P. 63

IN-DEPTH ANALISYS OF JURKAT T-CELL SECRETOME

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Inflammatory mediators secreted by leukocytes play a significant role in the progression of several diseases. These mediators are mainly proteins and their identification will help to better understand the complex signal transduction network of inflammation. Here, we present an in-depth analysis of proteins secreted by lymphoid Jurkat T-cells, using a combination of IEF coupled with liquid chromatography mass spectrometry. Cells were washed with PBS and incubated for 48h with serum-free medium. Cell-free supernatant proteins were concentrated on SDS-PAGE gels by running the sample 3 mm into the resolving gel. The concentrated gel bands were subjected to trypsin digestion and the resulting peptides were separated into 24 fractions by IEF using an OFF-Gel electrophoresis unit. Fractions were analyzed, under high peptide loading conditions, by RP-HPLC-MS/MS using a linear ion trap LTQ MS. More than 500 unique peptides were identified per fraction, resulting in the identification of more than 8,000 peptides at a FDR of 5%, corresponding to more than 2,800 proteins. Identified proteins were compared to the high confidence reference set of human plasma proteins (Schenck et al., BMC Medical Genomics, 2008). Also, after a SecretomeP 2.0 analysis, proteins were termed as potentially secreted via a classical pathway if they contained a signal peptide, whereas those with NN score exceeding 0.6 were classified as secreted by a non-classical pathway. The lack of transmembrane helices or intracellular localization signals was predicted by TMHMM server. By these means, more than 500 unique secreted proteins were identified. Our results may help understanding the secretory functions of resting T-cells.