Glycosylation profiling with mass spectrometry: method development and application to cancer biomarker studies

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English Summary

Glycosylation profiling with mass spectrometry

Clinical assays on body fluids are routinely performed for diagnostic and therapeutic purposes. Although an indispensable tool, it is widely acknowledged that such measurements of biomolecule concentrations leave room for improvement with regard to sensitivity and specificity. Moreover, for many diseases a detection modality based on a blood or urine specimen is not yet available and new biomarkers are urgently needed. In the case of cancer early detection often improves survival. Due to a globally increasing incidence of disease the screening for cancer turns more important. For pancreatic cancer, magnetic resonance imaging is currently considered the gold standard for high-risk patient screening, which is a relatively expensive technique. For breast cancer screening purposes, mammography is routinely applied although the diagnostic performance of this technique is rather limited, with for example a low sensitivity for dense breast tissues and low specificity.

Molecular markers are foreseen as a valuable addition to the imaging screening techniques currently used. Consequently, many studies have focused on method development for exploring biomarkers aiming for early detection. In a joint effort clinicians and researchers have been searching such markers since the early days of omics. Especially blood-based markers are of interest due to low invasiveness and therefore limited risk for the patient. Both for breast cancer and pancreatic cancer multiple protein candidate markers have been reported, however for various reasons these have not found their way into the clinic yet. An alternative strategy for finding new markers involves the exploration of post-translational modifications (PTMs) on proteins, or more generally profiling proteoforms. One of these PTMs is protein glycosylation, and is known that the expression of glycosyltransferases is altered in cancer, which has stimulated the biomarker field to search for candidate markers that relate to differences in protein glycosylation.

In the first part of this thesis the current clinical tests for the detection of pancreatic- and breast cancer are described to provide more insight into the need for new diagnostic methods (**Chapter 1**). Additionally, in this chapter the basics of human glycosylation and the biosynthetic pathway of glycans are outlined. Besides the biosynthesis, also the biological roles and the known relation with cancer are described. Moreover, the basic principles of glycosylation analysis and the analytical methods, for example

derivatization and mass spectrometry (MS), applied in this thesis are elaborated on.

The second part of this thesis is focused on the development of methods for glycosylation analysis (**Chapter 2, 3 and 4**). A previously established high-throughput serum/plasma protein glycosylation analysis method was further developed (**Chapter 2**). Side-reactions that can occur during the derivatization reaction were minimized, which resulted in a less complex mass spectrum. Additionally, the purification protocol was adjusted and glycan profiles were measured with matrix-assisted laser desorption/ionization (MALDI) Fourier transform ion cyclotron resonance (FTICR)-MS. This ultrahigh resolution mass analyzer is a highly sensitive and accurate platform for detection and assignment of glycans. The optimized method was extended to enable dried blood spot analysis in addition to serum- or plasma analysis (**Chapter 3**). From a clinical perspective, dried blood spots are easier to collect and convenient for shipment. The influence of storage time and temperature were evaluated. Sampling of dried blood spots is a promising method for specimen collection for released glycan analysis. In the last chapter of the method development section, the analysis of overlapping glycan signals with MALDI-FTICR-MS was explored (**Chapter 4**). The analysis of mono- and disaccharide glycan structures is generally difficult with MALDI-MS, as these signals are often located in the mass range where many matrix signals are present. These small glycans are also difficult to detect with other analytical platforms, such as porous graphitized carbon liquid chromatography-MS. Ultrahigh resolution MALDI-FTICR-MS combined with absorption mode readout resolves overlapping isobaric signals and successfully identifies such small glycans in complex cell line samples.

In the third part of this thesis, the developed serum glycan profiling method (**Chapter 2**) was applied to study the glycosylation profiles of pancreatic cancer and a similar method was used for breast cancer patients in order to evaluate glycan signatures as potential biomarkers for the detection of these cancers (**Chapter 5 and 6**). Released *N*-glycan profiles from two independent cohorts, both consisting of serum from pancreatic cancer patients and healthy controls, were measured and evaluated for the difference in glycosylation between the cases and controls (**Chapter 5**). Here, increases in branching and fucosylation were found and additionally a shift in sialic acid linkage was seen. The discriminating performance of the classification model built with these glycosylation features was good, which indicates the potential of glycosylation for detection of pancreatic cancer. In the breast cancer study, the released *N*-glycan profiles from breast cancer patients and healthy control were compared (**Chapter 6**). Interestingly, only three glycans were found to differ between cases and controls and also findings reported in literature were not replicated in this

study. However, it is noted that the findings reported in literature are very diverse and often contradicting each other. It is therefore hypothesized that the absence of a strong glycomic difference between cases and controls might be explained by the heterogeneity of the disease.

In the last part of this thesis a general discussion on biomarker research is provided (**Chapter 7**). Here, standardization of (pre-)analytical methods and the use of software for data-processing and interpretation is discussed. Additionally, the difficulties of translational research and cancer biomarker research are reflected on. Although the currently applied single biomarkers are an attractive starting point due to their straightforward cut-off values, the combination of multiple markers might allow to better distinguish between different diseases. In the future, this might be helpful in the development of blood tests for the early diagnosis of cancer and other diseases.