Chapter 6

Concluding remarks and Future perspectives

In this study, through two different approaches, genomic and proteomic, potential biomarkers for gastric cancer have been identified.

One of the major outcomes of the gene expression analysis study was the identification of a novel marker SPOCK1/Testican-1 that was significantly upregulated in gastric cancer. By large scale validation using tissue microarrays, we were also able to show that this protein was overexpressed in more than 60% of the cases tested. Testican-1 is an extracellular proteoglycan. Though it has been shown to be associated with other malignancies, its role in tumorigenesis is not clear. Further studies are needed to elucidate its function in the context of gastric cancer. siRNA-based knockout studies could be carried out in cell line models or animal models to elucidate its role in gastric carcinogenesis. In addition, through this high-throughput study, we could identify many candidates that have been discovered previously by other studies. This shows that these candidates are repeatedly identified by different studies and they could be pursued further and should be investigated in detail for their prevalence, role in carcinogenesis and whether they could be targeted for therapy.

Proteins that are secreted by tumor cells and not by normal cells are of particular interest in discovering prospective markers. However, it is challenging to identify such candidates. Cell line-based models are proven beneficial to study proteins that are secreted from diseased tissues which would otherwise be harder to detect by direct analysis of plasma from patients. Secretome from gastric cancer cells would mimic the fluid compartment that is proximal to the gastric tissues. In this study, the proteins secreted by gastric cancer cells were analyzed by SILAC labeling followed by mass spectrometric analysis. This analysis resulted in the identification of 1,733 proteins in the gastric cancer secretome, out of which 268 proteins were differentially expressed in tumor cells secretome as compared to secretome from normal cells. This depth of analysis has not been achieved by previous studies on secretome. We could accomplish this by using a high resolution mass spectrometer that is highly sensitive compared to other platforms. Interestingly, many proteins identified in this study have not been reported earlier to be present
in gastric cancer. We could validate few candidates, \textit{PCSK9}, \textit{LMAN2}, \textit{LGALS4} and \textit{PDAP1} in a larger cohort of patients. A larger majority of these proteins were known to be localized in the extracellular region or found to be detected in body fluids. Through this study, we have demonstrated that SILAC coupled with mass spectrometry serves as a robust pipeline to analyze secretome. We anticipate that proteins that are identified in secretome could potentially be identified in plasma of the patients with gastric cancer. As the next step, proteins identified from secretome will be tested in the blood samples of patients with gastric adenocarcinoma.

Considering the complexity of plasma, there is an increasing awareness of the use of other body fluids in biomarker detection, in particular urine\textsuperscript{214}. Though urine was routinely used in clinic for pregnancy test or to detect certain metabolic disorders, it was not viewed as a complex fluid. Until recently, it was believed that there is only handful of proteins in urine. Advancement in technologies has revolutionized this concept. Studies in the past ten years have revealed the potential of urine as alternative source of biomarkers with proteins present at detectable levels. Biomarkers have been detected for few cancers arising in prostate, bladder, kidney and ovaries\textsuperscript{214}. However, there are no studies carried out till date to discover gastric cancer biomarkers in urine. As a first step, we have carried out a comprehensive proteomic profiling of urine from healthy individuals. In this study, we have reported the maximum number of identifiable proteins in normal urine (\textit{Journal of Proteome Research}, 2011, 10: 2734-2743). The rationale behind this study was to create a reference list of proteins identifiable in urine under normal conditions without any disease. The putative biomarkers identified in gastric cancer can be compared to this reference list to choose urinary markers that could further be validated.