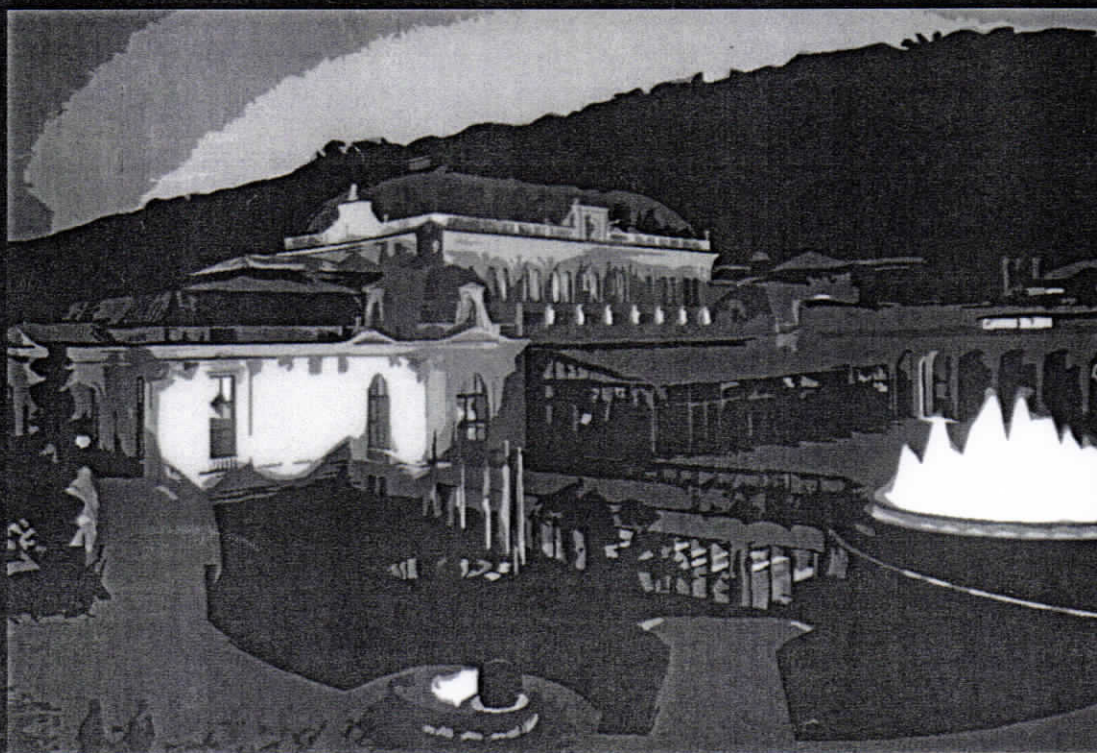


Europhosphatases 2011

Protein Phosphatases From Molecules to Networks

18. – 23. July, 2011
Congress Casino Baden
Baden near Vienna, Austria



Organizer: Egon Ogris

Co-Organizers: Irute Meskiene, Jeroen den Hertog, Ari Elson

EMBO CONFERENCE SERIES, THIRD IN A SERIES

Andrea Kiss^{1,2}, Dóra Dedinszki¹, Zsuzsanna Gaál¹, Ferenc Erdődi^{1,2}

¹ Department of Medical Chemistry, ² Cell Biology and Signaling Research Group of the Hungarian Academy of Sciences, Research Center for Molecular Sciences, University of Debrecen, Debrecen, Hungary

Phosphorylation of the retinoblastoma protein (pRb) is crucial for cell survival and protein phosphatase-1 (PP1) is involved in the mediation of this process. In THP-1 cells the basal level of pRb phosphorylation is relatively high, which may reflect increased effectiveness of PP1 inhibitory proteins. Hence, we tested the presence and phosphorylation of CPI-17, a PP1 (and myosin phosphatase) inhibitory phosphoprotein in these cells. Surprisingly, we found relatively low level of phospho-CPI-17, but identified a few other proteins in 20-100 kDa molecular mass range that cross-reacted with anti-phospho-CPI17 antibody. A search in the protein database based on CPI-17 sequence highlighted already known related proteins such as PHI and KEPI. In addition, LIM-kinase 1 and 2 (LIMK1 and LIMK2), and a protein termed as "similar to serologically defined breast cancer antigen" included significant sequence similarity with CPI-17.

Among these phosphorylated proteins LIMK2 was identified by immunoprecipitation in THP-1 cell lysate and was significantly phosphorylated at the sequence motif similar to the CPI-17 phosphorylation site. LIMK2 phosphorylation was completely diminished upon treatment of the cells with daunorubicin, a chemotherapy drug. Full-length Flag-LIMK2 was expressed in tsA201 cells and purified on Flag-affinity column. Unphosphorylated Flag-LIMK2 inhibited the myosin light chain phosphatase activity of PP1 slightly, whereas phosphorylation by PKC at the inhibitory site (verified using anti-phospho-CPI-17 antibody) increased profoundly the PP1 inhibitory potency.

Our results suggest that LIMK2 is a novel inhibitor of PP1 and it may mediate the phosphorylation level of proteins important in the regulation of the chemosensitivity of malignant cells.

This work was supported by grants from the Hungarian Scientific Research Fund OTKA K68416 and CNK 80709, and by the TÁMOP 4.2.2.-08/1-2008-0019 DERMINOVA project.