Septin Phosphorylation and Neuronal Degeneration; Role of Cyclin Dependent Kinase 5 (Cdk5)

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Abstract

It is evident from the literature that most cellular functions are mediated by protein phosphorylation. It has also been demonstrated that abnormal phosphorylation of neuronal cytoskeletal proteins often leads to the pathology of several neurodegenerative diseases. For example, hyperphosphorylation of microtubule associated tau protein by Cdk5 and other kinases such as GSK3 has been linked to the formation of neurofibrillary tangles (NFTs) associated with Alzheimer’s disease (AD). Septins, a family of cytoskeletal proteins, also expressed in mammalian neurons, are involved in synaptic function and have also been linked to various neurological disorders. Although Sept5 phosphorylation by Cdk5 at the synapse has been reported to modulate exocytic function, as yet, its phosphorylation has not been linked to hyperactivated Cdk5 as seen in AD. The present review begins with an overview of Cdk5 function in the nervous system, its role in neurodegeneration and its relationship to neuronal septins, their function and role in neurological disorders. In a summary speculation, we attempt to correlate synaptic function of Cdk5 phosphorylation of septins that may play a role in the etiology of neuronal disorders.

Keywords: Cdk5; Alzheimer’s disease; Parkinson’s disease; Amyotrophic Lateral Sclerosis (ALS); Septin; Phosphorylation; Neurodegeneration; Synapse

Introduction

Cellular function is tightly regulated by protein kinases that orchestrate cell signaling events [1]. During cell replication, kinases activated by cyclin family members (Cdkks) play an important role in regulating transitions through the cell cycle in most organisms. Cdkks are activated upon association with cyclin regulatory subunits coupled to a phosphorylation event at specific activation sites [2,3]. Cdk5, though a member of the Cdk family, plays an important role outside the cell cycle. It is activated by non cyclin proteins, p35 and p39, which are predominantly expressed in the brain [4-8]. Though there are few sequence similarities between p35/p39 and regulatory cyclins, structural studies have shown that p35 assumes a cyclin like fold [9-11]. Cdk5 plays a key role in neuronal development and function including neuronal migration, neurite extension, and synapse formation, as well as synaptic activities in mature neurons [12-15]. Cdk5 phosphorylates neuronal cytoskeletal proteins tau and neurofilaments [16,17]. In neuronal migration, Cdk5 modulates actin dynamics, microtubule and transport phenomena via phosphorylation of multiple substrates. For example, phosphorylation of FAK at S732 (serine 732) is necessary for microtubule organization, nuclear translocation, and neuronal migration [18]. Phosphorylation of double cortin (Dcx) at S297 modulates its association with microtubules and regulates their polymerization [19]. A most prominent target of Cdk5 is tau, a microtubule associated protein [20]. Excessive phosphorylation of tau by deregulated Cdk5/p25 is crucially implicated inopathogenesis of AD [21,22].

Synaptic functions of Cdk5 in mature neurons have been shown with p35 knockout mice, a conditional knockout of Cdk5, and conditional expression of p25, a C-terminal fragment of p35 [13,23-25]. Cdk5 phosphorylation modulates synaptic transmission, neuronal excitability, dendritic spine morphogenesis, and has been linked to learning and memory [13]. It is clear from its extensive repertoire of target substrates that Cdk5 activity is a key player in the nervous system; hence, its activity must be tightly regulated. Moreover, it has become increasingly clear that deregulation of Cdk5 activity promotes the pathological hallmarks of neurodegenerative diseases such as AD [22], Parkinson’s disease (PD) [26] and Amyotrophic Lateral Sclerosis (ALS) [27,28].

Cdk5 involvement in synaptic function and plasticity depends upon its regulation of intracellular transport processes such as endocytosis and exocytosis. Its role at the pre-synaptic compartment is complex; it is involved in endocytic recycling of synaptic vesicles by rephosphorylating dephospholipids like the GTPase dynamin C [29-31]. During exocytosis, several target proteins are phosphorylated by Cdk5 [32-35] among which is Sept5; its phosphorylation by Cdk5 has a profound effect on exocytosis. It is also evident that phosphorylation of other neuronal septins may contribute to some of the pathogenesis of neurodegeneration.

Septins

Septins are a conserved family of GTPases that were first found in yeast as being essential for the completion of cell division [36-38]. The name ‘Septins’ alludes to the widespread involvement of this family of proteins in cytokinesis and septum formation. The discovery of a complex world of cytoskeletal GTPases that play important roles in human diseases emerged from the original discovery of four septin proteins in the budding yeast Saccharomyces cerevisiae. Septins have been...
found in all eukaryotic organisms, with the exception of plants [39-41]. The most recent review describes septins as the fourth component of the cytoskeleton [42] because of their filamentous appearance and their association with cellular membranes, actin filaments and microtubules.

