Neuronal Cell Cycle Regulation of Cdk5 in Alzheimer’s Disease

Yaqiong Niu1, Huifang Li2, Karl Herrup3 and Jie Zhang1,3*

1Institute of Neuroscience, Xiamen University, Xiamen, Fujian, China
2Division of Life Sciences, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong
3Institute of Neuroscience, Xiamen University, SimingNanLu 422, Xiamen, Fujian, China

Abstract

Neuronal cell cycle dysregulation is closely related with the neuronal death in Alzheimer’s disease (AD), but the detailed mechanism linking the two processes is unclear. Cyclin-dependent kinase 5 (Cdk5) is described as an atypical Cdk, which has been shown to have no cell cycle promoting activity. Yet while Cdk5 may not promote the cycle, we have found that Cdk5 may play a role in maintaining the quiescent stage of post-mitotic neuron. In this chapter, we review recent findings concerning the cell cycle suppression activity of Cdk5, and relate this function to the initiation and progression of neurodegenerative diseases, in particular AD. Our data suggest that nuclear Cdk5 can block the cell cycle. When the post-mitotic neuron is subjected to β-amyloid stress, Cdk5 is translocated from nucleus to cytoplasm. Deprived of its nuclear Cdk5, the post-mitotic neuron will re-enter into cell cycle, ultimately leading the cycling neuron to die rather than divide. Our work has identified the molecular basis of the cell cycle suppression effect of Cdk5. Taken together, our data reveal that Cdk5 does indeed regulate cell cycle activity. These finding may provide new pharmacotherapeutic approach to treating brain disorders such as AD.

Keywords: (Cyclin-dependent kinase 5) Cdk5, Cell Cycle; Neuronal Death; Alzheimer’s disease

Cell Cycle Regulation

The cell cycle is a highly conserved mechanism that controls the cells decision to proliferate and regulate the process once it starts. Typically, the cell cycle is divided into four phases, namely G1 (first gap), S (DNA synthesis), G2 (second gap), and M (mitosis). The cell cycle process is regulated by the sequential expression, activation, and inhibition of Cyclin-dependent kinases (Cdks) associated with activating subunits, the cyclins, as well as two families of Cyclin-dependent kinase inhibitors (CKIs)–the Kip/Cip family of proteins (p21, p27 and p57) and the INK4 family (p16, p15, p19 and p18) [1-3]. There are ten Cdks and nine cyclins in mammal tissues that have been described to date. After mitogen initiation, synthesized D-type cyclins bind to and activate Cdk4 and Cdk6. These Cdks target several proteins, chief among them the tumor suppressor protein, retinoblastoma (RB). Phosphorylated RB releases the E2F1 transcription factor which binds to DNA and allows cells to enter G1. The cyclin E/Cdk2 complex is required for transition from G1 to S phase. Later, in M phase, Cyclin B/Cdk1 activation is triggered allowing the cell to proceed through cytokinesis. During all four stages of the cell cycle, the activity of both Cdks and CKIs are tightly controlled by transcription, translation, and ubiquitin-mediated proteolysis [4-7].

Cdk5: A typical Cyclin Dependent Kinase

Cyclin-dependent kinase 5 (Cdk5) is a unique member of the Cdk family, despite the fact that its cloning was based on its sequence homology to other Cdks [8]. Cdk5 expression can’t drive the cell cycle forward, nor is it necessary for normal cell cycle progression. Yet, as described in more detail below, Cdk5 has a potent cell cycle activity that is inhibitory in nature. It is a strong force in mature cells to hold the cell cycle in check. As a proline directed serine/threonine kinase, Cdk5 is structurally most similar to CDC2 (Cdk1), but it functionally differs from traditional Cdks [8]. Cdk5 is abundant in nerve cells, but evidence for its existence in other cells is well established [9,10]. Several of the traditional cyclins can bind with Cdk5, but none can activate it. Instead, Cdk5 is primarily activated by p35 and p39, which are highly expressed in the nervous system [11-14]. The p35 and p39 share no homology to cyclins at the amino acid level but have remarkable structural similarity all the same [8]. The association of Cdk5 with p35 or p39 is required in processes such as neurite outgrowth, axonal migration, and cortical lamination, control of cell adhesion, axonal transport and synaptic activity [8]. Consequently, the loss of Cdk5 give rise to a failure of cell cycle suppression and neuronal cell death, both in vivo and in vitro [15,16].

