Abstract:

Head and neck cancer is the sixth most leading cancer worldwide, both in men and women. In India, it is one of the leading cancers in both of the sexes. Tumorigenesis is asymptomatic and patients with head and neck cancer are often diagnosed at an advanced stage due to lack of suitable markers for early detection. Surgery, radiotherapy, chemotherapy, chemo radiotherapy and targeted therapy are the currently available treatment options in head and neck cancer. Any combination of the methods or a single method is used in the treatment. Surgery is preferred in early stages of the disease. In advanced lesions, failure of the tumor to respond to chemotherapy or radiotherapy contributes to poor outcome in HNSCC. Currently used biomarkers are not satisfactory due to their limited specificity and/or sensitivity, which is evident from the overall 5-year survival rate of less than 40%. With the aim to identify potential biomarkers in HNSCC we analyzed the differential proteomic profiles of HNSCC cell secretome and total proteome. For this purpose we had undertaken an in vitro isobaric tag for relative and absolute quantitation (iTRAQ)-based labeling approach followed by LC-MS/MS analysis. Through this approach, several differentially expressed proteins were identified in the HNSCC cell secretome compared to normal cell line secretome. Of the differentially expressed proteins, quite a few proteins were known to be involved in HNSCC development along with other novel proteins which were not known previously. Olfactomedin 4 (OLFM4) was one among the novel candidates that was found to be 12-fold abundantly secreted in HNSCC secretome. Immunohistochemical validation for OLFM4 and insulin like growth factor binding protein 3, (IGFBP3) was carried out in HNSCC tissues using tissue microarrays. OLFM4 and IGFBP3 were found to be overexpressed in 70% and 75% of the tested cases respectively. Western blot analysis confirmed overexpression of these proteins in HNSCC secretome. In another study, iTRAQ-based quantitative proteomics was carried out to identify proteins that were differentially expressed in HNSCC cell lines.
compared to normal cell line. This led to the identification of several known and novel proteins. Among other proteins HMGB2 was overexpressed across all the HNSCC cell lines used in the study. Overexpression of HMGB2 was further validated in HNSCC TMAs using immunohistochemistry. We addressed the role of HMGB2 in chemoresponsiveness of HNSCC cells to cisplatin and 5-FU. siRNA mediated silencing resulted in increased sensitivity to both cisplatin and 5-FU. Our study indicates that HMGB2 plays crucial role in chemoresponsiveness against these two major drugs. Further studied are needed to understand the exact molecular mechanism associated with role of HMGB2 in conferring resistance in HNSCC. Through this study, a robust pipeline to discover biomarkers has been established. The first phase includes discovery of biomarkers using mass spectrometry-based platforms followed by large scale validation using tissue microarrays. The second study reveals HMGB2 as a potential therapeutic target. The candidate markers identified in this study should be tested in larger cohort of patients if they were to be used in the clinic settings.