

THE STUDY OF THE OF HUMAN PRIMARY T-LYMPHOCYTE PHOSPHOPROTEOME

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Global phosphorylation profiles will be used for the characterization of biological markers of cell status that could be used as prognostic or diagnostic markers for hypersensitivity or immunodepression-related diseases. Protein kinase inhibitors are the second most popular drug target class in the pharmaceutical and biotech industries. Drugs like ciclosporin or rapamycin are kinase and phosphatase inhibitors (calcineurin and mTOR respectively) and are used to inhibit the immune response in transplants. The characterization of phosphorylation motifs will help in the search for new, physiologically relevant kinases as well as in the understanding of the mechanism involved in the regulation of protein function. This information is the first step towards the design of new therapies based on drugs controlling either kinase/phosphatase activities or the activity of other proteins in key points of the affected pathways.

In this work, we describe a large-scale phosphorylation analysis of primary T-cells using a multidimensional separation strategy involving preparative SDS-PAGE or SCX for prefractionation and sequential phosphopeptide enrichment using IMAC and titanium dioxide, followed by LC-MSⁿ analysis using a LTQ linear ion trap. In total, more than 300 different high-confidence phosphorylation sites were described mapping more than 600 possible phosphoproteins.

To organize the phosphopeptide data we created a relational database called LymphOS that currently comprising phosphopeptide sequences, p-sites and information about the proteins containing these phosphopeptides. Also, this information is linked to the mass spectrometric information and is publicly accessible on the net (www.lymphos.org). This constitutes the only phosphorylation map for human primary T-lymphocytes.