Department of Molecular Medicine and Surgery

Combining Mass Spectrometric Tissue Imaging with Conventional Histology Focus on Alzheimer's Disease

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Karolinska Institutet

offentligen försvaras i föreläsningssalen, Institutionen för Cell och Molekylärbiologi (CMB), Berzelius väg 21, Karolinska Institutet Solna

Fredagen den 16/3, 2012, kl 09.00

av

Santiago Solé Domènech

Msc.

Huvudhandledare:
   Asc. Prof. Björn Johansson
   Karolinska Institutet
   Molecular Medicine and Surgery

Bihandledare:
   Prof. Peter Sjövall
   SP Sveriges Tekniska Forskningsinstitut
   Chemistry and Materials

   Prof. Martin Schalling
   Karolinska Institutet
   Molecular Medicine and Surgery

   Prof. Lars Terenius
   Karolinska Institutet
   Clinical Neuroscience

   Asc.Prof. Xinghua Zhou
   Karolinska Institutet
   Inst. Medicin

   Docent Catharina Lavebratt
   Karolinska Institutet
   Molecular Medicine and Surgery

Fakultetsopponent:
   Prof. Gunnar Gouras
   Lunds Universitet
   Experimental Medical Science

Betygsnämnd:
   Prof. Maria Eriksdotter Jönhagen
   Karolinska Institutet
   Neurobiology, Care Sciences and Society

   Docent Per Andrén
   Uppsala Universitet
   Pharmaceutical Biosciences

   Prof. Anders Hamsten
   Karolinska Institutet
   Inst. Medicine

Stockholm 2012

ABSTRACT

Historically, most scientific discoveries have been accompanied by previous technological innovation. Representative examples are the introduction of gene sequencing technologies, which allowed for the mapping of the human genome, or the constant improvement in the microscopy field that lead to the introduction of modern multiphoton imaging techniques, allowing for live imaging of body organs in animal specimens. In this regard, the molecular imaging of analytes in tissue has traditionally been a challenging field. For instance, mass spectrometric techniques did not initially allow for the imaging of compounds in a given sample. Technological improvement lead to the introduction of instruments that could transform the spectra acquired from samples into images of the distribution of surface analytes. In the present thesis work, we approach the study of biological samples using Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS), a surface imaging technique specially suited for the imaging of lipids and lipid precursors in biological tissue. Our project started with the optimization of ToF-SIMS protocols for the study of mouse brain and fat adipose tissues, which allowed us to learn ways to systematically process biological material in a reliable and reproducible manner. Next, we focused our efforts on the application of ToF-SIMS to the study of a well established biological problem: Alzheimer's disease. We developed a methodology combining ToF-SIMS with fluorescence imaging techniques in order to visualize lipids, lipid precursors and cell populations in brain regions undergoing senile plaque deposition. This multiplexed imaging approach enabled us to simultaneously observe lipid and glial cell distributions in regions undergoing senile plaque deposition with great lateral (<500 nm) resolution, and revealed micrometer-sized cholesterol accumulations in hippocampal regions occupied with senile plaques likely to mirror an AD-related pathological event. The systematic study of brain tissue sections from mouse models of Alzheimer's disease followed; we investigated the cholesterol profile of brain regions occupied by senile plaques and compared it to that of wild-type, control brain. Regions undergoing plaque deposition displayed, often, severe cholesterol granulation, whereas regions devoid of plaques displayed a homogeneous sterol profile. Furthermore, investigation of glial profiles revealed increased astrocytic immunoreactivity in regions associated to cholesterol granulation, which was consistent with the already known role played by astrocytes during central nervous system injury. Besides cholesterol, other lipid compounds were studied, such as sulfatides and fatty acids, revealing specific surface distributions associated to distinct tissue structures. Given the growing attention paid to the involvement of lipids and lipid precursors in health and disease, tools that allow for the study of such compounds might play a vital role in the understanding of disease pathogenesis. The implementation of technological improvements that allowed for the study of biological samples, with reduced analyte fragmentation and increased efficiencies, lead to the modern "ToF-SIMS imaging" concept presented in this thesis work. The current sensitivity and resolution are sufficient for cellular imaging of many lipids of likely pathophysiological importance.

Stockholm 2012