Novel Prognostic and Therapeutic Targets for Oral Squamous Cell Carcinoma

March 18, 2013

VA Morris

Oral squamous cell carcinoma (OSCC) is diagnosed in 30,000 Americans every year. If detected and treated early with surgery or radiation, five-year survival is greater than 50% for OSCC localized to the tongue, floor of the mouth, or lips. If metastases are detected in neck lymph nodes, OSCC five-year survival decreases by 50%. Furthermore, therapies for advanced disease can lead to lifelong swallowing and speech impairments. By understanding the underlying molecular mechanism of tumor progression, novel targeted therapies could be developed for OSCC. In the first study to characterize the genomic aberrations in metastatic tumor cells, Staff Scientist Dr. Chang Xu and Assistant Member Dr. Eduardo Méndez from the Clinical Research Division identified biomarkers for OSCC progression and potential therapeutic targets by analyzing gene changes associated with metastatic versus non-metastatic OSCC primary tumors.

To identify molecular differences between primary and metastatic OSCC, DNA copy number changes were correlated with gene expression changes using DNA and RNA arrays (see figure). A signature of 95 genes was identified in tumor cells from lymph node metastases (n=20) compared to non-metastatic primary tumors (n=17). These genes were associated with survival and prognosis of patients by looking at an independent set of 133 OSCC patients, where 65 patients were still alive at the end of the follow up time (mean: 5.4 years, range 4.0-7.3 years). Using the gene signature and hierarchical clustering based on the gene expression variances determined by principal component analysis, patients were divided into two clusters that correlated with the stage of disease and prognosis. The 5-year overall survival for patients in cluster 1, who had mainly primary tumors, was higher than patients in cluster 2, who were more likely to have nodal metastasis (69.1±6.98% versus 41.7±5.45%, P = 0.000858). Using multivariate analysis adjusting for sex, stage, age, and human papillomavirus (HPV) status, hazard ratios (HR) for cluster 2 patients were 3.45 (95% confidence interval: 1.84-6.50) for overall survival and 4.75 (95% confidence interval: 2.03-11.11) for OSCC-specific mortality.

Next, the authors examined if the genes unique to metastases also affected tumor growth. They established three OSCC cell lines from metastatic tumors, and two derived from early-stage primary tumors. The metastasis derived cell lines displayed a more aggressive phenotype in vitro and in vivo. Finally, 26 of the 95 transcripts with amplification and overexpression were silenced by RNA
interference in the five OSCC cell lines. In at least one of the OSCC cell lines, 18 of the 26 genes (69%) reduced cell viability. On average, the early-stage OSCC cell lines were more sensitive to gene silencing. However, silencing expression of the gene GAP SH3 Binding Protein 1 (G3BP1) decreased cell viability and increased apoptosis in late-stage OSCC. Upon further characterization, the induction of cell death by G3BP1 silencing preferentially occurred in OSCC cell lines that also had a mutation in the tumor suppressor p53. The precise mechanism for why knockdown of G3BP1 expression induces cell death remains to be elucidated. Nevertheless, G3BP1 was identified as a novel therapeutic target previously not described for head and neck cancer.

According to Dr. Méndez, this study provides “a roadmap showing which genes may be essential for the survival advantage inherent in metastatic tumor cells, and this can be exploited to selectively target these cells.” Efforts are underway to develop the identified gene signature as a prognostic biomarker for OSCC, as well as to understand how these genes mediate metastasis and proliferation. Based on the current study, Dr. Méndez was awarded a $720,000 four-year Research Scholar Grant from the American Cancer Society to further study potential therapeutic targets in OSCC.

A signature of 95 genes differentially expressed between early-stage primary OSCC tumors and late-stage metastatic tumors was identified correlating gene expression changes with DNA copy number changes. Heat-map of 1,988 transcripts with differential expression between primary tumors (yellow) and metastatic tumors isolated from lymph nodes (red) as determined by mRNA expression arrays (top panel). DNA copy number differences between primary and metastatic OSCC were determined by single-nucleotide polymorphism arrays. Red bars highlight genes with significant Z-score DNA copy number differences (bottom panel, top). The correlation coefficients of copy number changes and gene expression are show by Manhattan plot (bottom panel, bottom). Each dot represents one of the differentially expressed genes, with significant genes highlighted in red.

Image adapted from manuscript, published in the open-access journal PLOS Genetics.