QUANTITATIVE CHARACTERIZATION OF P-SITES FROM THE HUMAN T-LYMPHOCYTE PHOSPHOPROTEOME USING ISOTOPIC LABELING AND MASS SPECTROMETRY.

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T lymphocytes mediate cellular and humoral defense against foreign bodies or autoantigens. Protein phosphorylation-dephosphorylation is involved in many aspects of lymphocyte activation. Thus, the global phosphorylation profiles in these cells could be the source for new biological markers for hypersensitivity or immunodepression-related diseases. In this context, we are involved in the qualitative characterization of phosphoproteins and p-sites in human primary T-cells and in the determination of the quantitative changes induced by different activators on the phosphoproteome of these cells.

Relative changes on phosphosite abundance after 15 min and 2-4 h activation with PMA-ionomicin are being studied using a shotgun approach with iTRAQ or TMT tag for quantitation. Labeled peptide mixtures are submitted to a SCX off-line fractionation followed by IMAC and TiO_2 phosphopeptide enrichment as described (Carrascal et al. J. Proteome Res. 2008). Purified fractions are analyzed by capLC-μESI-$ITMS^n$ usign a combination of PQD/CID or HCD/CID scans in a LTQ or an Orbitrap XL instrument, respectively.

Data is stored in our publicly available LymPHOS database (http://lymphos.org) (Ovelleiro et al. Proteomics 2009). We have implemented an automatic workflow for the annotation of the database that includes tools for MS data filtering and accurate phosphorylation site assignation.

This data constitutes the only phosphorylation map available for human primary T-lymphocytes. Several novel lymphocyte specific p-sites are described and quantified. This information could be the basis for future studies on the role of phosphorylation in T-cell functions and the effect of pharmacological and immunological agonists in T-lymphocyte activation.