2D-DIGE analysis of thrombin induced changes in the proteome and secretome of human vSMCs

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Under pathophysiological conditions like atherosclerosis and inflammation the mitogenic serine protease thrombin promotes transition of vascular smooth muscle cells (vSMCs) from a contractile to a migratious, proliferatious and secretory phenotype. The effects of thrombin are mediated by protease-activated receptors (PARs) which affect various cellular processes by engaging G protein-dependent signalling pathways. The aim of the present study was to identify new cellular target structures of thrombin in vSMCs. Human cultured venous SMCs were rendered quiescent and stimulated with thrombin for 0.25, 3 and 12 hours. Cells were lysed and the soluble protein fraction was concentrated by centrifugal ultrafiltration. The secreted protein content of the culture medium was also isolated. Thrombin-induced changes in the vSMC proteome and secretome were analysed by two-dimensional differential gel electrophoresis (2D-DIGE). To minimize costs and increase the number of possible experiments, the fluorescent dyes used for DIGE labelling were synthesized in our laboratory according to a protocol published by Jung & Kim (2006). Protein labelling and separation were performed according to the 2D-DIGE standard protocol. 2D gels were digitized and the gel images were analysed with Proteomweaver software. Protein spots of interest were identified by MALDI-TOF MS. Protein patterns of the DIGE gels were highly reproducible and showed excellent overlapping of differentially labelled protein spots. Based on this experimental approach we were able to detect variations in abundance of several proteins. Among the proteins of interest were Hsp27 (variation factor 1.7), Lasp1 (0.78) and Grp78 precursor (1.25). In this way, differential proteome analysis will allow us to gain further understanding of the complex response of vSMCs to thrombin stimulation.

Jung ME, Kim WJ: Practical syntheses of dyes for difference gel electrophoresis. Bioorg Med Chem. 2006;14(1):92-97.