P. 119

DIFFERENTIAL PROTEIN EXPRESSION ANALYSIS OF ACTIVATED E2F2$^{-/-}$ T LYMPHOCYTES BY MEANS OF A LABEL-FREE QUANTIFICATION METHOD

M. Azkargorta[1], K. Aloria[2], A.M. Zubiaga[3], J.M. Arizmendi[1], A. Fullaondo[3]

[1]Department of Biochemistry and Molecular Biology,
University of the Basque Country, Leioa, Bizkaia;
[2]Proteomics Unit, SGIker, University of the Basque Country, Leioa, Bizkaia;
[3]Department of Genetics, Physical Anthropology and Animal Physiology,
University of the Basque Country, Leioa, Bizkaia

Differential proteomics has traditionally relied on two-dimensional electrophoresis (2DE). 2DE is a powerful technique that allows the separation and analysis of hundreds of proteins in a single gel, leading to the analysis of global expression patterns. 2DE, however, has a number of inherent limitations and disadvantages. To overcome these limitations several MS-based gel-free techniques have been developed. Among these, a label-free absolute quantification method based on the three most intense peptides at each protein developed by Waters (Hi3) is very simple and affordable.

E2F family of transcription factors (E2F1-8) are crucial regulators of cell-cycle and proliferation. Characterization of mice lacking functional E2F2 transcription factor (E2F2$^{-/-}$) showed the development of an autoimmune syndrome due to the presence of hypersensible and hyperreactive peripheral T lymphocytes. These T lymphocytes hyperproliferated upon antigenic stimulation, and showed an accelerated entry to the S phase of cell cycle, suggesting a role for E2F2 in the repression of G1/S transition. Previous effort in our lab has allowed us the characterization of E2F2-lacking T lymphocyte's expression patterns on a 2DE-based approach.

In this work we characterize E2F2$^{-/-}$ activated T lymphocyte's protein expression patterns by Hi3 absolute quantification method. Hi3 analysis of these extracts revealed the deregulation of some interesting cell-cycle and E2F-related proteins, proving the usefulness of this technique for differential expression analysis. Moreover, results obtained by 2DE and Hi3 analysis of activated E2F2$^{-/-}$ T lymphocytes are compared, suggesting that both approaches can provide complementary viewpoints in differential proteomics analyses.