Is there a role of the cyclin-dependent kinase 5 activator p25 in Alzheimer's disease?

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Sporadic Alzheimer's disease is the leading cause of dementia, but the underlying molecular processes are still unknown. Several studies have observed an accumulation of the protein fragment p25 in sporadic Alzheimer's disease brain. p25 derives from proteolysis of p35, and overactivates the τ kinase cyclin-dependent kinase 5. Transgenic mice expressing high levels of p25 exhibit hyperphosphorylation of τ as seen in Alzheimer's disease, and neurodegeneration. In contrast, low-level p25 expression, less than half of endogenous p35 expression, has a sex-specific effect on hippocampal synaptic plasticity and improves spatial learning in female but not in male mice. Therefore, p25 formation may initially be a compensatory response for early learning deficits in Alzheimer's disease, but continued formation could contribute to detrimental changes in Alzheimer's disease. *NeuroReport* 00:000–000 © 2005 Lippincott Williams & Wilkins.

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Introduction

The neuropathological hallmarks of Alzheimer's disease include neurofibrillary tangles (NFTs) composed of hyperphosphorylated microtubule-associated protein τ, and amyloid plaques formed by fibrillary amyloid β peptide. In NFTs, the protein τ is hyperphosphorylated at proline-directed serine/threonine sites. Therefore, proline-directed serine/threonine kinases including glycogen synthase kinase 3 (GSK3) and cyclin-dependent kinase 5 (Cdk5) have been implicated in mediating τ hyperphosphorylation [1]. The roles of GSK3 in Alzheimer's disease have been reviewed elsewhere [2]. A recent study suggests that Cdk5 regulates GSK3 activity regarding axonal transport [3]. Cdk5 is thought to be a prime candidate for mediating τ hyperphosphorylation, as it colocalizes with NFT [4], and its activity is increased in Alzheimer's disease [5]. Cdk5 belongs to the cyclin-dependent kinase family and has a broad range of substrates [6]. As with all members of the cyclin-dependent kinase family, Cdk5 alone shows no enzymatic activity, and requires association with a regulatory subunit for activation [6]. Several Cdk5 activators have been identified including p35 and p39. The calcium-dependent protease calpain can cleave p35 into the more stable protein fragment p25, which overactivates Cdk5 [7]. It has been suggested that formation of p25 is specific for Alzheimer's disease [8,9], although this is currently controversial [10,11]. Recent mutant mouse studies, in particular with transgenic expression of p25, provide support for a role of p25 in Alzheimer's disease (Tables 1–3).

The proteolytic fragment p25

In Alzheimer's disease, it has been proposed that an alteration in intracellular calcium homeostasis occurs. Changes in cellular calcium levels can induce calpain activation, and the subsequent cleavage of p35 leads to the formation of protein fragments p25 and p10 [7]. So far, p25 formation has been observed under pathological conditions. Like p35, p25 is a Cdk5 activator; however, p25 overactivates Cdk5 because it is more stable than p35. p35 has a short half-life and is rapidly degraded via the ubiquitin–proteosome pathway. The degradation of p35 is directly regulated by Cdk5 phosphorylation, which results in negative feedback regulation of the active p35/Cdk5 complex. In addition, formation of p25 has been suggested to alter the localization of active Cdk5 [8]. p35 is myristoylated at the N-terminus, which confers membrane localization. p25 lacks the myristoylation site, and consequently it is distributed throughout the cell. Finally, the active complexes p25/Cdk5 and p35/Cdk5 may have discrete substrate specificities, because differential phosphorylation of the amyloid precursor protein and microtubule-associated protein 1B has been demonstrated, depending on which of the two activators regulate Cdk5 [12,13].

