

**NEURONAL CYCLIN-DEPENDENT KINASE 5(CDK5) AND ITS  
SPECIFIC INHIBITOR (CIP): ITS PHYSIOLOGICAL AND  
PATHOLOGICAL ROLE IN THE NERVOUS SYSTEM**

YALI ZHENG, SASHI KESAVAPANY and HARISH C. PANT  
NINDS, NIH, Bethesda, MD, USA

Cdk5, a multifunctional kinase involved in a wide range of neuronal behavior, is regulated by its neuron-specific activators, p35 and p39. It is becoming clear that cdk5, like the MAP kinases, is a key player in signal transduction networks underlying neuronal cell survival, growth and differentiation. Our studies of cdk5 KO mice (-/-) have shown that they express a lethal phenotype with abnormal corticogenesis and embryonic death [1]. Recently, we have also demonstrated that reconstitution of cdk5 expression with a cdk5 transgene under a neuronal specific promoter of p35, completely rescued such null mice; a wild type phenotype was obtained. This clearly demonstrated that neuronal and not glial cdk5 activity is necessary and sufficient for normal development and survival [2]. The p35<sup>-/-</sup>-mouse, which exhibits significant reduced cdk5 activity [3, 4], and a similar disruption of corticogenesis, is not lethal, but develops into a fertile adult with some behavioral abnormalities. However, the p35 and p39 double knockout mice show similar phenotypes as cdk5 [5]. Moreover, in the cdk5<sup>-/-</sup> mice, we observed the presence of hyperphosphorylated cytoskeletal proteins in swollen brain stem and spinal cord perikarya. This led us to look for other kinases affected by the absence of cdk5 in cdk5<sup>-/-</sup> mice [6]. Since cdk5 is down regulated in p35<sup>-/-</sup>-mice, we used brain extracts from these mice and found that MAPK (Erk1/2) was hyperactivated. Indeed, we have shown that MEK1 is an *in vitro* and *in vivo* target for cdk5/p35 phosphorylation; MEK1 catalytic activity was inhibited by phosphorylation at a specific site, Thr 286. This down regulation of the MAP kinase pathway results sustained MAP (Erk1/2) kinase activity in NGF stimulated PC12. These results suggest that cdk5 is involved in 'cross talk' with other signal transduction and apoptotic pathways.

Finally, in an effort to find the other cdk5 targets, we discovered that cdk5 directly phosphorylates c-Jun N-terminal kinase 3 (JNK3) on threonine 131 and inhibits its activity, leading to reduced c-jun phosphorylation [7]. In addition, other laboratories have shown that deregulation of cdk5 is involved in neurodegeneration (e.g. AD, ALS) [8-10]. Thus, cdk5 may turn out to function as the "Yin and Yang" in neuronal survival, exhibiting protective or destructive roles, depending on its mechanisms of 'cross talk' and regulation within the network of signal transduction pathways.

Cdk5 activity is tightly regulated in the nervous system. Recent studies suggest that the deregulation, (hyperactivation) of cdk5 activity by p25, a proteolytic fragment of p35, induces the hyperphosphorylation of tau and neurofibrillary tangle formation leading to neuronal death in neurodegenerative diseases such as AD and ALS.

Recently, we have discovered a specific inhibitor of cdk5. The inhibitor, termed cdk5 Inhibitory Peptide-CIP, derived from p35, specifically inhibits cdk5 activity in vitro. The CIP peptide (amino acid residues 154-279) also inhibits the kinase activity in p25/cdk5/CIP co-transfected HEK293 cells but had no effect on endogenous Cdc2 activity [11]. CIP has a high affinity for cdk5 and might act as a specific inhibitor of cdk5 in the nervous system. Additionally, CIP reduces p25/cdk5 mediated tau phosphorylation in HEK293 cells and indicates that efficient transfection of CIP, specifically inhibits p25/cdk5 activity and in cell lines, reduces tau phosphorylation. Currently, we are involved in investigating whether aberrant cdk5 hyperactivity caused by its association with p25 can be inhibited by CIP in neuronal cells. Once we show that CIP specifically inhibits cdk5 deregulation, we plan to extend these studies using AD and ALS transgenic models.

#### REFERENCES

1. Ohshima, T., Ward, J.M., Huh, C.G., Longenecker, G., Veeranna, Pant, H.C., Brady, R.O., Martin, L.J. and Kulkarni, A.B. Targeted disruption of the cyclin-dependent kinase 5 gene results in abnormal corticogenesis, neuronal pathology and perinatal death. **Proc. Natl. Acad. Sci. USA** 93 (1996) 11173-11178.
2. Tanaka, T., Veeranna, Ohshima, T., Rajan, P., Amin, N.D., Cho, A., Sreenath, T., Pant, H.C., Brady, R.O. and Kulkarni, A.B. Neuronal cyclin-dependent kinase 5 activity is critical for survival. **J. Neurosci.** 21 (2001) 550-558.
3. Tsai, L.H., Delalle, I., Caviness, V.S., Jr., Chae, T. and Harlow, E. p35 is a neural-specific regulatory subunit of cyclin-dependent kinase 5. **Nature** 371 (1994) 419-423.
4. Lew, J., Huang, Q.Q., Qi, Z., Winkfein, R.J., Aebersold, R., Hunt, T. and Wang, J.H. A brain-specific activator of cyclin-dependent kinase 5. **Nature** 371 (1994) 423-426.
5. Ko, J., Humbert, S., Bronson, R.T., Takahashi, S., Kulkarni, A.B., Li, E. and Tsai, L.H. p35 and p39 are essential for cyclin-dependent kinase 5 function during neurodevelopment. **J. Neurosci.** 21 (2001) 6758-6771.
6. Sharma, P., Veeranna, Sharma, M., Amin, N.D., Sihag, R.K., Grant, P., Ahn, N., Kulkarni, A.B. and Pant, H.C. Phosphorylation of MEK1 by cdk5/p35 down-regulates the mitogen-activated protein kinase pathway. **J. Biol. Chem.** 277 (2002) 528-534.
7. Li, B.S., Zhang, L., Takahashi, S., Ma, W., Jaffe, H., Kulkarni, A.B. and Pant, H.C. Cyclin-dependent kinase 5 prevents neuronal apoptosis by negative regulation of c-Jun N-terminal kinase 3. **EMBO J.** 21 (2002) 324-333.

8. Lee, M.S., Kwon, Y.T., Li, M., Peng, J., Friedlander, R.M. and Tsai, L.H. Neurotoxicity induces cleavage of p35 to p25 by calpain. **Nature** 405 (2000) 360-364.
9. Nguyen, M.D., Lariviere, R.C. and Julien J.P. Deregulation of Cdk5 in a mouse model of ALS: toxicity alleviated by perikaryal neurofilament inclusions. **Neuron** 30 (2001) 135-147.
10. Patrick, G.N., Zukerberg, L., Nikolic, M., de la Monte, S., Dikkes, P. and Tsai, L.H. Conversion of p35 to p25 deregulates Cdk5 activity and promotes neurodegeneration. **Nature** 402 (1999) 615-622.
11. Zheng, Y.L., Zukerberg, L., Nikolic, M., de la Monte, S., Dikkes, P. and Tsai, L.H. A peptide derived from cyclin-dependent kinase activator (p35) specifically inhibits Cdk5 activity and phosphorylation of tau protein in transfected cells. **Eur. J. Biochem.** 269 (2002) 4427-4434.