Activated Human Umbilical Vein Endothelial Cells express adhesion molecules: a comparative quantification using Parallel Reaction Monitoring (PRM) and Data Dependent Analysis (DDA)

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Introduction: In endothelial cells, membrane adhesion molecules have an essential role during leukocyte migration in inflammatory scenarios. Endothelial membrane molecules are crucial to allow leukocyte migration from peripheral blood to tissues, through the interaction with proteins present on leukocytes. Therefore, the quantification of those membrane proteins is important to evaluate the inflammatory scenario. In this context, proteomics presents itself as a powerful tool capable of detecting and quantifying those molecules and alterations on their abundance.

High-Density Lipoprotein (HDL) Apolipoprotein M (ApoM) carries one bioactive sphingolipid denominated Sphingosine-1-Phosphate (S1P). The Apo M-bounded S1P is associated to a vascular protective role of HDL, responsible for decreasing the expression of adhesion molecules.

Material and Methods: Primary Human Umbilical Vein Endothelial Cells (HUVEC) were cultivated until confluency for experiments. Cells were plated in 12 well plates and pre-treated with 50 ug/mL HDL from apparently healthy subjects (2h, 37°C, 5% CO_2) and stimulated with 10 ng/mL of TNFa (4h, 37°C, 5% CO_2). Total cell lysates were obtained using 0.2% of Sodium Deoxycolate in 100 mM of Ammonium Bicarbonate.

For FASP protocol, 30 kDa cutt-off filters were passivated overnight with 0.05% Tween-20 solution. The samples were washed with Urea 8M in Ambic 25 mM, reduced and alkylated. Trypsin was added for the digestion of the proteins (1:25 w/w enzyme:protein, 16h, 37°C, under agitation). In the next day, peptides were eluded, dried and resuspended with formic acid 0.1% for the final concentration of 0.1 ug of peptides/uL.

For the data acquisition, Data Dependent Analysis (DDA) and Parallel Reaction Monitoring (PRM) were used aiming the detection and quantification of adhesion molecules. For data analysis, after DDA runs MaxQuant software was used to identify and perform label-free quantification of the proteins for each experimental group; for PRM runs, the area under the curve for each individual peptide was integrated using Skyline software.

Results: In both data acquisition methods, the detection and quantification of E-Selectin, VCAM-1 and ICAM-1 was possible. In HUVEC cells, the expression of E-Selectin and VCAM-1 were detected only in the TNFa treated samples, whereas ICAM-1 was detected in all samples but strongly induced by TNFa treatment. Accordingly to literature, a reduction profile of adhesion molecules was observed on HDL-treated samples in comparison with only TNFa treated samples: 21% of reduction for E-Selectin (T-test p-value = 0.24), 33% of reduction for VCAM-1 (T-test p-value = 0.16) and 27% of reduction for ICAM-1 (T-test p-value = 0.16). More experiments aiming to reduce variability are underway.

To compare the results obtained by DDA (LFQ Intensity quantification) with those obtained by PRM, Pearson and Spearman correlation coefficients were obtained for those proteins. Pearson and Spearman coefficients were respectively equal to: 0.88 and 0.78 for E-Selectin, 0.77 and 0.75 for VCAM-1, and 0.85 and 0.88 for ICAM-1.

Conclusion: With these results, it is possible to conclude that mass spectrometry-based quantification is suitable to evaluate the participation of inflammatory adhesion molecules in cell-based studies, although specific techniques for sample preparation may be needed.

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