Neuronal Cell Cycle Dysregulation in Alzheimer’s Disease

In the adult mature brain, once the neurons of the central nervous system leave the ventricular, they will be permanently post mitotic, and never complete a full cell cycle again. But despite this non-mitotic state, a neuron is still capable of initiating a cell cycle. This leads to the provocative question of what would happen if a neuron loses its control of the cell cycle and attempted to divide? It has been shown in numerous studies that neurons that re-enter the cell cycle are fated to die rather than divide. For example, in areas where neurons are likely to die in neurodegenerative diseases such as Parkinson’s or Alzheimer’s disease (AD) there is substantial evidence from many labs that nerve cells are expressing cell cycle proteins and have completed at least one round of DNA replication. The induction and activation of CDC2 is found in degeneration neuron in AD brain, as is Cyclin B [17-19]. Other cell cycle proteins such as Cyclin D, Cdk4, Cdk6 and Ki-67 (a DNA binding protein that is found only in dividing cells) are also found in the neurons of the AD brain [20-22]. Prior to their death,
gene-specific DNA replication has been demonstrated by fluorescent in situ hybridization (FISH) [23]. These experiments offer direct evidence that AD neurons probably complete enough of the cell cycle to enter G2 phase after completing G1 and S phase. The assignment of specific cell cycle phases to these neurons is probably not completely correct. For example, several cell cycle proteins are found in unnatural locations in the ‘cycling’ neurons. PCNA and Cyclin B, both normally nuclear proteins during the cell cycle are found in aberrant cytoplasmic locations. Yet despite these anomalies, numerous laboratories studying this abortive neuronal cell cycle re-entry have found a tight connection between evidence of cell cycle events in neurons and sites of apoptosis in AD and other neurodegenerative diseases.

**Does Cdk5 Play a Role in Cell Cycle Regulation?**

Data from our lab supports the suggestion that Cdk5 functions actively in cell cycle regulation. Unlike other Cdns, however, its role appears to be to suppress the cell cycle, both in post-mitotic neurons and neuronal cell lines. We have proposed that to perform this cell cycle suppression function Cdk5 must be located in the nucleus. Here it plays an active role in allowing neurons to remain post-mitotic as they mature. Consistent with this hypothesis, we have found that loss of nuclear Cdk5 leads to cell cycle re-entry, even if the levels of cytoplasmic Cdk5 remain significant. The shift in sub-cellular location and cell cycle re-entry is accompanied by neuronal death. Significantly, we have found evidence for this phenomenon in non-neuronal cell as well. By synchronizing NIH 3T3 cells and neuronal N2a cells, we find low levels of nuclear Cdk5 in proliferating fibroblasts, as well as neuroblastoma cells. Here again, in cells that are actively proliferating, the nuclear/cytoplasmic ratio is low during S-phase of the cell cycle [16].

Historically, the first evidence to hint at the cell cycle regulatory effect of Cdk5 was the discovery that neurons in the Cdk5<sup>-/-</sup> mouse brain cortex re-express cell cycle proteins, such as cyclin D and incorporate BrdU before apoptotic death [15]. Experiments *in vitro* showed that the nuclear location of Cdk5 is the key to cell cycle arrest rather than its total amount [16]. Even in the AD brain, neurons that express cell cycle proteins (in the regions where neuronal death is prevalent) also lose their nuclear fraction of Cdk5, attesting to the generalizability of this phenomenon. Indeed, results in both human AD and its mouse models further prove that Cdk5 localization and cell cycle re-entry are intimately linked. In both the R1.40 AD mouse and human AD brain, neurons in vulnerable regions re-enter a cell cycle and lose their nuclear Cdk5. We suggest that without nuclear Cdk5, the neuronal cell cycle is released followed by an ultimately lethal series of subsequent events.

