DERIVATIZATION STRATEGIES FOR SENSITIVE MONITORING OF SMALL MOLECULES ON TISSUE SAMPLES

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Study of small molecules (mostly known as metabolites) for biomarker discovery consists of a complete set of metabolites in complex biological systems (databases contain ~ 115 000 metabolites). Metabolome is much smaller than proteome (1.8 million different proteins) making it relatively simple for data analysis. On the other hand, metabolites are chemically diverse compounds that occur at vide range of concentrations (ten orders of magnitude) which makes metabolomics analysis challenging.

Gas/Liquid Chromatography-Mass Spectrometry (GC/LC-MS) has become the gold standard for metabolome analyses of clinical samples. However, these approaches require tissue homogenization prior to analysis and they are only hardly able to provide information concerning the spatial localization molecules of interest. MALDI MSI offers a label-free, unbiased visualization of the spatial distributions of biomolecules without almost any sample preparation prior to analysis. This method easily correlates with different histopathological features of the tumor tissue, allowing further insights into the tumor environment.

Various chemical derivatization procedures have been extensively studied to enhance ionization efficacy, modify retention time, and shift molecular mass to a higher value. Functional groups including aldehyde, primary amines, carboxylic acid, and hydroxyl group have been targeted using derivatization strategies. Derivatization process can differ regarding sample types: in-solutions derivatization for the MS homogenous biological solutions (cell/tissue extract, plasma, and other biological fluids) or on-tissue derivatization concerning the spatial localization of biological markers - Matrix Assisted Laser Desorption/Ionization-Mass Spectrometry Imaging (MALDI-MSI).

Herein, the method for the on-tissue derivatization of various small molecules has been tested and optimized for the salivary gland tissue. Three derivatization reagents have been used: Girard reagent T (GirT) – carbonyl group, coniferyl aldehyde (CA) – primary amine group, and 2-fluoro-1-methylpyridinium p-toluenesulfonate (FMP-TS) – hydroxy group. Spatial distribution of derivatized/non-derivatized molecules detected in MALDI-MSI has been visualized using commercial software as well as using in-house developed script in R and Python programming language. Mass spectra processing and exploratory analysis using commercial software were compared with in-house developed pipelines. MALDI-MSI spatial segmentation maps were compared with the histopathological annotation. These results underline the potential of on-tissue derivatization MALDI-MSI for sensitive detection of metabolite's spatial distribution on tissue samples.

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