Thirteen septin genes have been identified in humans (Sept1-Sept12 and Sept14, as Sept13 is now defined as a pseudo gene of Sept7 and Sept11) [43]. Many of these septin genes have alternative splice variants [44] and have been subdivided into four groups based on phylogeny. The septin 2 group (septins 1, 2, 4, and 5): the septin 3 group (septins 3, 9, and 12): the septin 6 group (septins 6, 8, 10, and 14) and the septin 7 group consist only of septin 7. Figure 1 shows the basic architecture of the longest known form of each of the 13 known human septins and their structural diversity.

Directly adjacent to the variable N-terminal tail is a short polybasic sequence followed by the highly conserved central GTPase domain that spans most of the septin sequence; then a series of highly conserved amino acids termed the septin unique element. In most septins, the septin unique element is followed by a coiled-coil domain of variable length [45]. The polybasic sequence is responsible for binding of septins to membrane phospholipids [46]. Since all septins possess a conserved GTP-binding domain, septins function as GTPase-signaling proteins related to their ability to bind and hydrolyze GTP.

**Neuronal Expression and Functions of Septin**

Based on the original yeast studies, septins play an important role in cytokinesis [39,50-52]. Mammalian septins have been associated with other cellular functions including apoptosis, vesicle trafficking, cytoskeletal dynamics, cell polarity, and sperm motility [53-56]. Multiple septins discovery in adult brain and post-mitotic neurons suggests that septins may have critical cellular functions in neurons. Among all the septins, Sept2, Sept3, Sept4, Sept5, Sept6, Sept7, Sept8 and Sept11 are highly expressed in central nervous system [57]. Two septins, Sept3 and Sept5 express primarily in the brain [58-60]. However Sept5 is also present in platelets that suggest that Sept3 is the only septin to date that is specific to the brain, as it is not found in platelets or any other non neuronal cell lines [60,61]. Nine of the 13 septins in mammals (Sept2 to Sept9 and Sept11) have been found in rat brain PSD fractions by mass spectrometry [57,62,63]. It has been suggested that these septins are involved in dendritic maturation, including spine morphogenesis and synaptic connectivity in cultured hippocampal neurons [64-66]. A number of septins that are found in post mitotic neurons have potential roles associated with synaptic transmission. Sept7 is present in postsynaptic density fractions [67] suggesting a role in cell architecture. One group, Sept6, Sept7, Sept2, and Sept4, associates with the exocyst complex, which is involved in polarized vesicle secretion function [50]. Sept5 and Sept2, have a role in exocytosis and synaptic vesicle dynamics as both co-immunoprecipitate with the snare protein syntaxin and synaptic vesicles [58,68,69]. Sept2 is a component of the exocyst complex [50] and ablation of its GTP binding activity leads to altered neurite sprouting [70]. The Sept3/S/7 complex was identified in the mammalian brain and Sept3 specifically is developmentally regulated and enriched in pre-synaptic nerve terminals, suggesting a role for these septins in neuronal biology [71,72]. Sept2 and Sept6 are associated with neurotransmitter release due to their interaction with glutamate transporter (Glast) and the synaptic vesicle protein synaptobrevin 2(Vamp2) [73,74]. Expression of several septins is developmentally regulated in cerebral cortex; protein levels of Sept2, Sept5, Sept6, Sept7 and Sept8 gradually increase at late embryonic stages through early postnatal days [73,65]. Recently, Sept14 expression has been reported in the developing mouse brain [75].

**Phosphorylation of Septins**

Protein phosphorylation, one of the fundamental mechanisms governing diverse cellular function has a profound effect on septin function. In the published reports several septins have shown to be phosphorylated in vitro and/or in vivo [76-78,72]. For instance Sept1 is phosphorylated in vitro by aurora-B kinase [77]. Sept2 is a substrate of casein kinase 2, and its phosphorylation on S218 is crucial for the proliferation of hepatoma carcinoma cells [78]. Moreover, Sept2 is also an in vitro substrate for protein kinase C and cAMP dependent protein kinase A [59]. Sept3 phosphorylation on S91 by CGMP dependent protein kinase G may contribute to the regulation of its subcellular localization in neurons [72]. The in-depth research into Sept5 function revealed that over expression of wild-type Sept5 in HIT-T15 cells resulted in inhibition of regulated secretion, whereas over expression of a dominant negative GTPase mutant S58A of Sept5, which cannot bind to GTP, resulted in potentiation of exocytosis [58]. The dominant negative phenotype of S58A Sept5 binds syntaxin more efficiently compared to wild-type Sept5.

