Expanding the proteome: Advancing proteomics methodologies to uncover new insights into cancers

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The dynamic interconnection between proteomics and cancer biology necessitates sensitive and reproducible methodologies to decode intricate molecular mechanisms, especially in clinically relevant scenarios. Mass spectrometry (MS)-based proteomics is a powerful analytical instrument for profiling and quantifying proteins in biological samples. This thesis focuses on developing and broadening the application of MS-based proteomics technologies to advance the understanding of cancer biology in order to improve the diagnosis and treatment of cancer. The first part of the thesis describes the application of a microscale proteomics method, microdroplet processing in one pot for trace samples (microPOTS), which can be used to identify and quantify proteins from small numbers of cells. microPOTS was applied to identify proteomic changes in Barrett's esophageal cells following physiologic and radiation stress exposure. From a small population of cells, microPOTS demonstrated relatively high proteome coverage with the identification of >1500 protein groups and achieved high quantitative reproducibility.

The second part of the thesis focuses on developing and applying a data-independent acquisition (DIA) MS proteomics workflow to identify and quantify proteins from formalin-fixed paraffin-embedded (FFPE) tissues. This workflow was applied to glioblastoma (GBM) FFPE tissue microdissections, and more than 1700 proteins were detected, and over 1400 proteins were quantified. GBM-relevant proteins (e.g., GFAP, FN1, VIM, and MBP) were quantified with high precision (median coefficient of variation <12%). In addition, immune-related proteins (e.g., ILF2, MIF, and CD38) were consistently detected and quantified. The strategy holds great potential for routinizing protein quantification in FFPE tissue samples.

The third part of the thesis focuses on investigating the impact of hypoxia on antigen presentation in GBM using an integrated approach combining MS-based proteomics and immunopeptidomics strategies. Hypoxic stress induces significant changes in the GBM proteome. Key enzymes (ERAP1 and ERAP2) in the antigen processing and presentation

machinery (APM) were downregulated in hypoxia. Moreover, the findings revealed that HLA-I-associated antigen peptide repertoire was reduced under hypoxia. Overall, these results open new horizons to further explore the interplay between hypoxia and antigen presentation.

Collectively, this thesis presents a series of studies that have advanced the field of proteomics and its application to cancer biology, especially GBM tumors. The development of new proteomics technologies and their application to cancer research has the potential to improve the understanding of cancer biology and to develop new diagnostic and immunotherapeutic strategies.