

## REGULATION OF STATHMIN PHOSPHORYLATION IN LIVER PROLIFERATING CELLS DURING PROTEASOME INHIBITION

**Enrique Santamaría, Maria I. Mora, Javier Muñoz,  
Joaquín Fernández-Irigoyen, Jesús Prieto, Fernando J. Corrales**

Division of Hepatology and Gene Therapy, Proteomics Unit.  
Center for Applied Medical Research (CIMA). University of Navarra. 31008 Pamplona, Spain.

Proteasome inhibitors are novel therapeutic agents which might be used for the treatment of hepatocarcinoma and other liver diseases as they are able to induce cell death in proliferating cells specifically. The analysis of alternative protein phosphorylation states might contribute to elucidate the underlying mechanisms of proteasome inhibitor-induced apoptosis. In the present study we have investigated the response of MLP-29 liver cells to MG132 using a combination of phosphoprotein affinity chromatography, Differential in Gel Electrophoresis (DIGE), and nanoLC-MS/MS. Seventeen spots corresponding to 13 unique deregulated phosphoproteins were unambiguously identified, which are involved in chaperone activity, stress response, mRNA processing and cell cycle control. Some of these protein species, including NDRG1 protein, hnRNP A2/B1, and stathmin suggest new mechanisms associated to proteasome inhibitor-induced apoptosis in proliferating liver cells. Particularly, a transient modification of the phosphorylation state of Ser<sup>16</sup>, Ser<sup>25</sup> and Ser<sup>38</sup>, which are involved in the regulation of stathmin activity, was detected in three distinct isoforms upon proteasome inhibition. The parallel deregulation of calcium/calmodulin-activated protein kinase II (CaMKII), extracellular regulated kinase (ERK1/2) and cyclin-dependent kinase (CDK2), catalyzing the phosphoryl group addition to these Ser residues, might explain the modified phosphorylation pattern of stathmin. Interestingly, stathmin phosphorylation profile was also modified in response to epoxomicin treatment, a more specific proteasome inhibitor. In summary, besides impairment of proteins participating in central cellular pathways, we report here novel mechanisms involved in proteasome inhibition-induced apoptosis that regulate the stathmin phosphorylation status in liver proliferating cells.