Table I Mouse models with p25 expression

Mouse line; reference	Transgene; promoter; mouse strain	Expression pattern; expression levels; Cdk5 activation	Appearance/ behaviour/synaptic plasticity	AD-like molecular phenotype; neuro-degeneration	Other remarks
p25 Transgenic mice [21]	Human p25; PDGF B chain promoter; FVB/N strain	Brain and spinal cord; ratio of p25/p35 is 5:1; 1.5 to 2-fold increase in Cdk5 activity	Hindlimb semiparalysis and mild forelimb dyskinesia beginning at 3 months of age	Hyperphosphorylated τ, axonal swelling; no NFTs	Sciatic nerve and leg muscle do not show axonal abnormalities
p25 Transgenic mice [24]	Human p25; rat NSE promoter; FVB/N strain	Brain (tested only); ratio of p25/p35 is 1:1 (lower in cerebellum); upto 2-fold increase in Cdk5 activity	At 4–9 weeks of age, whole-body exertion tremors/increased locomotor activity, more time spent on open arms of elevated plus-maze test	Hyperphosphorylation of τ and NF detected by IHC, but not IB, positive Bielschowsky staining; axonal swelling in amygdala	Axonal swelling in spinal cord
p25 Transgenic mice [33]	Bovine p25; pCMV or PDGF promoter	Cortex, cerebellum and pituitary gland; 3 to 5-fold increase in p25 expression; 2 to 5-fold increase in Cdk5 activity	Unable to stand upright at 5 months, died at 6 months	No τ hyperphosphorylation (Ser202, Ser396, and Ser404 tested); no neuronal cell loss and apoptosis	Enlarged pituitary gland with high level of p25 expression, possibly inducing cell proliferation
p25/mutant τ double transgenic mice [25]	p25 Mouse line [24] crossed with TG mice expressing mutant (P30IL) human τ	Amygdala (tested only); ratio of p25/p35 is about 1:2 to 1:4; approx. 2-fold increase in Cdk5 activity	Pre-paralysis and early-stage degeneration mice were analyzed	Tau hyperphosphorylation, τ aggregates in sarkosyl-insoluble fractions; NFT as detected by silver stain	P30IL TG mice develop NFTs in the brainstem, spinal cord, telencephalon and diencephalons; neuronal cell loss in spinal cord
Inducible p25 transgenic mice [23]	Human p25, GFP and cmyc tagged; TetO-p25 mice crossed with CamKII-tTA TG mice; C57BL/6 strain	Inducible, restricted to forebrain; ratio of p25/p35 is about 2:1 to 3:1; 2.3-fold increase in Cdk5 activity	Not assessed	Tau hyperphosphorylation, accumulation of sarkosyl-insoluble τ, NFTs; neurodegeneration and gliosis tested with antibodies in IHC and IB	Raised under presence of doxycycline for 4–6 weeks; leaky transgene; different substrate specificity of p25/Cdk5 detected
p25 Transgenic mice [22,29]	Mouse p25; CamKIIα; C57BL6 or I29B6Fl strain	Restricted to forebrain; ratio of p25/p35 is 1:3 in hippocampus; 2-fold increase in Cdk5 activity	Normal appearance/altered fear conditioning and spatial memory formation/altered hippocampal LTP	Hyperphosphorylation of NF-M and age-dependent increase in τ hyperphosphorylation (unpublished); no neurodegeneration or gliosis	Sexual dimorphisms in behavioral phenotype and hippocampal LTP

Abbreviations: AD, Alzheimer's disease; CamKIIα, calcium/calmodulin kinase II-α; CamKII-tTA, tetracycline-sensitive transactivator driven by CamKII-α promoter; Cdk5, cyclin-dependent kinase 5; CMV, cytomegalovirus; GFP, green fluorescence protein; IB, immunoblot; IHC, immunohistochemistry; LTP, long-term potentiation; NF, neurofilament; NFTs, neurofibrillary tangles; NSE, neuron-specific enolase; PDGF, platelet-derived growth factor; TetO, tetracycline operon; TG, transgenic.