The consistency of the cell cycle suppression role of Cdk5 in a variety of cell types, in stressful situations both *in vivo* and *in vitro*, proves that the nuclear localization of Cdk5 that plays an unexpected but widespread role in neuronal cell cycle suppression. The types of stress that can induce a cell cycle related neuronal death are wide ranging and include oxidation (H₂O₂), DNA damage (camptothecin, etoposide), as well as the Alzheimer’s peptide (Aβ).

**How does Cdk5 Suppress Neuronal Cell Cycle?**

In exploring the mechanism by which Cdk5 suppresses neuronal cell cycle, our attention was drawn to the transcriptional factor E2F1. E2F1 is a well-known transcription factor that is involved in the regulation of cell proliferation, differentiation, and apoptosis through transcriptional regulation [24-26]. Just as Cdk5 needs its cyclin-like p35 protein to be fully active, E2F1 requires a co-factor, DP1, to bind DNA appropriately and drive cell cycle protein expression. Without DP1, E2F1-dependent transcriptional activation reduces to a large

![Diagram of neuronal cell cycle regulation of Cdk5](image_url)

**Figure 1: Neuronal cell cycle regulation of Cdk5.**

**Panel A:** In post-mitotic neurons, Cdk5 was located both in nuclear and cytoplasm. Nuclear Cdk5 will disrupt the interaction between E2F1 with DP1 in the present of p35. Without DP1 binding, the cell cycle driving capacity of E2F1 is limited and the neurons were kept in post-mitotic stage.

**Panel B:** Nuclear Cdk5 localization is dependent on its binding with p27. Mitotic signals appear to dissociate the interaction between Cdk5 with p27 and trigger Cdk5 to shuttle from nucleus to cytoplasm by NES-CRM1 pathway.

**Panel C:** As soon as nuclear Cdk5 was transported into cytoplasm in S phase, the cytoplasmic Cdk5 will be ubiquitinated by the E3 ligase APC-CDH1. Ubiquitinated Cdk5 is degraded then by the proteasome.
extent [27]. We first explored the interactions among Cdk5, E2F1, DP1, and p35 at a physical level. We found that Cdk5 performs as a cell cycle suppressor by way of its participation in a multi-protein complex that contains E2F1. Electromobility shift assays proved that the DNA binding ability of E2F1 was significantly attenuated by overexpression nuclear Cdk5. The association of Cdk5 with E2F1 and its cofactor, DP1, provides evidence for this inhibition [28,29]. After binding with E2F1 and preventing the interaction of DP1 with E2F1, Cdk5 suppress cell cycle by decreasing the occupancy of the E2F1 promoter element. Unexpectedly, a kinase-dead Cdk5 produced the same results. This suggested that Cdk5 does not need its normal kinase activity to suppress the neuronal cell cycle [28,29]. But Cdk5 does need the binding of its activator p35 to inhibit the cell cycle. This p35-dependency is supported by lack of effect on E2F1 promoter occupancy in the Cdk5 (S159T) mutant that lacks the ability to bind p35, and by the observation that this mutant cannot function as a cell cycle suppressor. The p35 protein contains a myristoylation signal motif, which has been speculated to give it the ability to be anchored to the plasma membrane. Yet, cell fractionation and immunocytochemistry reveal that in addition to the membrane-bound form of p35, substantial amounts of the protein also exist in the nucleus of cultured neurons and other cells [30,31]. Here it is well positioned to participate in the E2F1/Cdk5/p35 complex. We also directly visualized the interaction between Cdk5 and p35 in the nucleus using BIFC (bimolecular fluorescence complementation).

It is the location in the nucleus or the cytoplasm that is the critical determinant of the function of the Cdk5-E2F1 complexes. Our data suggested that each protein of complex has a potential binding site for each of the other three proteins. They can bind each other as heterodimers in all combinations. If all of them gather at once, Cdk5 and DP1 apparently compete to bind a p35–E2F1 complex. However, the DP1–p35–E2F1 trimer is most often observed in the cytoplasm while the Cdk5–p35–E2F1 trimer is more often seen in the nucleus. The nuclear Cdk5–p35–E2F1 complex keeps DP1 from binding with E2F1, which will dramatically decrease the cell cycle driving ability of E2F1. The direct consequence will be inhibiting the cell cycle (Figure 1).

