RESEARCH ARTICLE

Identification of proteins secreted by head and neck cancer cell lines using LC-MS/MS: Strategy for discovery of candidate serological biomarkers

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Received: March 20, 2010
Revised: February 13, 2011
Accepted: February 21, 2011

In search of blood-based biomarkers that would enhance the ability to diagnose head and neck/oral squamous cell carcinoma (HNOSCC) in early stages or predict its prognosis, we analyzed the HNOSCC secretome (ensemble of proteins secreted and/or shed from the tumor cells) for potential biomarkers using proteomic technologies. LC-MS/MS was used to identify proteins in the conditioned media of four HNOSCC cell lines (SCC4, HSC2, SCC38, and AMOSIII); 140 unique proteins were identified on the basis of 5% global false discovery rate, 122 of which were secretory proteins, with 29 being previously reported to be overexpressed in HNOSCC in comparison to normal head and neck tissues. Of these, five proteins including α-enolase, peptidyl prolyl isomerase A/cyclophilin A, 14-3-3 ζ, heterogeneous ribonucleoprotein K, and 14-3-3 σ were detected in the sera of HNOSCC patients by Western blot analysis. Our study provides the evidence that analysis of head and neck cancer cells' secretome is a viable strategy for identifying candidate serological biomarkers for HNOSCC. In future, these biomarkers may be useful in predicting the likelihood of transformation of oral pre-malignant lesions, prognosis of HNOSCC patients and evaluate response to therapy using minimally invasive tests.

Keywords:
Biomarkers / Cell biology / Head and neck cancer / 1-D LC-MS/MS / Secretome / Secretory proteins

1 Introduction

The interactions between cancer cells and the host's dynamic microenvironment play vital roles in tumor growth, invasion, and metastasis [1]. The cancer cells and the host's microenvironment secrete and shed proteins or their fragments extracellularly and into bodily fluids, including blood. These proteins and their fragments have been described to constitute the ''cancer secretome'' [2]. As about a quarter of all cellular proteins are secreted, many proteins relevant to carcinogenesis may be detectable in the blood or other bodily fluids [3, 4]. Sampling of bodily fluids is relatively straightforward, is minimally invasive, and can

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Abbreviations: 14-3-3 σ, stratifin; **CE,** collision energy; **FDR,** false discovery rate; **HNOSCC,** head and neck/oral squamous cell carcinoma; **hnRNPK,** heterogeneous nuclear ribonucleoprotein K; **IDA,** information-dependent acquisition; **IPA,** Ingenuity Pathway Analysis; **OPLs,** oral pre-malignant lesions; **PPIA,** peptidyl prolyl isomerase A/cyclophilinA

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