Differential Proteomic Analysis of the Reactivated p53 via Nutlin-3A, in 3 Different Types of Human Lymphomas

Konstantina Psatha[1], Elias Drakos[2], George Z. Rassidakis[1,3], Michalis Aivaliotis[3,4]

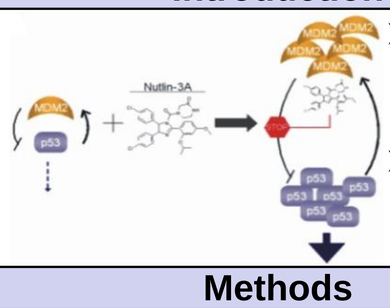
[1]First Department of Pathology, National and Kapodistrian University of Athens, Athens, Greece.,[2] Department of Morphology, Medical School, University of Crete, Greece, [3]Department of Haematopathology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, USA, [3] Max-Planck-Institute of Biochemistry, Department of Membrane Biochemistry, Martinsried, Germany, [4]Institute of Molecular Biology and Biotechnology, FORTH, Heraklion, Greece.

Overview

- **Purpose:** The identification and quantification of protein expression levels of nutlin-3A-induced p53 stabilization and activation in human lymphoma.
- **Methods:** The Isotope Coded Protein Label (ICPL) technique was followed by nano-Liquid Chromatography coupled on-line with Mass Spectrometry (nLC-MS/MS).
- **Results:** Reliable identification & differential quantitative determination of human lymphoma proteome profile, revealing alterations in the HSPs relative expression levels.

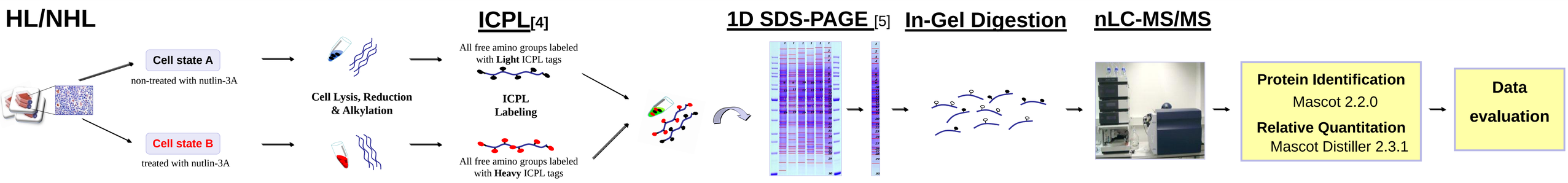
Introduction

- p53, a master tumor suppressor gene is impaired in >50% of all human cancers [1]. However, >80% of haematological malignancies express wt p53, including Hodgkin's (HL) and Non-Hodgkin's lymphomas (NHL).
- MDM2 (HDM2 in humans) binds to wt p53 and negatively modulates its transcriptional activity and stability [2].



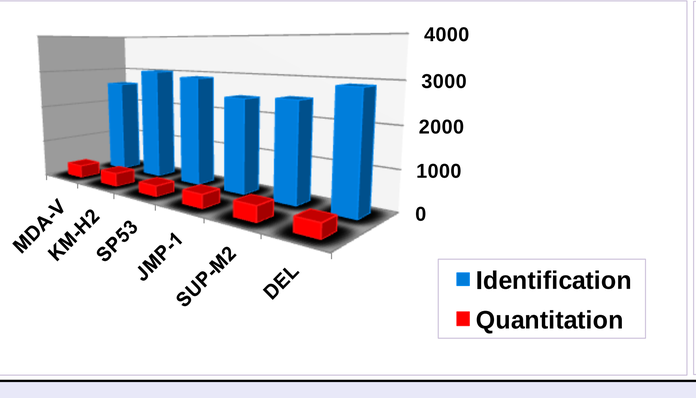
- Nutlin-3A, an MDM2-antagonist, induces stabilization and reactivation of the wt p53 pathway in cancer cells, followed by the infliction of cell cycle arrest and apoptosis [3].
- Scope of the study is the mode of action of nutlin-3A-mediated wt p53 stabilization and reactivation, in human cell lines of HL (2) and NHL, Anaplastic Large Cell Lymphoma (ALCL, 2) and Mantle Cell Lymphoma (MCL, 2).

