Cyclin-Dependent Kinase 5 (Cdk5): A Potential Therapeutic Target for the Treatment of Neurodegenerative Diseases and Diabetes Mellitus

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Abstract: Cyclin-dependent kinase 5 (Cdk5) is a serine/threonine protein kinase, which forms active complexes with p35 or p39 expressed predominantly in neurons. Cdk5 is indispensable for the development of the central nervous system through regulation of neuronal migration. In mature neurons, Cdk5 has been implicated in various signaling transduction pathways, which contribute to functional neuronal activity. It has been widely accepted that aberrant Cdk5 activity induced by the conversion of p35 to p25 plays roles in the pathogenesis of neurodegenerative diseases. Cdk5 also contributes to adaptive changes in the brain related to drug addiction. Moreover, recent studies suggest that Cdk5 plays crucial roles in physiological functions in non-neuronal cells such as glucose-stimulated insulin secretion in pancreatic -cells. The present evidence indicates that Cdk5 might be a potential drug target for the treatment of neurodegenerative diseases, drug abuse and diabetes mellitus. This review focuses on the implication of Cdk5 in the signaling pathways of both neurodegenerative diseases and drug abuse, and the mechanism of Cdk5 involvement in insulin secretion. This review also discusses the possibility of using Cdk5 inhibitors as therapeutic drugs.

AN OVERVIEW OF CDK5

Biochemical Features of Cdk5

Cyclin-dependent kinase 5 (Cdk5) is a serine/threonine protein kinase that was first purified from bovine brain as a protein sharing the same substrates with a cell cycle regulating kinase, Cdk2 [1,2]. Indeed, Cdk5 has many common features with all other members of the Cdk family. The protein sequence of Cdk5 shows 60% identity with Cdk2, as well as excellent homology of the crystal structures of these proteins. Similar to other Cdks, which need cyclins to activate the kinase activity, Cdk5 also needs cyclin-like proteins, which have been identified as p35 and its isoform p39 [3-6]. Despite these similarities, the regulation of Cdk5 kinase activity is somewhat different from that of other Cdks. It is well established that phosphorylation of Thr160 within Cdk2 by CAK and dephosphorylation of Tyr15 by cdc25 are necessary for the maximum activation [7-9]. In contrast, activation of Cdk5 does not require the phosphorylation of the corresponding threonine residue. Although there are contradictory results regarding the effect of tyrosine phosphorylation on Cdk5 activity, it seems that tyrosine-dependent regulation is significant for Cdk5 [10,11]. At present, it is generally thought that binding of p35 or p39 to Cdk5 is both necessary and sufficient to activate Cdk5 kinase.

Cdk5 Activators

Although Cdk5 is expressed in all tissues, Cdk5 kinase activity is predominantly observed in neurons. The restriction of Cdk5 activity to neurons is due to the fact that the Cdk5 activators p35 and p39 are predominantly expressed in neurons. However, there is some recent evidence that p35 and p39 are also expressed in some non-neuronal cells, such as pancreatic β cells and Sertoli cells in the testis [12-14]. In neurons, the expression of p35 and p39 is regulated in both temporal and spatial manners. The mRNA of p35 appears at an early embryonic stage and is maintained at a high level for 2 weeks after birth and then declines to a low level in the rodent brain [15,16]. Immunohistochemical studies show that p35 protein is located in neurons in all regions throughout the rodent brain. In contrast, the level of p39 mRNA is low in the embryonic stage [17]. Just as p35 mRNA starts to decline, p39 reaches a high level in rodents from 1 week to 3 weeks after birth. Moreover, p39 is abundant in Purkinje and granule neurons in the cerebellum after 3 weeks of age. These findings suggest that p35 and p39 regulate Cdk5 activity in different manners during the development of the brain.