Formation of p25 in Alzheimer's disease and other diseases

Several post-mortem studies have suggested that the protein fragment p25 accumulates in patients with sporadic Alzheimer's disease, but not in normal individuals [8,9]. These studies are confounded by the fact that p25 is formed spontaneously in post-mortem tissue, triggered by pathological activation of calpain [14]. Consequently, some studies could not replicate p25 formation in Alzheimer's disease [10,11]. Consistent with the idea that p25 could be formed in Alzheimer's disease, however, increased activities of both calpain and Cdk5 have been observed in post-mortem Alzheimer's disease brains [5,15]. Further support for p25 formation in Alzheimer's disease comes from studies of the Tg2576 transgenic mice in which Alzheimer's disease is modeled by expressing a mutated form of the amyloid precursor protein [16]. In these mutant mice, formation of p25 has been demonstrated. Additionally, formation of p25 occurs in conditional presenilin knockout mice [17].

Formation of p25 has also been suggested to occur in diseases other than Alzheimer's disease; for example, in patients with Niemann–Pick type C disease [18]. Additionally, p25 formation was detected in mutant mice modeling Niemann–Pick type C disease and amyotrophic lateral sclerosis [18,19]; however, p25 does not contribute to the amyotrophic lateral sclerosis-like pathogenesis [20]. Finally, induction of ischemia in rats leads to p25 formation in the hippocampal area CA1, and p25-mediated activation of Cdk5 contributes to the ischemic insult [7]. For a summary, see Table 3.

Modeling p25 formation in transgenic mice

Several transgenic mouse lines expressing p25 have been generated to study the physiological effects of p25 formation (Table 1). These mouse lines differ in level and distribution of p25 expression, and some are not suitable for investigating the role of p25 formation in Alzheimer's disease. For example, in one mouse line expressing relatively high amounts of p25 in the spinal cord, semiparalysis of the hind limbs and premature death occurred [21]; this severe phenotype precludes an analysis of memory formation and age-dependent processes. Thus, to fully evaluate the potential physiological role of p25 formation in Alzheimer's

Table 2 Mouse models with altered expression of Cdk5 or p35

Mouse line; reference	Mutation; mouse strain	Phenotype/appearance	AD-like molecular phenotype	Remarks
Cdk5 (−/−) [34]	KO mouse line; C57BL/6 strain	Lesions in brain and spinal cord, not in other tissues, lack of cortical laminar structure and cerebellar foliation; perinatal death	Accumulation of NF immunoreactivity in neuronal cell bodies suggesting defective transport of NF	Heterozygous Cdk5 (+/−) mice appear normal
p35 (−/−) [35]	KO mouse line; I29/Sv and C57BL/6 strain	Cortical lamination defects, altered cell orientation, dendritic and axonal trajectories; sporadic adult lethality and seizures	Not assessed	Study links the Cdk5 activator p35 to proper neuronal migration
p39 (−/−) and p35/ p39 (−/−) [36]	p39 KO mouse line and p35/ p39 double KO mouse line; C57BL/6 strain	p39 KO mice have no phenotypic abnormalities, loss of p39 produced no changes in p35 and Cdk5 levels and Cdk5 activity; double KO mice are perinatal leathal and display similar phenotype as Cdk5 KO mice, double KO display no Cdk5 activity in the brain	No changes in τ phosphorylation during development; phosphorylation of NF-H is unaltered; changes in cellular NF-H localization in the spinal cord	Study shows distinct roles for p35 and p39; regulation of p39 levels by p35 expression; p35 and p39 seem to be the only Cdk5 activators important during CNS development
p35 (−/−) [37]	KO mouse line; C57BL/6 strain	Cortical lamination defects, axonal derangement, redistribution of Cdk5 and cytoskeletal proteins, Cdk5 activity reduced by approx. 60%	Increased phosphorylation of NF, MAP2B and τ at Ser202, Ser235, Ser396/ 404; increased GSK3β activity	Results indicate a role in neuronal trafficking for CdK5/p35
p35 (−/−) expressing p25 [38]	Crossing of transgenic p25 mice [24] and p35 (−/−) mice [35]; C57BL/6 strain	Partial rescue of the p35 (−/−) phenotype; phosphorylation of disabled-I is partially restored	No τ hyperphosphorylation, p25 levels increase with age (spongiform degeneration in the cortex at 2 years)	Study shows that p25 can substitute some but not all functions of p35