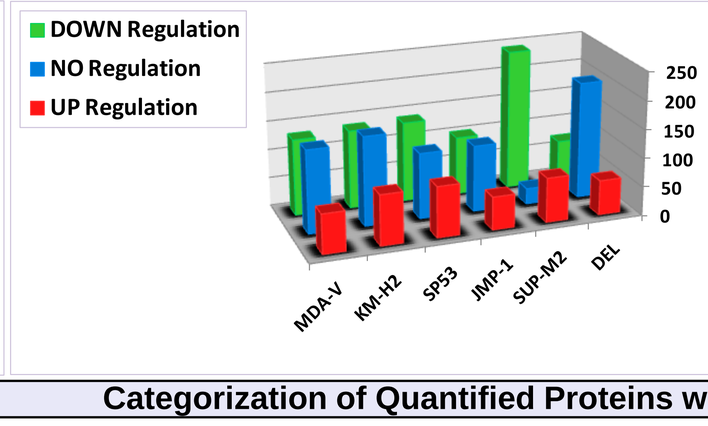
Methods



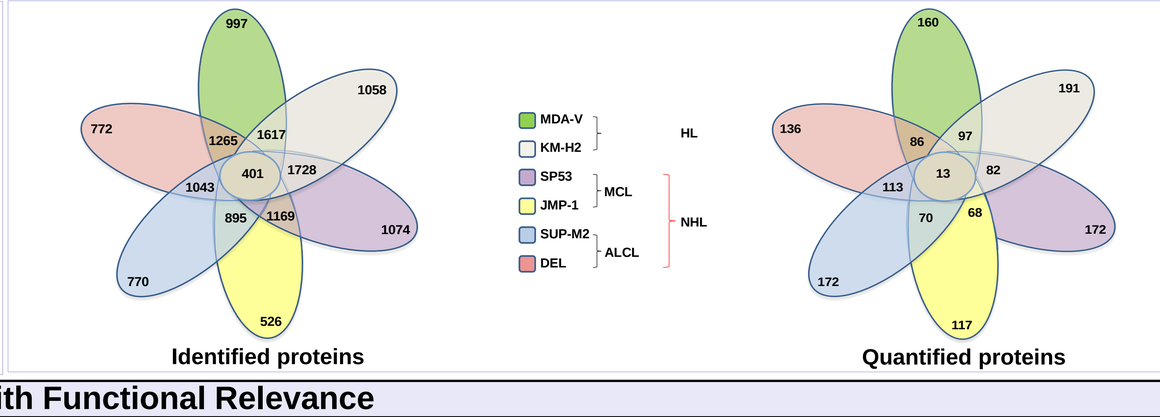
Results

Protein Identification & Quantitation-Differential protein expression

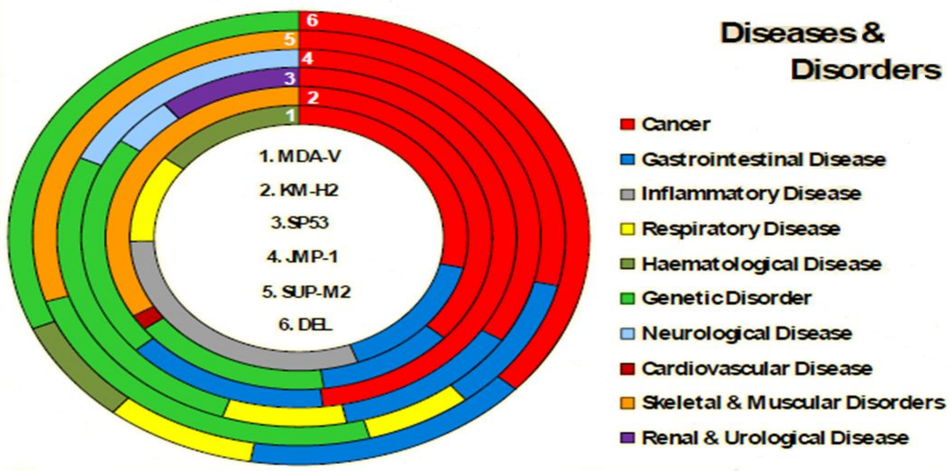


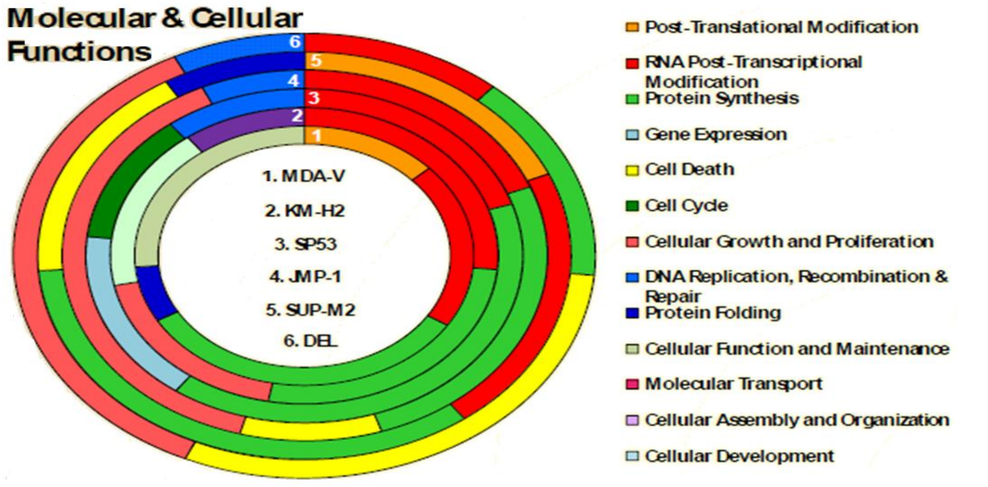


