A 2D and 3D MALDI FT-ICR imaging strategy to investigate metabolic changes in sperm maturation during epididymal transit

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Introduction

Over the past years, MSI has demonstrated its potential to construct 3D models from 2D data acquired from successive sections of an organ. Although mass spectrometers are now faster, the key limitations of 3D-MSI remains the time-consuming acquisition and processing of the huge amount of data generated, particularly when a high-resolution mass spectrometer is used. In this context, the METASPACE European project has led to a complete workflow combining specific statistical methods with m/z annotation engines and 3D reconstruction tools. In the rat, the epididymis is a single narrow tightly-coiled tube divided into 3 main parts (caput, corpus and cauda) and in 19 intra-regional segments defined by histology. Spermatozoa released by the testis are morphologically complete but they acquire their mobility and fertilizing capacity during their transit through the epididymis. The specific mechanisms underlying the male gamete maturation are based on sequential modifications of the spermatozoa surface and of the epididymal sperm micro-environment. As we suspect that a large number of metabolic events occurs in the epididymis, we used the METASPACE workflow to investigate the post-testicular sperm maturation by MALDI imaging.

Methods

Whole rat epididymides were collected and wrapped loosely in an aluminum foil filled with 2% CMC to preserve the organ shape. The epididymis was then cut in two parts named “head” and “tail”(Figure 1A). MALDI images were acquired at a spatial resolution of 50 µm using a FT-ICR Solarix XR 7 Tesla. SCiLS Lab Premium 3D (version 2018b) was used to perform spatial segmentation and to determine m/z-values discriminating each of the clusters observed. Analyzed sections were further exported under the imzML format to the METASPACE molecular annotation engine [1]. FDR-controlled metabolite annotations against HMDB were assigned by METASPACE (on the basis of accurate mass and matched isotope distributions measured with the high-resolving mass spectrometer). Finally, we used 3D-MALDI MSI to access and determine in a large-scale approach the volumetric distributions of metabolites identified in the rat epididymis with SCiLS Lab Premium 3D (version 2018b).

Results

Spatial Segmentation

21 spectral clusters were found and compared to the 19 intraregional segments.

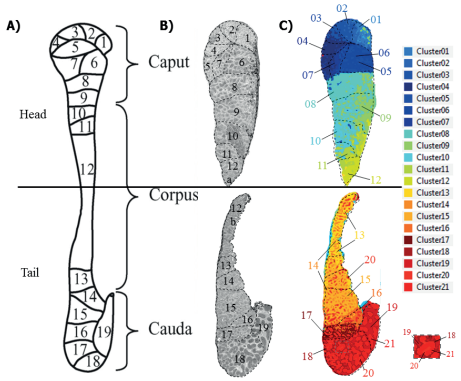

Figure 1: Spatial segmentation map obtained from 2D-MALDI MSI data.

Identification of discriminative m/z ratios

Statistical ROC curve analyses were used to assess the discrimination quality of all m/z-values detected in each cluster of the spatial segmentation map.

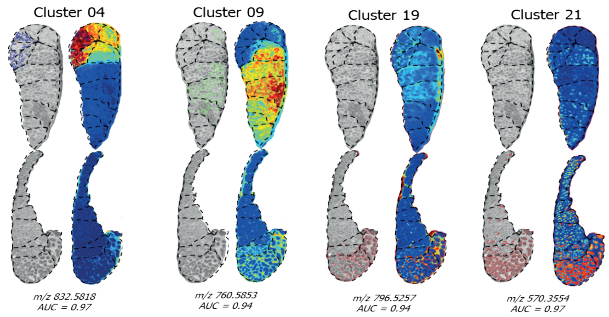

Figure 2: Examples of discriminative m/z ratio in clusters.

Annotation of discriminative m/z

In epididymis head, discriminative m/z corresponded mainly to PC (85%).while a significant diversification of lipids annotations was observed in epididymis tail with the apparition of LysoPC, TG and LysoPE and the increase in the number of SM.

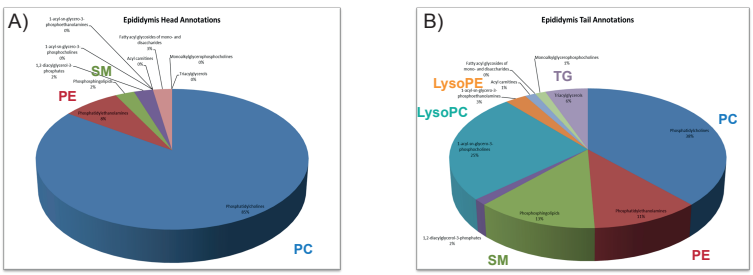

Figure 3: Annotations of discriminative m/z in A) epididymis head (clusters 1-12) and B) tail (clusters 13-21).

3D Visualization

When visualizing lipids within the 3D volumes, we can observe that their location varies considerably within the entire volume and in the depth of the tissue (z dimension).

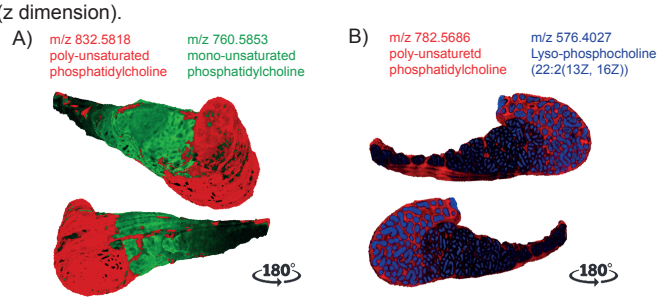

Figure 4 : 3D distribution of A) m/z 832.5818.(Segment 4)and m/z 760.5853(segment 9) in epididymis head and 3D distribution of B) m/z 576.4027(Segment 19) and m/z 782.5686 (segment 21) in epididymis tail.

Conclusions & Perspectives

The segmentation obtained from molecular features appears to be quite different from histological segmentation.

The lipids diversification observed between Head and Tail is known to play a major role in spermatozoa membranes fluidity, the mobility and fertilizing ability of sperm, progressive motion acquisition, and the capacitation process before the spermatozoa arrive at the oocyte and undergo the acrosome reaction.

3D images allowed us to explore the changes in metabolites composition along the epididymal tube. 3D allows for the visualization of analyte in the context of an important part of the organ and the evolution of this analyte through it and in relation, or not, to other specific levels in the volume.

Reference :[1] Palmer et al., Nature Methods, 2017. ;
Abbreviations : HMDB=Human Metabolome Database ; phosphatidylcholines=PC ; 1-acyl-sn-glycero-3-phosphocholines=LysoPC ; triacylglycerols=TG ; 1-acyl-sn-glycero-3-phosphoethanolamines=LysoPE; sphingolipids=sphingomyelins=SM