or evolution); (3) to investigate genetic polymorphisms associated with metabolic pathways which are involved in tumor progression; (4) to validate emerging molecular markers by quantitative PCR, immunohistochemistry and genotyping in a large number of specimens.

To achieve these aims, samples collected by the team of project will be analyzed. This series includes clinically well-characterized tumor samples, surgical margins and blood samples from 1,547 patients and blood samples from 554 controls.

The data may elucidate the mechanisms governing genetic changes during tumor progression and their relation to the tissue genetic background, resulting in new insights into signalling and metabolic pathway abnormalities that could be useful therapeutic targets.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Combining genomic and proteomic techniques, differences in gene and protein expression profiles from HNSCC and apparently normal counterparts and their relations to clinical and laboratory parameters were investigated as well as genetic polymorphisms associated with metabolic pathways involved in tumor progression. Epidemiological analysis according to demographical variables and risk factors showed a higher average age in cases than controls. Most patients were males, Caucasian and smokers and more than 70% are alive 20 months after the diagnosis. Oral cavity and larynx were the most frequent sites of tumor, the latter showing the highest survival rate. There were statistical significant differences between cases and controls for all variables studied, indicating possible environmental factors to be explored in their relation with HNSCC in future analysis.

The analysis of gene expression by SAGE methodology identified subsets of differentially expressed tags between laryngeal carcinomas and normal tissues, and between metastatic and non-metastatic samples. Differential expression of a subset of genes was confirmed by quantitative polymerase chain reaction (qPCR). The product of one of these genes, ANXA1, was also investigated at the subcellular level by immunohistochemical analysis and the results showed down-regulation in dysplastic, tumoral and metastatic lesions, providing evidence for the progressive migration of ANXA1 from the nucleus towards the membrane along the laryngeal tumorigenesis.

A microarray analysis was also performed in oral squamous cell carcinomas and identified punctual differences between subsites and TNM classified samples, suggesting that oral tumors may respond differently to therapies currently under development.

Still analyzing data of HNSCC transcriptome, more than four hundred splicing events were evaluated. A subset of 43 new splicing isoforms was validated by qPCR and DNA sequencing and differences were detected between normal and metastatic samples. With respect to polymorphisms, the DNA of >250 samples was investigated for 45 single nucleotide polymorphisms. Differences of genotype frequencies between aggressive and less-aggressive oral tumors could be detected for a number of SNPs. The most significant were located on the genes *Mmp*7, *Mmp*14, *Gstp*1 and *Cdkn*1*A*. The proteomic analysis explored cancer-related proteins in HNSCC. Qualitative and quantitative variations in aggressive and less-aggressive tumors were identified by bidimensional electrophoresis and mass spectrometry, including cytokeratins, heat shock proteins and proteins involved in cell signaling, adhesion, transport, and apoptosis, indicating their potential as cancer-related markers.

One of the hallmarks of the Head and Neck Genome Project is the close interaction among the groups that perform different analyses. The continuous update and exchange of results enable a more comprehensive view of the results. By using this approach, consistent data on gene and protein expression and SNP frequencies related to aggressive and non-aggressive tumors may be achieved and the data used for predicting outcome and rational targets in HNSCC.

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