Protein Comparison in the 6 Different Lymphoma Cell Lines (HL & NHL)

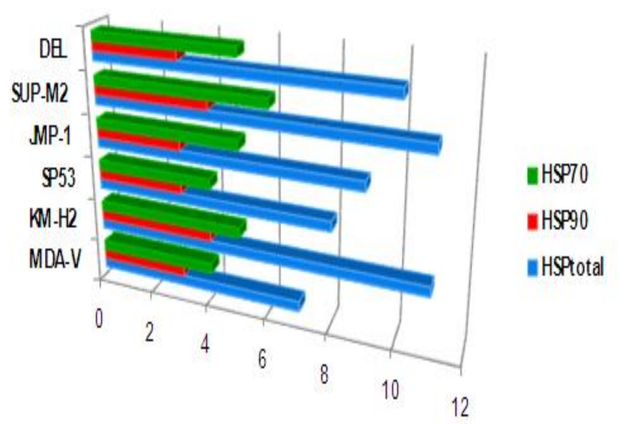


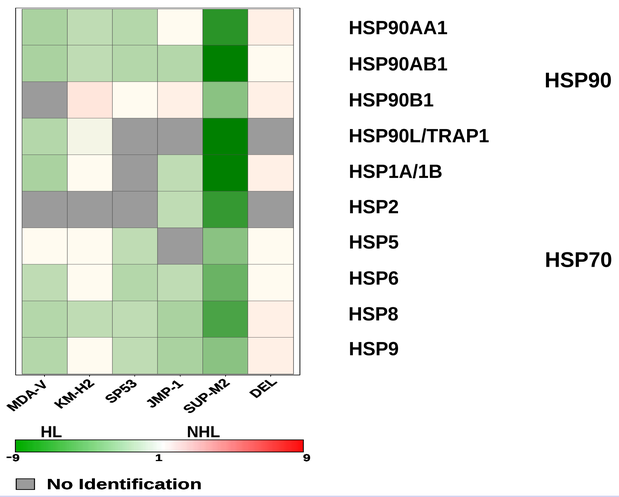
Categorization of Quantified Proteins with Functional Relevance



