Biomarker discovery for cervical cancer
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Chapter VII.

Summary and perspectives

This thesis describes the analysis of serum samples of cervical cancer patients before and after treatment using LC-MS of trypsin-digested serum followed by data processing and statistical data analysis. Paired samples from the same patients before treatment and after remission were selected in order to optimize the chance of discovery cancer-related changes in the proteome. While we did not discover major changes in the serum proteome, some changes in protein composition were found in samples taken before and after treatment. It is thus demonstrated that the described methods are applicable to highly complex body fluids such as serum and that further studies into the relevance of the discovered changes of the serum proteome are warranted.

At present, we are able to discriminate serum proteins in healthy (patients after successful treatment) and cancer patients at a concentration which is still considered to represent “classical serum or plasma proteins” [1]. Changes in those proteins reflect the condition of patients in general. We have to reach lower concentration ranges for disease-specific biomarkers (ng/mL range). For this purpose, protein enrichment will be very helpful (e.g. an enrichment strategy for less abundant serum glycoproteins).

Proteins such as cytokines, growth factors, cycling dependent kinases (CDKs) and inhibitors of cell cycling belong to the very low-abundance proteins in the cell. Although DNA microarray studies demonstrate that they are very important in the diagnosis of malignant disease [2], the staging of cancer and the characterization of the cell cycling in general, these proteins are not readily detectable in proteomics studies. Part of the problem originates from the source of the samples: cell cycle controlling proteins are localized in
the nucleus. It may thus be of interest to select the most appropriate type of tissue and to perform subfractionation of cell organelles prior to MS analysis.

The problem of biological variance of protein concentrations that are unrelated to disease is very common and well recognized in cancer biomarker studies. In our case it is necessary to analyze additional patient samples and to perform more extensive longitudinal studies to discriminate between actual cancer markers and unrelated confounding effects.

The importance of genome-wide searches for disease markers in cancer has been proven with DNA microarray studies [3]. Microarrays are increasingly important tools for the diagnosis and classification of various cancers [4-6]. Expression profiles with microarrays in their present form are capable to detect nanogram quantities of mRNA, but say very little about the corresponding protein levels in normal and malignant tissues.

The technology of oligonucleotide-based arrays is well developed and well understood, which is not the case for attempts to create similar protein microarrays [7]. In this respect, the LC-MS approach offers additional possibilities to the protein-chip concept. The performance of mass spectrometers has been steadily improving in recent years and present modern LC-MS systems are capable of analyzing complex protein/peptides samples generating thousands of peaks. Whether these analyses are comprehensive is still doubtful (limitation of proteomics methods with respect to concentration sensitivity) but it is fair to assume that a considerable part of the proteome can be covered. It has become increasingly clear that the successful integration of sample preparation, protein/peptide separation and data analysis is key to an overall successful biomarker discovery strategy.

Data pre-processing and statistical analysis of comprehensive LC-MS profiles is a rather new field. In collaboration with other groups, we are working on further improving our statistical analysis platform, since it is a crucial step in the discrimination of samples and the search for potential biomarkers.

By nature, cervical cancer is a localized disease at its early stage and will therefore quite often escape the general immune response mediated by natural killer cells and T-cells circulating in blood. Because of such a limited exchange between proteins of the cervical epithelium and blood, finding specific biomarkers seems more promising in comparative studies of cervical cancer and healthy tissue. Work along this line has been initiated using Laser Dissection Microscopy in conjunction with mass spectrometry.

Although an effective vaccine against Human Papillomavirus (HPV) will most probably decrease the incidence of cervical cancer significantly, early diagnosis still remains relevant. Moreover, finding biomarkers for cervical cancer can help in other cases where HPV virus seem to be involved. As an example, HPV 16 was found in lung carcinoma cases in Chile [8] and Korea [9].
It is thus quite likely that this virus can infect other kinds of epithelia than the cervix alone.

Finally, I would like to stress that only well-integrated, multi-disciplinary studies will have the potential to discover relevant and new biomarkers in cervical cancer, resulting in earlier diagnosis and thereby better treatment of patients with cervical neoplasia.
References