Cdk5 in Neuronal Development

Cdk5 is indispensable for brain development. Disruption of Cdk5 in mice causes perinatal mortality and results in extensive deficiency in neuronal migration in the cerebral cortex, hippocampus and cerebellum [18-20]. The Cdk5-null brain lacks a cortical laminar structure and cerebellar foliation. The crucial role of Cdk5 in development is further supported by studies of p35 and p39 knockout mice. Mice lacking p35 show abnormal lamination in the cerebral cortex, like Cdk5-null mice, but exhibit only mild defects in the hippocampus and cerebellum [21-23]. While Cdk5-null mice show perinatal lethality, p35-null mice are viable and fertile. The milder phenotype of p35-null mice has been attributed to the presence of p39. Double knockout mice of p39/p35 exhibit a phenotype identical to that of Cdk5 knockout mice [24]. These results support the notions that p35 and p39 are the principle activators of Cdk5, and Cdk5 activity is essential for brain development.

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CDK5 IN NEURODEGENERATIVE DISEASES

Alzheimer's Disease and Cdk5

In Alzheimer's disease, tau protein has been intensively studied because tau is a component of paired helical filaments (PHFs), which form neurofibrillary tangles (NFs) in the Alzheimer's disease brain [25,26]. The insoluble PHF has been considered to be a trigger for the induction of neuronal cell death and subsequent onset of Alzheimer's disease. Since tau protein in PHFs is abnormally hyperphosphorylated, it has been proposed that aberrant phosphorylation accounts for the tau-toxicity [27,28]. Historically, Cdk5 was firstly purified as a microtubule-associated cdc2-like protein kinase and named tau protein kinase II because of its ability to phsophorylate tau [29]. Cdk5-dependent phosphorylation sites in tau are detected by antibody recognizing the abnormal phosphorylation state of tau observed in the Alzheimer's brain [30,31]. To date, Cdk5 is a major kinase (together with MAPK and GSK3) shown to be responsible for the abnormal phosphorylation of tau in the Alzheimer's brain [32-35].

The precise mechanism of Cdk5-dependent tau phosphorylation in Alzheimer's disease remained unknown until the discovery of cleavage of p35 to p25 [36-38]. p35 is associated with the cell membrane through myristoylation at its N-terminus [38]. Upon toxic stimulation, which allows excess elevation of the intracellular calcium concentration, p35 is cleaved by a protease, calpain, between amino acids 98 and 99. The resulting C-terminal fragment of p35 is designated p25, which can still bind to Cdk5 (Fig. (**1**)). Surprisingly the biochemical characteristics of Cdk5/p25 are rather different from those of Cdk5/p35 [39-41]. Firstly, p25 is more stable than p35. p35 is an unstable protein that is degraded by the ubiquitin-proteasome system with a half-life as short as 30 minutes. Conversion of p35 to p25 can extend the half-life to several hours. Secondly, since p25 does not contain the myristoylated residue in the N-terminus, Cdk5/p25 is pre-ferentially located in the cytoplasmic compartment. Thirdly, the substrate preference of Cdk5/p25 is somewhat different from that of Cdk5/p35 [42,43]. Several lines of evidence showed that Cdk5/p25 phosphorylates tau more potently than Cdk5/p35. All these discoveries support the elegant hypothesis that deregulation of Cdk5 activity through cleavage of p35 to p25 causes abnormal phosphorylation of tau and thus induces neuronal cell death and the subsequent onset of Alzheimer's disease.

Pathology of p25 Transgenic Mice

Cdk5/p25 has been implicated in tau phosphorylation as well as neuronal cell death in primary cultures. Recent studies have focused on whether p25 expression directly causes Alzheimer-like symptoms in model mice. One line of transgenic mice with overexpression of p25 shows hyperphosphorylation of tau and an Alzheimer-like immunohistochemical staining pattern [44]. In another line of p25 overexpressing transgenic mice, despite the failure to detect phosphorylated tau, the mice display axonal degeneration and severe limb paralysis [45]. In mice in which overexperssion of p25 is precisely controlled by the tet-off system, moreover, hyperphosphorylation of tau and formation of neurofibrils are observed [46]. Although there are some results which indicate that p25 is not related to the phosphorylation of tau, these conflicting findings may have been the result of the different genetic backgrounds of the transgenic mice [47]. Nevertheless, the overexpression of p25 in neurons has consistently been shown to contribute to pathological changes that are considered to resemble Alzheimer-like neurodegeneration [48].

