Identification of cell surface proteins susceptible to oxidation during the cell adhesion process in endothelial cells using a proteomic redox

Danielle Fernandes Vileigas[1], Beatriz Pereira da Silva[1], Railmara Pereira da Silva[1], Mariana Pereira Massafera[1], Flavia Carla Meotti[1]

[1.] IQ-USP, Department of Biochemistry, Institute of Chemistry, University of São Paulo, Av. Prof. Lineu Prestes, 748 - Butantã, São Paulo - SP, 05508-000;

Cell adhesion is essential for various biological processes, such as cell survival, formation of organs, cellular migration, immune response, vascular remodeling, and communication with target cells and the extracellular matrix. The involvement of redox regulation during cell adhesion has been evidenced, mainly by producing reactive oxygen species. However, this topic has been scarcely studied so far, and the number of recognized cell surface proteins targeted by oxidative modification is limited. Studies have focused on the major family of cell adhesion molecules, i.e., integrins; nevertheless, a variety of other adhesion molecules are involved in the cell adhesion process, which could also be the target of oxidation. Thus, this study aimed to identify cell surface thiol-containing proteins susceptible to oxidation at different times (0, 1.5, 3.5, and 24 hours) of cell adhesion in human umbilical vein endothelial cells using redox proteomics. The identification of oxidized proteins was based on a thiol-reactive cell-impermeable biotin label, maleimide-PEO2-biotin, and purification by affinity chromatography with monomeric avidin agarose beads. For proteomics, the protein digestion was based on the FASP method (Filter-Assisted Sample Preparation). The peptides were analyzed by LC-MS/MS with label-free quantification. The proteomic analysis identified 1,062 cell surface-related proteins, with 977, 962, 988, and 947 proteins detected at 0, 1.5, 3.5, and 24 hours, respectively. Substantial overlap was observed across all time points, with over 80% of the total (865 proteins) being common, and only a few proteins being exclusive to specific time points. Statistical analyses revealed 66 differentially abundant proteins across the time points, with most of the differences concentrated at 24 hours compared to the other time points. Both increases and decreases in protein abundance were noted, suggesting variations in oxidation levels throughout the cell adhesion process. Moreover, these proteins represent channels, receptors, enzymes, and cytoskeleton, which play a crucial role in several signaling pathways. In conclusion, the study identified significant cell surface proteins with distinct redox switches during the cell adhesion, highlighting its dynamic nature. These findings provide valuable insights into extracellular redox signaling and may have future therapeutic implications for major vascular disorders involving redox signaling.

Agradecimentos: We thank Professors Paolo Di Mascio and Graziella E. Ronsein for the Mass spectrometry analyses performed at the Redox Proteomics Core of the Mass Spectrometry Resource at Chemistry Institute, University of Sao Paulo (FAPESP grant numbers 2012/12663-1, 2023/00995-4, CEPID Redoxoma 2013/07937-8). We also thank FAPESP for financial support (grant numbers 2018/14898-2 and 2020/01394-6)