The Nucleocytoplasmic Trafficking of Cdk5

As mentioned above, Cdk5 is normally located in both nucleus and cytoplasm, yet it is only nuclear Cdk5 that is effective in serving as a cell cycle suppressor. It is not surprising, therefore, that in cultured postmitotic neurons, the export of Cdk5 from the nucleus to the cytoplasm is pivotal for cell cycle re-entry, a first step on a pathway that leads to neuronal cell death. This places a premium on understanding the forces that regulate its location and in particular its nuclear retention. It is the location in the nucleus or the cytoplasm that is the critical determinant of the function of the Cdk5-E2F1 complexes.

The control of Cdk5 localization also depends on nuclear export processes. Unlike the lack of NLS sequences in Cdk5 itself, we have been able to show that endogenous NES signals direct the export of Cdk5 [32,33]. Similar to other nucleocytoplasmic proteins, nuclear export of Cdk5 is dependent on its ability to bind to CRM1 [35-37]. Truncation mutation studies allowed us to show that the export of Cdk5 relies on two atypical NES motifs; one is located between amino acids 64 and 83 and the other is between amino acids 128 and 147. We have just begun to explore this export process, yet already we have discovered several intriguing results. The most unexpected of these is that when Cdk5 is exported to the cytoplasm in post-mitotic neuron under stress, we find that the extra cytoplasmic Cdk5 shows every sign of being neuroprotective. The extra cytoplasmic Cdk5 attenuates the caspase-3 cleavage and degradation of BCL-2. Even though the mechanism behind this neuroprotection is unclear, the distinct nuclear and cytoplasmic function of Cdk5 points to the critical importance of the factors that regulate Cdk5 location.

Cdk5 is Degraded in Cycling Cells

The timely degradation of cyclin proteins is important for the progression of the normal cell cycle process. Cyclin degradation is usually mediated by a highly specific ubiquitin-dependent proteolysis [38]. In contrast to the cyclins, the levels of the Cdk proteins themselves are normally maintained unchanged during the cell cycle. Our recent data show that Cdk5 is a relatively unstable protein, in neuronal cell lines and, surprisingly, its levels oscillate during the neuronal cell cycle [39]. This oscillation is not due to transcriptional regulation as mRNA levels remain relatively level. Rather, we have shown that Cdk5 is significantly degraded when cells enter S phase.

The mechanism behind this cells cycle stage-specific degradation is the UPS (ubiquitin-proteosome system). Cdk5 is ubiquitinated by the E3 ligase APC-Cdh1 after it is transported to the cytoplasm. The ubiquitin tag targets the protein for proteasomal degradation in a relatively short time. We used truncation mutations to identify the region containing the ubiquitination site on Cdk5, and found it to be in close proximity to the protein pocket that forms the binding site for p35. We validated the importance of this location by showing that the ubiquitination of Cdk5 was blocked when high levels of p35 were present. And when ubiquitination was blocked, the degradation of Cdk5 in S phase was also attenuated. This S-phase specific degradation of a Cdk once again marks Cdk5 as an atypical Cdk. In this behaviour, it is more similar to a cyclin than a true Cdk. As we know that the substrate recognition of ubiquitin-ligases relies on the E3 component [40], we identified that the Cdh1 is the E3 ligase to mediate the degradation of Cdk5 [39].
been explored for more than 2 decades. Almost all Cdk5 research has been focused on the localization and transportation of Cdk5 to investigate its neuronal cell cycle blocking ability for the first time to provide evidence that Cdk5 may perform neuronal cell cycle suppressor in post-mitotic neurons. This new finding will contribute to better understanding the involvement of Cdk5 in brain disorders including Alzheimer's disease.