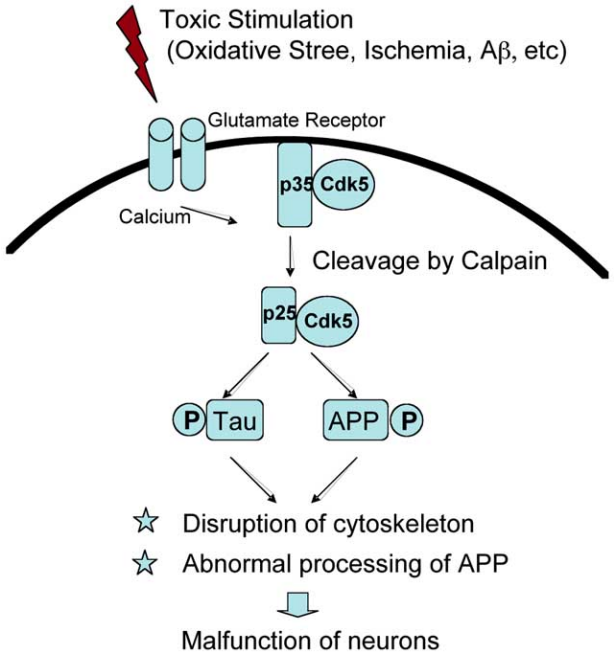


Fig. (1). Pathological pathway of Cdk5/p35 in neurodegenerative diseases. Toxic stimulation causes excessive influx of calcium into cytosol and results in generation of Cdk5/p25 by proteolysis. Cdk5/p25 abnormally phosphorylates a number of substrates including Tau and APP, which might trigger on-set of neurodenegerative diseases.

Amyloid Hypothesis and Cdk5

In Alzheimer's disease, β-amyloid-containing neuritic amyloid plaques are another important pathological hallmark. The insoluble β-amyloid peptide is an abnormal product of the cleavage of amyloid precursor protein (APP) by β-secretase (BACE) and gamma-secretase (presenilin) [49-51]. A body of evidence shows that β-amyloid triggers a cascade of pathogenic signaling pathways underlying its extreme neurotoxicity, resulting in widespread cell death both in cultured neurons and *in vivo*. At present, β-amyloid is considered the most critical pathogen in Alzheimer's disease. Accumulating evidence shows that Cdk5 is also involved in a β-amyloid-dependent signaling pathway [52]. Cdk5 directly phosphorylates APP at Thr668 both in cultured neurons and *in vivo* [53]. Overexpression of p25 in cultured cells and transgenic mice results in an increase of the immature form of APP as well as phosphorylation at Thr668 [54,55]. Phosphorylation of APP at Thr668 is preferentially found in the fragment generated by BACE-dependent cleavage and the mutation of Thr668 to Ala reduces the production of β-amyloid [56]. Cdk5/p25-dependent production of β-amyloid stimulates additional cleavage of p35 to p25, resulting in fur-

ther aberrant processing of APP and hyperphosphorylation of tau. Furthermore, phosphorylated APP is accumulated in the brains of patients with Alzheimer's disease. Given these results, it is apparent that the Cdk5/p25-dependent pathway is crucial in the pathogenesis of Alzheimer's disease.

