The control of protein phosphatase-1 by phosphorylation of the regulatory subunit and binding of inhibitors to the catalytic subunit

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SUMMARY

Protein phosphatase-1 (PP1) is involved in the regulation of numerous cellular processes by dephosphorylation of phospho-Ser/Thr side chains in proteins. We studied the myosin phosphatase (MP) holoenzyme in which PP1 catalytic subunit (PP1c) is associated with a 110-130 kDa regulatory subunit, termed myosin phosphatase target subunit (MYPT1). Our aims were to investigate how the phosphorylation of MYPT1 subunit influences PP1 activity and what could be the significance of this phosphorylation in the control of cell viability. In addition, we studied the role of PP1c binding tannin components in the regulation of phosphatase activity.

We show that Rho-associated kinase (ROK), beside Thr695 in MYPT1, also phosphorylates Thr850, and this phosphorylation (similarly to Thr695) results in inhibition of the phosphatase activity. Phosphorylation of Thr850 in MYPT1 by ROK occurs in smooth muscle cells under physiological (i.e. stimulation by lisophosphatidic acid, LPA) or pathological (i.e. treatment with calyculin-A (CL-A), a phosphatase inhibitory toxin) conditions and this phosphorylation may represent a novel regulatory mechanism in the control of MP activity by various signalling pathways.

Treatment of leukemic cells with CL-A proved that PP1 and PP2A enzymes play important roles in the regulation of cell viability. CL-A attenuates the extent of daunorubicin (DNR) induced cell death and this effect is accompanied with increased phosphorylation of the retinoblastoma protein (pRb) and its decreased DNR-induced degradation. We proved that MP is involved in the dephosphorylation of pRb, and MYPT1 targets the PP1c to the pRb substrate. CL-A treatment of the cells resulted in inhibitory phosphorylation and translocation of MYPT1 from the nucleus to the cytoplasm. It is concluded that MP, by dephosphorylation of pRb, is implicated in the regulation of both the cell cycle and the chemoresistance of leukemic cells.

Our results imply that tannin components such as penta-O-galloyl-D-glucose (PGG) and epigallocathecin-3-gallate (EGCG), inhibit the activity of protein phosphatases and they are partially selective inhibitors of PP1c. The interaction of PP1cδ with PGG or EGCG are studied by surface plasmon resonance and NMR based binding experiments, as well as by computer based molecular modelling, and it is concluded that these molecules exert their inhibitory effect by binding to the hydrophobic groove of the PP1c catalytic centre. PGG treatment decreases the viability of tumor cells more profoundly than does EGCG. The tannin components represent a new family of phosphatase inhibitory compounds and their structural modifications by chemical synthesis may result in the development of pharmacologically important phosphatase inhibitory molecules.