PROTEOMICS OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS: DEVELOPMENT OF A PROTOCOL FOR PROTEIN EXTRACTION

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Endothelial cells form a continuous monolayer lining the inside faces of all blood vessels, and present the ability to selectively control vascular permeability. The endothelium is involved in a wide variety of normal physiological and pathological processes. The endothelial dysfunction occurs under activation conditions, with the acquisition of many new functional, inflammatory, and immune properties, and as a consequence, endothelial cells display many different transcription profiles. We describe here the isolation and culture of the most useful model of human umbilical vein endothelial cells (HUVECs), and undertake the proteomic analysis of a whole cell extract using two extraction buffers: Tris-HCl 25mM, NaCl 250mM, EDTA 5mM, Igepal 1%, PMSF 1mM and leupeptin 40µM (A) and Urea 8M, Tris-HCl 30mM, Chaps 4%, PMSF 1mM and leupeptin 40µM (B). Series of two-dimensional electrophoresis have allowed us to detect a total of close to 210 polypeptide spots with solution A and 235 with solution B using 3.0-11.0 pH range in both conditions. Cross-matching the gels, we found that 89 polypeptide spots in common. The protein identification was performed by MS/MS analysis. The profile of proteins expressed under controlled culture conditions will be used for further studies on activated endothelial cells.

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