Amyotrophic Lateral Sclerosis (ALS) and Cdk5

ALS is an adult-onset neurodegenerative disease with selective loss of motor neurons in the spinal cord, brainstem and cerebral cortex [57,58]. The precise mechanism causing motor neuron loss is still unknown. However, among ALS patients, approximately 10% patients are familial cases, 20% of whom have missense mutations in the gene encoding superoxide dismutase 1 (SOD1). Therefore, extensive studies have been conducted to clarify how mutant SOD1 induces neuronal cell death. There are several pieces of evidence which implicate a Cdk5/p25-dependent pathway in the pathogenesis of ALS [59-62]. In ALS model mice overexpressing a mutant SOD1 (G37R), that has been identified in a familial case of ALS, Cdk5 activity is augmented because of the cleavage of p35 to p25. The mice display Cdk5/p25-dependent hyperphosphorylation of tau and have a shorter life span. Interestingly, in double transgenic mice in which the SOD1 mutant is expressed in a neurofilament heavy subunit-overexpressing background, the life span is extended, suggesting that the overexpressed neurofilaments trap Cdk5/p25, and thereby prevent its functioning in the pathogenic pathway. Indeed, colocalization of Cdk5/p25 with overexpressed neurofilaments is observed in the double transgenic mice. These results not only indicate that Cdk5/p25 is an important mediator in the pathogenesis of ALS, but also suggest that suppression of Cdk5/p25 activity is a possible approach for the treatment of ALS.

Other Neurodegenerative Diseases and Cdk5

In addition to the extensive studies of Alzheimer's disease and ALS, emerging evidence suggests that the Cdk5/p25 pathway is also involved in various types of neurodegenerative diseases such as Parkinson disease and Niemann-Pick Type C disease [63-67]. In these diseases, cleavage of p35 to p25, mislocalization of Cdk5/p25 and alteration of substrate preference are observed. The deregulation of Cdk5 upregulates the pathogenic pathway to the hyperphosphorylation of tau and results in the onset or development of neurodegenerative disease. The Cdk5/p25 hypothesis raises the question of whether treatment with Cdk5 inhibitors would prevent or ameliorate neurodegenerative disease. Previous studies have shown that inhibition of Cdk5 attenuates the progression of disease in model mice and highlight the potential role of Cdk5 inhibitors as therapeutic agents [67]. Cdk5 may be a potential target for the treatment of patients with neurodegenerative diseases.

CDK5 IN THE PANCREAS

Regulation of Insulin Secretion

Both p35 and p39 are predominantly expressed in neurons, and Cdk5 has been extensively investigated to clarify its neuronal functions. However, emerging evidence shows that p35 and p39 are also expressed in non-neuronal cells, such as pancreatic β-cells [14]. This discovery highlights a novel physiological function of Cdk5.

Cdk5 has been shown to be associated with exocytosis machinery and to be involved in the regulation of neurotransmitter release in neurons [69]. Because both neurons and pancreatic β-cells share similar secretion machinery, it is natural to imagine that Cdk5 would regulate insulin secretion in β-cells. It has been reported that inhibition of Cdk5 by selective inhibitors increases insulin secretion in β cell-derived cell lines and mouse pancreatic islets. Furthermore, elimination of p35 expression by siRNA or by genetic manipulation of β-cells augments insulin secretion. These results suggest that Cdk5 negatively regulates insulin secretion in β-cells and that Cdk5 inhibitors might improve hyperglycemia in patients with type 2 diabetes. The mechanism of Cdk5-dependent regulation of insulin secretion is thought to be as follows (Fig. (**2**)). Insulin secretion is triggered by calcium influx through L-type voltage-dependent calcium channels (L-VDCCs) in response to elevation of the extracellular glucose level. Cdk5 phosphorylates loop II-III of the α1c subunit of L-VDCC and inhibits the channel activity, resulting in inhibition of glucose-stimulated insulin secretion.

