**Metabolomics of Exosomes from Uterine Aspirates and Plasma Samples of Endometrial Cancer Patients**

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**Introduction:** Endometrial cancer is the most frequent gynecological cancer diagnosed in the United States, accounting for 76,000 deaths annually. Mortality is associated with presentation of poor prognostic factors and/or advanced disease at diagnosis. Exosome-like vesicles (ELV) obtained from easy-to-access biofluids are an untapped resource for discovery and validation of clinically relevant biomarkers in health and disease. Although the proteomic and transcriptomic profile of ELV have been broadly described, the metabolomic profile is uncharacterized. Thus the goal of this study is to characterize the metabolome of ELV derived from human uterine fluids and plasma, and identify metabolomic signatures that could be used for risk stratification and diagnosis of endometrial cancer patients.

**Methods:** Uterine aspirate and plasma samples from 5 endometrial cancer and 8 benign patients were collected and processed in Vall Hebron Hospital (Barcelona, Spain) in accordance with approved institutional consent and review protocols. ELVs were isolated by standard ultracentrifugation, characterized by immunoblot against known exosomal markers and size and concentration estimated using Nanoparticle Tracking Analysis. We analyzed the metabolomic and lipidomic profile of the ELVs by ultra-performance liquid chromatography coupled with electrospray quadrupole time of flight mass spectrometry (UPLC-ESI-QTOF-MS). Data were pre-
processed using the XCMS software while the database search was performed using the Madison Metabolomics Consortium Database (MMCD), the Human Metabolome Database (HMDB), LIPID MAPS and Metlin for putative metabolite identification.

**Preliminary Results:** As a first step, we optimized the starting volume of biofluid for exosome isolation that would yield high spectral data quality. Thus, ELVs from different starting volumes ranging from 200 to 800µL of uterine fluid and from 250 to 2000µL of plasma were isolated, and confirmed by determining the expression of known exosomal markers Flotilin 1, TSG101, CD63, CD9, Rab5, and CD81. The average size of the exosomes was found to be 140nm±10nm. Comparative analyses showed that 400µL of uterine aspirates and 200µL of plasma as a starting was sufficient for ELVs isolation to produce high quality metabolomic and lipidomic data. The metabolome and lipidome of ELVs derived from uterine fluid and plasma samples contained a high percentage of phospholipids, peptides and nucleotides within ELVs, and interestingly, some other less common molecules such as Vitamin D derivatives. A comparative analyses of metabolites derived from plasma and uterine aspirate exosomes suggested unique features for each matrix although a number of metabolites were common to both matrices. These findings once relocated could have broad application for clinical and translation studies that focus on biomarker development. Furthermore, a metabolomic and lipidomic profile that differentiates endometrial cancer from benign patients was obtained; validation of these findings is ongoing.

**Novel aspects:** To our knowledge this is a first report that attempts to optimize an exosomal metabolomics workflow and examines the metabolomics profile of uterine aspirates and plasma. Our findings represent a novel approach to identify predictive biomarkers that can be used for a broad range of applications.