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Analysis of histone deacetylase drug target activation by whole-cell-MALDI-TOF mass spectrometry fingerprinting and imaging

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Targeted therapy approaches have prompted a rethinking in cancer combat, and more and more such targets have been identified and become the focus of current research. Epigenetic modulators such as Histone Deacetylases (HDAC) play an essential role in maintaining the epigenetic balance, and their dysregulation can contribute to neoplasia. Epigenetic drugs targeting HDACs have evolved as a promising drug class for cancer treatment, and the development of potent and selective small-molecule inhibitors (HDACi) is of major interest for clinical oncology and industry. However, analytical workflows are required to confirm drug binding to and activation of their enzyme targets, generally referred to as target engagement. Monitoring of target activation after drug binding (target engagement) is not self-evident and represents a crucial step for estimating drug efficacy during the drug discovery process and requires high analytical and technical know-how. Due to its simplicity and analytical speed whole-cell-MALDI-TOF-MS has evolved as the standard technique for classification and identification of microorganisms in clinical chemistry. Therefore, the establishment of an analytical workflow based on WC-MALDI-TOF fingerprinting and MALDI imaging allowing specific measurements of drug target engagement (e.g HDACi and Tyrosine Kinase Inhibitors TKis) would have an essential impact on the development of future targeted therapeutics. Therefore, this thesis focuses on the following objectives:

1. Development and evaluation of a sensitive, robust and automated WC-MALDI-TOF MS method for the classification of primary blood cells and continuous cell lines
2. Application of this method for quantitative and qualitative studies of cell responses upon cell treatment with targeted drugs (HDACi and TKi).
3. Use of MALDI-imaging mass spectrometry to study target engagement of HDACi in murine tumor models

The main results of this dissertation are:

- Application of WC-MALDI TOF MS method for biotyping of mammalian cell lines and primary blood cells shows high technical and biological reproducibility reflecting long term fingerprint robustness, high sensitivity and specificity. This could allow in the long term the generation of cell fingerprint databases, in concordance to the bacterial fingerprint analysis.
- Fingerprints of TKi treated CML cell line revealed high specificity, indicating the possibility of a drug response-specific fingerprint.
- Use of WC-MALDI-MS biotyping to study the proximal response of cultured cells to HDACi by specifically monitoring the hyperacetylation status of histone core proteins: Development of a quantitative histone biotyping-based cell based assay to determine HDAC inhibitory potency in whole cells.
- First demonstration that the pharmacodynamic response of a drug, Panobinostat, could be specifically determined in murine tumor regions by MALDI imaging MS, supporting the tumor specificity of HDACi.

In summary, this study suggested that mammalian cell biotyping by MALDI-MS has a high potential for analyzing targeted therapeutics. In addition, MALDI-MS biotyping could become a complementary tool during the drug discovery process.