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**INFLUENCE OF PROTEIN PHOSPHATASE-1 INHIBITORY PROTEINS ON THE
DEPHOSPHORYLATION OF RETINOBLASTOMA PROTEIN**

The phosphorylation level of retinoblastoma protein (pRb) plays an important role in the regulation of cell cycle, and therefore has a major impact on the survival of malignant cells. 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 in pRb-dephosphorylation.

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

The silencing of PP1c resulted in increased phosphorylation level of pRb. Both KEPI and LIMK-2 include a phosphorylatable peptide sequence that is necessary for the potent 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 pRb-phosphorylation.

Our results indicate the essential role of PP1c in determining the phosphorylation level of pRb, which is also regulated by PP1 inhibitory proteins. Thereby PP1, LIMK-2 and KEPI could be considered as potential drug targets with therapeutic means in different kinds of malignancies.