So far the phosphorylation of septins by Cdk5 has only been reported for two isoforms of Sept5. In mice, Cdk5 phosphorylates S17 residue of adult type Sept5 [78]. However, that residue is not present in the human fetal type Sept5. Human Sept5 has been phosphorylated at the S327 residue instead [76]. Differences in secondary structure...
may account for a different phosphorylation site in the two isoforms. Nonetheless, phosphorylation of either site was reported to result in decreased interaction of Sept5 with syntaxin 1 [78,76]. A S327A mutation at the Cdk5/p35 phosphorylation site of human Sept5 results in a more efficient binding to syntaxin 1 [76]. Furthermore, when this non-phosphorylatable S327A mutant of Sept5 is over expressed in PC12 cells, it yields an increased S327A mutation at the Cdk5/p35 phosphorylation site of human Sept5 resulting in a more efficient binding to syntaxin 1 [76]. Therefore, it appears that more efficient interaction of Sept5 with syntaxin 1 results in potentiation of exocytosis, whereas over expression of the wild type Sept5 inhibits secretion, and this interaction is likely to be controlled by the phosphorylation state of Sept5 [79]. These studies of Sept5 and its phosphorylation suggest a strong link between Sept5 and the regulation of exocytosis. Though Cdk5 phosphorylation of other septins awaits future experiments it is appropriate to speculate that it may phosphorylate other septins possessing a proline-directed consensus sequence as evident from table 1. The phosphorylation of Cdk5 may show its role in synaptic function and disruption in synaptic function may lead to neurodegeneration.

**Septins in Neurodegeneration**

Septins are abundant in the central nervous system and are associated with many neurological diseases such as Parkinson’s, Alzheimer’s, Schizophrenia, and hereditary neuralgic amyotrophy (HNA) [80-84]. The brain specific expression of septins, their differential regulation in neural development, their role in exocytosis and their association with some disease states [85-88] suggest that abnormal septin function may contribute to neurological disorders. Possible roles for septins in neurological disorders have emerged based on the observation that Sept2, Sept1 and Sept4 have been found in Alzheimer specific NFTs [89]. Using proteomics, abnormalities of septin expression have also been reported in Down’s syndrome [85]. The Sept5 interaction with Parkin, a pathogenic protein in Parkinson’s disease [90,91], provides evidence for the involvement of another subset of septins in neuronal disease. Sept5_v2 has been reported to be a Parkin binding protein, which may function as an E2-dependent ubiquitin ligase capable of degrading Sept5. Sept5 accumulates in the brains of individuals with autosomal recessive juvenile Parkinsonism [92]. Sept4 has also been implicated in brain pathogenesis by its association with cytoplasmic inclusions found in Parkinson’s disease and other synucleinopathies. Sept2 and Sept8 are associated with neurotransmitter release due to their interaction with glutamate transporter (Glast) and the synaptic vesicle protein synaptobrevin 2(Vamp2) [73,74]. Other septin complexes have been associated with myelin formation in the peripheral nervous system. Recently a Sept9 point mutation and duplication within the gene have been identified in patients with the autosomal dominant neuropathy, hereditary neuralgic amyotrophy (HNA) [83,93,94]. It should be pointed out that the role of septin phosphorylation in these neuronal disorders has not been adequately investigated.

**Conclusion**

The literature reviewed above links septins to several neurodegenerative disorders including AD. Although a few studies, as we have seen, implicate septins in neurodegeneration, a correlation between septin phosphorylation and pathology, as is the case for tau, another cytoskeletal protein, has not been reported. Nevertheless, in view of the well established role of abnormally hyperactivated Cdk5/p25 in AD and ALS [95,22], it is not inappropriate to speculate that in disorders in which Cdk5 or other proline directed kinases are abnormally activated, several septin target substrates located at the synapse, are abnormally phosphorylated, contributing to the pathological phenotype. Considering the high incidence of proline directed consensus sequences in the various human septin families (Table 1), it is not unlikely that they are targeted for phosphorylation in neuronal disorders. The defects may manifest themselves not as tangles, nor plaques, but as more subtle synaptic malfunctions leading to defects in motor behavior, memory and learning. Those few studies focused on septin phosphorylation by Cdk5 [76,78] have identified dramatic effects on synaptic function and should stimulate a more intensive exploration of the relationship between hyperactive Cdk5/p25 and septins in various neurodegenerative disorders.

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**References**


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Table 1: Human septins having proline directed kinase consensus Sequence: (ST) PX(A/K/R)}