References
1. Malumbres M, Barbacid M (2005) Mammalian cyclin-dependent kinases. Trends Biochem Sci 30: 630-641.
2. Lees E (1995) Cyclin dependent kinase regulation. Curr Opin Cell Biol 7: 773-780.
3. Liu DX, Greene LA (2001) Neuronal apoptosis at the G1/S cell cycle checkpoint. Cell Tissue Res 305: 217-228.
4. Grana X, Reddy EP (1995) Cell cycle control in mammalian cells: role of cyclins, cyclin dependent kinases (CDKs), growth suppressor genes and cyclin-dependent kinase inhibitors (CDKIs). Oncogene 11: 211-219.
5. Morgan DO (1997) Cyclin-dependent kinases: engines, clocks, and microprocessors. Annu Rev Cell Dev Biol 13: 261-291.
6. Sherr CJ (1994) G1 phase progression: cycling on cue. Cell 79: 551-555.
7. Sherr CJ, Roberts JM (1999) CDK inhibitors: positive and negative regulators of G1-phase progression. Genes Dev 13: 1501-1512.
8. Dhavan R, Tsai LH (2001) A decade of CDK5. Nat Rev Mol Cell Biol 2: 749-759.
9. Philipotta P, Porro EB, Kirchner MW, Tsai LH (1997) The role of cyclin-dependent kinase 5 and a novel regulatory subunit in regulating muscle differentiation and patterning. Genes Dev 11: 1409-1421.
10. Gao CY, Zakeri Z, Zhu Y, He H, Zelenka PS (1997) Expression of Cdk5, p35 and Cdk5-associated kinase activity in the developing rat lens. Dev Genet 20: 267-275.
11. Lew J, Huang QQ, Qi Z, Winkfein RJ, Aebersold R, et al. (1994) A brain-specific activator of cyclin-dependent kinase 5. Nature 371: 423-426.
12. Tang D, Yeung J, Lee KY, Matsuhashita M, Matsui H, et al. (1995) An isoform of the neuronal cyclin-dependent kinase 5 (Cdk5) activator. J Biol Chem 270: 26897-26903.
13. Tsai LH, Delalle I, Caviness VS Jr, Chae T, Harlow E (1994) p35 is a neural-specific regulatory subunit of cyclin-dependent kinase 5. Nature 371: 419-423.
14. Ko J, Hambur S, Bronson RT, Takahashi S, Kulkarni AB, et al. (2001) p35 and p39 Are Essential for Cyclic-Dependent Kinase 5 Function during neurodevelopment. J Neurosci 21: 6758-6771.
15. Cicero S, Herrup K (2005) Cyclin-dependent kinase 5 is essential for neuronal cell cycle arrest and differentiation. J Neurosci 25: 9568-9585.
16. Zhang J, Cicero, SA, Wang L, Romito-Digiaccomo RR, Yang Y, et al. (2008) Nuclear localization of Cdk5. Cdk5 is a key determinant in the postmitotic state of neurons. Proc Natl Acad Sci USA 105: 8772-8777.
17. Busser J, Geldmacher DS, Herrup K (1998) Ectopic cell cycle proteins predict the sites of neuronal cell death in Alzheimer’s disease brain. J Neurosci 18: 2801-2807.
18. Vincent I, Jicha G, Rosado M, Dickson DW (1997) Abruption expression of mitotic cdc2/cdc12 kinase in degenegrating neurons of Alzheimer’s disease brain. J Neurosci 17: 3588-3598.
19. Pei JJ, Braak H, Gong CX, Grundke-Iqbal I, Iqbal K, et al. (2002) Up-regulation of cell division cycle (cdc) 2 kinase in neurons with early stage Alzheimer’s disease neurofibrillary degeneration. Acta Neuropathol 104: 369-376.
20. Nagy Z, Esiri MM (1998) Neuronal cyclin expression in the hippocampus in temporal lobe epilepsy. Exp Neurol 150: 240-247.
21. Nagy Z, Esiri MM, Smith AD (1997) Expression of cell division markers in the hippocampus in Alzheimer’s disease and other neurodegenerative conditions. Acta Neuropathol 93: 294-300.
22. Nagy Z, Esiri MM, Smith AD (1998) The cell division cycle and the pathophysiology of Alzheimer’s disease. Neuroscience 87: 731-739.
23. Yang Y, Geldmacher DS, Herrup K (2001) DNA replication precedes neuronal cell death in Alzheimer’s disease. J Neurosci 21: 2661-2668.
24. Muller H, Bracken AP, Vernell R, Moroni MC, Christians F, et al. (2001) E2Fs regulate the expression of genes involved in differentiation, development, proliferation, and apoptosis. Genes Dev 15: 267–285.