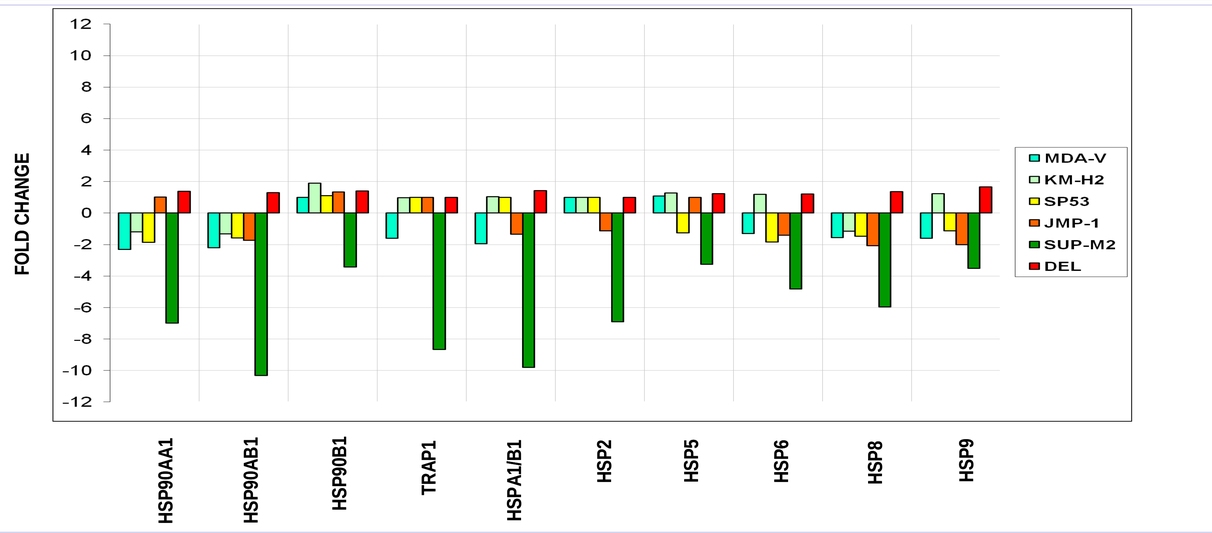


HSP90 & HSP70 Identification & Relative Expression Levels





Distribution of HSP90 & HSP70 Regulation



Conclusions – Future Plans

- ICPL approach coupled with 1D-SDS-PAGE & nLC-MS/MS was applied for a large-scale proteomics analysis of 3 types of human lymphoma, providing successful identification & relative quantitation of differentially expressed proteins.
- Our findings provide important advances in understanding the biology of nutlin-3A treatment in wt p53 lymphoma cells, demonstrating substantial decrease in HSPs levels .
- This study stresses out the biological similarities as well as differences between HL, MCL & ALCL. Further data processing will uncover far more comprehensive & thorough information on the participating pathways in haematological malignancies.
- Validation studies using genetic approaches are being performed, confirming a selection of abundantly expressed proteins of interest.

References

1. Hollstein et al. Science 1991;253:49–53.
2. Moll UM et al. Mol Cancer Res. 2003;1: 1001-1008
3. Vassilev et al. Science. 2004;303: 844-848.
4. Schmidt A et al. A novel strategy for quantitative proteomics using isotope-coded protein labels. Proteomics 2005, 5:4-15
5. Shevchenko et al. (1996) Mass spectrometric sequencing of proteins from silver stained polyacrylamide gels. Anal. Chem. 68, 850-858.

Acknowledgements

1. Prof. Dieter Oesterhelt, Dr. Frank Siedler, Dr Matthias Schlesner, Max-Planck-Institute of Biochemistry, Department of Membrane Biochemistry, Martinsried, Germany
2. ProFI lab & Prof. Anastassios Economou, Institute of Molecular Biology & Biotechnology, Foundation of Research and Technology, Heraklion, Crete, Greece

European Union
European Social Fund
OPERATIONAL PROGRAMME EDUCATION AND LIFELONG LEARNING
investing in knowledge society
MINISTRY OF EDUCATION, LIFELONG LEARNING AND RELIGIOUS AFFAIRS
MANAGING AUTHORITY
NSRF 2007-2013
EUROPEAN SOCIAL FUND
Co- financed by Greece and the European Union

This research has been co-financed by the European Union (European Social - ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) – Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund.