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Proteomic analysis of different stages of head and neck cancer

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For early diagnosis and prevention of primary head and neck squamous cell carcinoma (HNSCC) and recurrent disease, it is highly relevant to detect genetically altered fields of pre-malignant cells and characterize encoded sets of proteins. In order to identify changes in protein expression occurring at different stages of tumorigenesis, a large selection of clinical frozen biopsies (n=281) including tumors, tumor-adjacent, tumor-distant, and healthy mucosae were analyzed by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS). Protein profiling generated a total number of 78 informative peaks among the groups analyzed, of which 48 were differentially expressed between HNSCC and healthy mucosa. From these peaks, 14 candidate biomarkers in the mass range between 3 and 40 kDa were further enriched and submitted to protein identification. Most of the identified proteins were down-regulated in HNSCC and included the calgranulins A and B, annexin 1, cystatin A, calgizzarin, acyl-CoA-binding protein, stratifin, and beta-hemoglobin. The expression of alpha-hemoglobin and histone H4 remained stable in all groups analyzed. Proteins that showed up-regulated expression in HNSCC were identified as the α-defensins 1-3 and as the c-terminal fragment of beta-hemoglobin. Cystatin B and annexin 2 were also identified but the peaks representing these proteins could not be detected.

Independent approaches were used for supervised class comparison and class prediction analysis. The first approach applied a selection of peaks with a >4 signal-to-noise ratio and with a high power to discriminate between the groups of tissues. HNSCC and normal mucosae were used as training set in this approach, and the additional tumor-adjacent mucosae and tumor-distant mucosae were combined as test set. The predictor allowed excellent classification of both healthy mucosa and HNSCC samples (> 90% of the samples of each group were correctly classified), and distinct and increasing changes in the tumor-distant and tumor-adjacent mucosal biopsies. In the second approach, all data points were evaluated independently but different classifiers were trained and applied to different test sets. A classifier trained on HNSCC and healthy mucosa samples was tested on the tumor-distant mucosa group only, and approximately 11% of the tumor-distant mucosae were misclassified as tumors. Another classifier trained on all mucosa samples predicted correctly the healthy mucosa group and showed an overlap between the tumor-distant and tumor-adjacent mucosa samples, with the latter again showing increased changes in protein expression. Thus, both approaches confirmed the hypothetical axis normal-distant-adjacent-tumor, and very accurately distinguished healthy mucosa samples taken from healthy patients from healthy appearing mucosa samples taken from tumor patients. Finally, immunohistochemistry on tissue microarrays containing HNSCC (n=156) and tumor-adjacent mucosae (n=35) was applied to validate the results of proteomic profiling. The expression patterns of the newly identified candidate biomarkers cystatin A, cystatin B, annexin 1, stratifin, alpha-depensins1-3, calgranulin A, calgranulin B was in full accordance with the profiling results. The comparison with some well known markers of cell cycle control, regulation of proliferation and apoptosis (ATM, ki-67, p27, p63, pRb, p53, p16, cdk2, c-myc), differentiation (cytokeratin 14, vimentin), cell adhesion and extracellular matrix (E-cadherin, fibronectin) revealed some interesting and partly unexpected interactions. These findings indicate the existence of genetically altered fields, both in close vicinity to and at considerable distance from the primary tumors. It is expected that the procedure may be clinically useful to a rapid and more reliable cancer-risk assessment. We conclude that proteomic profiling in conjunction with protein identification outperforms histopathological diagnosis and may have significant predictive power for clinical outcome and personalized risk assessment.