SELECTIVE PHOSPHORYLATION OF CRMP2 AT SER-522 BY CDK5: GENE SILENCING STUDIES


Drug Discovery, Noscira S.A., Tres Cantos, Spain

Introduction: Cdk5 is a cyclin-dependent Ser/Thr kinase whose deregulation promotes hyperphosphorylation of proteins critical for microtubule assembly, hence axonal growth and stability, such as tau or CRMP2 and ultimately leads to neuronal death. As with other kinases the identification of therapeutic regulators is hampered by the lack of cellular assays selectively monitoring its activity since phosphorylation sites are shared by several kinases.

Aims: To demonstrate that CRMP Ser-522 is selectively phosphorylated by Cdk5 in order to validate the measurement of phospho-Ser522 levels as an indicator of cellular Cdk5 activity that might be used to identify potential therapeutic regulators.

Methods: N2A cells were transfected with Cdk5 siRNA and the levels of Cdk5 and phospho-Ser522 were monitored by fluorescence microscopy using suitable antibodies labelled with fluorescent tags. Selected compounds identified as Cdk5 inhibitors were then tested in a cellular assay monitoring phospho-Ser522 levels.

Results: Transfection of N2A cells with Cdk5 siRNA was achieved as observed by fluorescence microscopy. The levels of phospho-Ser522, but not those of total CRMP2, decreased in parallel to the levels of Cdk5 detected in the cells and were fully abolished in those cells showing complete silencing of the Cdk5 gene. An ELISA-based assay was developed and used in the evaluation of several compounds.

Conclusions: CRMP2 is phosphorylated at Ser-522 exclusively by Cdk5. Hence this measurement can be used to monitor the activity of Cdk5 inhibitors in a cellular context as demonstrated with the evaluation of several compounds.