

BIOPOLYMERS & **C**ELL

Volume 28

2
supplementary

2012

RECOOP HST Association

Bridges in Life Sciences

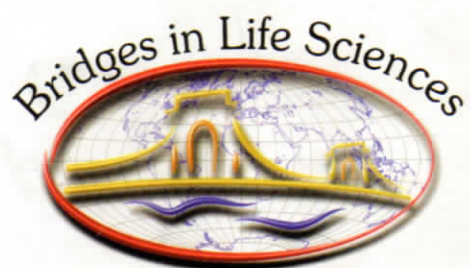
7th Annual Conference

Science and Art

for the Advancement in Medicine

March 30 – April 1, 2012

Budapest, Hungary



PP1 dephosphorylates the retinoblastoma protein

Gaál Zs., Kiss A., Dedinszki D., Erdődi F.

Department of Medical Chemistry, Medical and Health Science Center, University of Debrecen
zsuzsanna.gaal.46@gmail.com

Aim: The phosphorylation level of retinoblastoma protein (pRb) plays an important role in the regulation of cell proliferation and cell cycle progression, and therefore has a major impact on the survival of malignant cells. While the phosphorylation of pRb by kinases is well characterized, our knowledge on the dephosphorylating phosphatases is limited. Protein phosphatase-1 (PP1) has been considered as one of the pRb phosphatases, however its direct role in the dephosphorylation has not been fully established. The goal of our study was to investigate the role of PP1 and possible PP1 inhibitory proteins on pRb phosphorylation.

Methods: The catalytic subunit of PP1 (PP1c) was silenced by transfection of siRNA in HeLa cells. Vectors coding for Flag-tagged KEPI (kinase enhanced phosphatase inhibitor) and LIM-kinase-2 (LIMK-2) were transfected into MCF-7 and HeLa cells, respectively. The efficacy of the transfections was checked by Western blot experiments and immunofluorescence using anti-KEPI, anti-LIMK-2 and anti-Flag antibodies. The viability of cells was determined by MTT assay.

Results: Silencing of PP1c by pan-siRNA reduced markedly the amount of PP1c and in turn it resulted in increased phosphorylation level of pRb. Both KEPI and LIMK-2 include a phosphorylatable peptide sequence implicated in the inhibition of PP1. Expression of KEPI in MCF-7 cells led to its phosphorylation by endogenous kinases and inhibition of PP1 reflected in enhanced pRb phosphorylation. Similarly, expression of LIMK-2 in HeLa cells decreased protein phosphatase activity leading to increased phosphorylation of pRb.

Conclusions: Our results indicate the essential role of PP1c in determining the phosphorylation level of pRb and suggest that PP1 is implicated in the regulation of cell cycle progression and survival of malignant cells, thereby PP1 could be considered as a drug target with therapeutic means.

Keywords: Protein phosphatase-1; retinoblastoma protein (pRb); pRb dephosphorylation; LIM-kinase-2; kinase enhanced phosphatase inhibitor (KEPI).