INTACT CELL MALDI-TOF MASS SPECTROMETRY AND TOP-DOWN PROTEOMIC TO IDENTIFY BIOMARKERS

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Over the last fifteen years, MALDI-TOF MS has been used directly on intact cells to obtain protein and peptide fingerprints, in 2-30 kDa mass range. This fast, reproducible and sensitive method known as Intact Cell MALDI-TOF-Mass Spectrometry (ICM-MS) has been widely used on microorganisms for their identification[1]. Also, on prokaryotes, several differential and quantitative applications of ICM-MS have been developed to display biomarkers. Recently, some experiments using ICM-MS were reported on mammalian cells.

In the field of reproductive physiology, our aim was to develop ICM-MS profiling on follicular and germinal cells from various species (bovine, human, equine, ovine, porcine, chicken), and to obtain new information about low molecular weight proteins' content in these cells. We demonstrated the application of differential and quantitative ICM-MS on male and female gametes and follicular cells, rising up to the scale of a single cell (oocyte)[2] in order to characterize biomarkers related to maturation stage of the gametes, their quality or oocyte competence to develop an embryo, or to evaluate the effect of sperm cryopreservation.

However, by this approach the biomarkers of interest remain to be identified. A bottom-up proteomic approach using SDS-PAGE/nanoLC-MS/MS can be performed but due to post-translational modifications no correspondence can be made between the theoretical mass of identified proteins and the experimental peak. Therefore, a Top-down proteomic approach was carried out to obtain peptide sequences and structural information. Enriched fractions were infused and analyzed by tandem high resolution mass spectrometry (LTQ Velos Orbitrap) with CID/HCD fragmentation modes. Identification and characterization were done using ProSight PC software. Several major peaks revealed by ICM-MS were formerly identified in different biological samples. Moreover, post-translational modification was displayed for some of them. We evidenced that the protease activities were, in part, at the origin of the numerous peptides constituting these peaks but not only.

In conclusion, the differential and quantitative ICM-MS on small quantities of isolated mammalian cells was successfully realized and revealed to be a sensitive and repeatable approach. ICM-MS proved to be a tool of choice for scarce and precious biological samples. Moreover, the use of complementary technology as Top-down HRMS allowed identification and further characterization of biomarkers.

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[1] *Holland RD, Wilkes JG, Rafii F, Sutherland JB, Persons CC, et al. (1996) Rapid identification of intact whole bacteria based on spectral patterns using matrix-assisted laser desorption/ionization with time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 10: 1227-1232.*

[2] *Labas V, Spina L, Uzbekova S. Single cell MALDI-TOF mass spectrometry analysis: mammalian oocyte profiling could reflect its quality. 10th World congress HUPO. 3-7 september 2011, Geneva.*