25. Ren B, Cam H, Takahashi Y, Volkert T, Terragni J, et al. (2002) E2F integrates cell cycle progression with DNA repair, replication, and G2/M checkpoints. Genes Dev 16: 245–256.

26. Trimarchi JM, Lees JA (2002) Sibling rivalry in the E2F family. Nat Rev Mol Cell Biol 3: 11–20.

27. Zhang J, Li H, Yabut O, Fitzpatrick H, D’Arcangelo G, et al. (2010) Cdk5 suppresses the neuronal cell cycle by disrupting the E2F1-DP1 complex. J Neuroscience 30: 5219-5228.

28. Bandara LR, Buck VM, Zamanian M, Johnston LH, La Thangue NB (1993) Functional synergy between DP-1 and E2F-1 in the cell cycle-regulating transcription factor DRTF1/E2F. EMBO J 12: 4317–4324.

29. Zhang J, Herrup K (2008) Cdk5 and the non-catalytic arrest of the neuronal cell cycle. Cell Cycle 7: 3487-3490.

30. Nikolic M, Dudev H, Kwon YT, Ramos YF, Tsai LH (1998) The cdk5/p35 kinase is essential for neurite outgrowth during neuronal differentiation. Genes Dev 10: 816–825.

31. Qu D, Li Q, Lim HY, Cheung NS, Li R, et al. (2002) The protein SET binds the neuronal Cdk5 activator p35nck5a and modulates Cdk5p35nck5a activity. J Biol Chem 277: 7324–7332.

32. Zhang J, Li H, Herrup K (2010) Cdk5 nuclear localization is p27-dependent in nerve cells: implications for cell cycle suppression and caspase-3 activation. J Biol Chem 285: 14052–14061.

33. Zhang J, Herrup K (2011) Cdk5 nuclear localization is p27-dependent in nerve cells: implications for cell cycle suppression and caspase-3 activation. Cell Cycle 10: 1208-1214.

34. Görlich D, Kutay U (1999) Transport between the cell nucleus and the cytoplasm. Annu Rev Cell Dev Biol 15: 607–660.

35. Fornerod M, Ohno M, Yoshida M, Mattaj IW (1997) CRM1 is an export receptor for leucine-rich nuclear export signals. Cell 90: 1051–1060.

36. Fukuda M, Asano S, Nakamura T, Adachi M, Yoshida M, et al. (1997) CRM1 is responsible for intracellular transport mediated by the nuclear export signal. Nature 390: 308–311.

37. Ossareh-Nazari B, Bachelerie F, Dargemont C (1997) Evidence for a role of CRM1 in signal-mediated nuclear protein export. Science 278: 141-144.

38. Peters JM (2006) The anaphase promoting complex/cyclosome: a machine designed to destroy. Nat Rev Mol Cell Biol 7: 644-656.

39. Zhang J, Li H, Zhou T, Zhou J, Herrup K (2012) Cdk5 levels oscillate during the neuronal cell cycle: Cdh1 ubiquitination triggers proteosome-dependent degradation during S-phase. J Biol Chem 287: 25985-25994.

40. Kerscher O, Felberbaum R, Hochstrasser M (2006) Modification of proteins by ubiquitin and ubiquitin-like proteins. Annu Rev Cell Dev Biol 22: 159–180.

41. Harper JW, Burton JL, Solomon MJ (2002) The anaphase-promoting complex: it’s not just for mitosis any more. Genes Dev 16: 2179-2206.

42. Fang G, Yu H, Kirschner MW (1998) Direct binding of CDC20 protein family members activates the anaphase-promoting complex in mitosis and G1. Mol Biol Cell 2: 163-171.

43. Vasilint R, Prinz S, Amon A (1997) CDC20 and CDH1: a family of substrate-specific activators of APC-dependent proteolysis. Science 278: 460–463.

44. Huang X, Summers MK, Pham V, Lill JR, Liu J, et al. (2011) Deubiquitinase USP37 is activated by CDK2 to antagonize APC(CDH1) and promote S phase entry. Mol Cell 42: 511-523.