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Proteome-wide analysis of head and neck squamous cell carcinomas using laser-capture microdissection and tandem mass spectrometry

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Summary Remarkable progress has been made to identify genes expressed in squamous cell carcinomas of the head and neck (HNSCC). However, limited information is available on their corresponding protein products, whose expression, post-translational modifications, and activity are ultimately responsible for the malignant behavior of this tumor type. We have combined laser-capture microdissection (LCM) with liquid chromatography–tandem mass spectrometry (LC–MS/MS) to identify proteins expressed in histologically normal squamous epithelium and matching

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SCC. The protein fraction from approximately 10,000–15,000 normal and tumor cells was solubilized, digested with trypsin, and the resulting peptides were analyzed by LC–MS/MS. Database searching of the resulting sequence information identified 30–55 proteins per sample. Keratins were the most abundant proteins in both normal and tumor tissues. Among the proteins differentially expressed, keratin 13 was much lower in tumors, whereas heat-shock (Hsp) family members were highly expressed in neoplastic cells. Wnt-6 and Wnt-14 were identified in both normal and tumor tissues, respectively, and placental growth factor (PIGF) was detected only in tumors. Immunohistochemical analysis of HNSCC tissues revealed lack of keratin 13 in tumor tissues, and strong staining in normal epithelia, and high expression of Hsp90 in tumors. Our study, by combining LCM and proteomic technologies, underscores the advantages of this approach to investigate complex changes at the protein level in HNSCC, thus complementing existing and emerging genomic technologies. These efforts may likely result in the identification of new biomarkers for HNSCC that can be used to diagnose disease, predict susceptibility, and monitor progression in individual patients.
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Introduction

Annually, it is estimated that there are close to 500,000 cancer-related deaths in the United States alone, and of these approximately 13,000 are attributed to squamous cell carcinomas of the head and neck (HNSCC), making it the sixth most common cause of cancer deaths.[1] Even though risk factors for HNSCC, such as the use of tobacco and alcohol, are well documented, a distinctive lack of suitable pre-malignant markers for early detection and risk assessment is clearly reflected by the fact that more than 50% of all HNSCC patients have advanced disease at the time of diagnosis.[2–4] Indeed, the five year survival rate of HNSCC patients is in general poor, less than 50%, and the prognosis of the advanced HNSCC cases have not changed much over the past three decades.[5] This limits the treatment options and renders management of HNSCC extremely challenging.[6] Thus, the ability to identify and confidently predict malignant progression of HNSCC lesions will result in a reduction in mortality, by aiding in early diagnosis and treatment of this disease.

Expression profiling using various microarray platforms, and large-scale cDNA sequencing projects, such as CGAP (cancer genome anatomy project), have led to a plethora of publicly available information on gene transcripts, which has proven to be fundamental for research efforts related to understanding both human biology and disease states.[7–9] Despite this, there is only limited information available on the gene products, currently estimated to be over 1 million proteins in a single cell, which play vital roles in most key cellular processes.[10] Until recently, the analysis of a cell proteome using two-dimensional gels (IEF and SDS-PAGE) and mass spectrometry, was deemed technologically challenging.[11] For instance, improved instrumental advances and the coupling of HPLC to electrospray mass spectrometry combined with the rapid growth in genomic databases amenable to searching with mass spectrometry data, now affords the opportunity to develop high-throughput proteomic approaches to identity minute amounts (typically femtomoles) of proteins present in complex samples.[10,12,13] In that context, comparative analysis of the proteome in disease and normal cells, selectively procured by the use of laser-capture microdissection (LCM), is a critical step in the validation of the results because of the inherent clonal heterogeneity of most human cancers and the presence of host cells (fibroblast, endothelial and inflammatory cells).[14]

In this study, we have used LCM to isolate 10,000–15,000 normal and tumor epithelial cells from clinical samples of HNSCC, combined with mass spectrometry, to explore the feasibility of establishing a pattern of expressed cancer-related proteins for HNSCC. Our findings indicate that these approaches generate large proteomic datasets from minimal clinical samples that is likely to lead to the identification of novel HNSCC protein biomarkers. Indeed, some of the emerging protein information has already provided evidence of the expression of molecules that might be involved in tumor