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Investigation of Cancerous Tissue by MALDI Mass Spectrometry Imaging

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Multiple alterations of a normal cell can lead to a localised tumour that potentially develops the ability to become invasive and metastasize. Proteolysis of the surrounding tissue environment is driven by proteases, as they are involved in degradation of e.g. extracellular matrix. Proteases are a class of tightly regulated enzymes that hydrolyse peptide bonds. To investigate the spatial distribution of enzyme activity, typically fluorescence-based substrate reporters are developed for *in vivo* and *in vitro* applications. The design of such chemical probes is complex and requires knowledge about the target enzymes' specificity. The topical application of fluorescent probes to frozen tissue has rarely been done but enables the visualization of specific proteolytic activity. Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) is an analytical tool with increasing acceptance for tissue analysis in pre-clinical applications. The technique offers the spatially resolved analysis of hundreds of molecules at a time by their mass-to-charge ratio (*m/z*). Enabling the visualization of protease activity by MALDI MSI, without the necessity of extensive probe design, would have a major impact on the investigation of proteases in cancerous tissue. An absolute requirement for all newly developed methods, but especially if they should be transferred to clinical applications, is repeatability and reproducibility. However, MALDI MSI studies currently still lack a rational, score-based evaluation of repeatability. Thus, this thesis had the following aims:

1. Develop a method for monitoring of tissue protease activity by using MALDI MSI on different porcine tissues.
2. Validate the developed method by their time-dependence, concentration-dependence of chemical inhibition, as well as by the identification of specific protein degradation products. Furthermore, use a gastric tumour mouse model to evaluate differences in protease activity for tumorous tissue compared to non-tumourous tissue.
3. Demonstrate the transferability of the developed MALDI MSI method to relevant clinical samples, by analysing proteolytic activity in human tissue biopsies from gastroscopy.
4. Develop scores for the analysis of repeatability by using clinical material and compare published on-tissue digestion workflows for MALDI MSI in order to identify the best method.

The main results of this dissertation are:

1. Development of a method for visualizing the degradation of a universal protease substrate (substance P) on porcine tissues. An optimization system using water-sensitive paper and the dye rhodamine B was developed to ensure homogeneous substrate application and minimised delocalization effects.
2. Time- and protease inhibitor-concentration-dependent substance P degradation was shown in time-course experiments based on the proteolytic activity of different porcine tissues. Transiently occurring C-terminally cleaved peptides from substance P were identified by mass spectrometry. In addition, their spatial distribution could be imaged. With these lines of evidence, the decrease of the substance P signal could be correlated to a proteolytic digestion process. Pancreatic tissue showed the fastest degradation, whereas muscle tissue showed no degradation of substance P. The different proteolytic activities of porcine tissues were confirmed by adapting a photometric method using protein extracts and therewith quantifying protease activity. Furthermore, the method was applied to a gastric mouse tumour model which showed higher protease activities for tumorous compared to non-tumorous tissue.

3. The method was shown to be transferable to human gastric tumour biopsies. In order to get significant biological results, clinical studies with large and more defined patient cohorts are required.
4. Scores for evaluating the homogeneity of the application system as well as repeatability of on-tissue digestion protocol for MALDI MSI were developed. With the scores, one out of several published methods was identified to be superior to the others. The main difference was a prolonged incubation time that might be responsible for good repeatability.

In summary, the developed MALDI MSI method for visualising proteolytic activity in frozen tissue sections holds a high potential to become an important tool in pre-clinical studies.