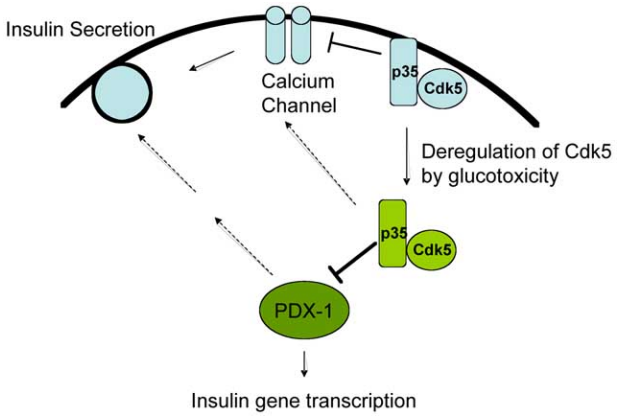


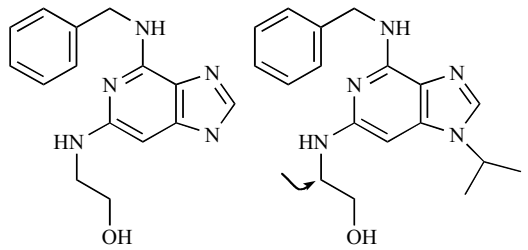
Fig. (2). Regulation of insulin secretion by Cdk5. Under normal condition, Cdk5 negatively regulates insulin secretion by phosphorylation of voltage-dependent calcium channel. Inhibition of Cdk5 can enhance glucose-stimulated insulin secretion. When b-cells are exposed to high glucose for a long time, Cdk5 might inhibit insulin gene transcription through regulation of PDX-1 protein.

A recent study showed that Cdk5/p35 is involved in glucotoxicity in pancreatic β-cells [69,70]. Transient elevation of extracellular glucose promotes pancreatic β cell function and survival, whereas chronic elevation of glucose has the opposite effect, impairing β cell function and survival. The deleterious effects of chronically elevated glucose are referred to as glucotoxicity. Glucotoxicity is a critical component of the pathophysiology of type 2 diabetes because it impairs both the effects of insulin on peripheral tissues and the secretion of insulin from β cells. An increase in p35 expression is observed in β cells subjected to glucotoxicity. Chronic exposure of β cells to high glucose reduces both the insulin mRNA level and the activity of an insulin promoter reporter gene. Inhibition of Cdk5 prevents the decrease of insulin gene expression through the inhibition of nuclear translocation of PDX-1, which is a transcription factor for the insulin gene (Fig. (**2**)). Although the precise mechanism of Cdk5-mediated regulation of glucotoxcity is still un-

known, Cdk5 plays a role in the loss of pancreatic β cell function in type 2 diabetes. These findings suggest that Cdk5 might be a potential therapeutic target for the treatment of type 2 diabetes.

CDK5 INHIBITORS

Two types of Cdk5 inhibitor, chemical compounds and peptides, have been identified to date. The most frequently utilized chemical compounds are purine analogues such as roscovitine and olomoucine (Fig. (**3**)) [71-73]. Cdk5 is inhibited by roscovitine and olomoucine with IC50s of 0.2 μM and 3 μM, respectively. These inhibitors compete with ATP for binding to Cdk5, and form hydrogen bonds with Cdk5. However, due to the high homology of the primary amino acid sequence and the 3D structure of Cdk5 and other Cdks, particularly in the kinase domain, roscovitine and olomoucine can also inhibit other Cdks to some extent. For example, a derivative of roscovine (R-roscovitine) inhibits Cdk1 and Cdk2, and the effect contributes to the inhibition of the growth of cancer cells [74]. R-roscovitine is now entering a phase II clinical trial against cancer [75].



Olomoucine **(R)-rescovitine**

Fig. (3). Structures of olomoucine and roscovitine.

To overcome this problem, Cdk5 inhibitory peptides have been generated [76,77]. These peptides are derived from a specific region in p35 protein and are highly specific for Cdk5. Studies using such Cdk5 inhibitory peptides suggest that these peptides could prevent Cdk5-mediated cell death. Nevertheless, the IC50s of these peptides are higher and the membrane permeability of these peptides is lower than those of roscovitine and olomoucine. It is important to develop the specific inhibitor of Cdk5 for therapeutic applications in neuronal degeneration and diabetes.

CONCLUSION

It has been over a decade since Cdk5 was discovered. Extensive studies have been conducted to clarify the role of Cdk5. Physiologically, Cdk5 has been implicated in the regulation of neuronal development and synaptic transduction. Pathogenically, Cdk5 has been recognized as a critical mediator in various neurodegenerative diseases, including Alzheimer's disease. Besides the neuronal functions of Cdk5, recent studies also suggest that Cdk5 might regulate insulin secretion in pancreatic β-cells. Given these findings, it is obvious that Cdk5 might be a useful target for the treatment of neurodegenerative diseases as well as diabetes.

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