Abbreviations: AD, Alzheimer's disease; Cdk5, cyclin-dependent kinase 5; CNS, central nervous system; GSK3β glycogen synthase kinase 3β; KO, knockout; MAP2B, microtubule-associated protein 2B; NF, neurofilament.

disease, it is important to restrict p25 expression to relevant brain areas such as the forebrain. Additionally, it is important to avoid embryonic expression of p25, because Cdk5 is important for neuronal development (Table 2). Thus, p25 expression during embryonic development may impair developmental processes, and these impairments could produce artifacts. Accordingly, two mouse lines fulfill these criteria, having the CaM kinase II (α-isoform) promoter to drive p25 expression [22,23]. This promoter is not active during embryonic development and it drives expression predominantly in the hippocampus, a brain area affected in the early stages of Alzheimer's disease.

Expression of p25, hyperphosphorylation of tau, and neurodegeneration

The expression of p25 leads to an overactivation of Cdk5 in all transgenic mouse lines tested [21,23–25]. As a result of this, most studies used the mutants expressing p25 as a tool to investigate whether overactivation of Cdk5 causes τ hyperphosphorylation and neurodegeneration; however, inconsistent results have been obtained (Table 1) [22–25]. The best interpretation of these results is that the level of p25 expression is an important factor. Transgenic mice expressing p25 at the same level or less than p35 do not have τ hyperphosphorylation, whereas transgenic mice expressing p25 at higher levels than p35 do exhibit τ hyperphosphorylation. Furthermore, high-level p25 expression leads to aggregation of τ, as demonstrated by immunoblots of sarkosyl-insoluble proteins [23]. It remains controversial,

however, whether high levels of p25 expression are sufficient to cause NFT formation, or whether additional factors are required [21,23]. In support of the requirement of additional factors, a recent study showed that p25 expression in combination with the expression of a mutated human τ isoform leads to NFT formation [25]. These results indicate that p25/Cdk5 does play a role in τ phosphorylation. It remains to be tested, however, whether p25/Cdk5 directly phosphorylates τ, or whether it indirectly regulates τ phosphorylation via other kinases, as proposed by studies with p35 knockout mice (Table 2) [26].

Interestingly, in most studies, the presence of p25 led to an approximately two-fold increase in Cdk5 activity, independently of the p25 level (Tables 1 and 3). Higher p25 expression did not correlate with higher Cdk5 overactivation in these transgenic mice. Consequently, the differential phenotypes between low and high-level expressing p25 transgenic mice cannot be solely attributed to the Cdk5 overactivation. High levels of p25 may also be toxic *per se*, as suggested by a study of transgenic mice not exhibiting NFT but showing axonopathy [21].

p25 has a role in synaptic plasticity and memory formation

Cdk5 is a multifunctional kinase. In addition to regulating τ phosphorylation, Cdk5 also phosphorylates synaptic proteins such as the *N*-methyl-D-aspartate receptor subunit NR2A [7]. Accordingly, blockers of Cdk5 impair long-term synaptic plasticity and associative memory formation

Table 3 Disease models in which p25 formation occurs

Mouse line; reference	Mutation; mouse strain	p25 levels; Cdk5 activity	AD-like phenotype; neurodegeneration	Phenotype	Other remarks
Amyotrophic lateral sclerosis model [19]	Mutant SOD (G37R); C57BL/6 strain	Ratio of p25/p35 is about 1:2 to 1:3; 2-fold increase in Cdk5 activity in spinal cord	Hyperphosphorylation of τ and NF proteins	Mislocalization of Cdk5 in motor neurons; accumulation of NF in the somata	Recent studies indicate p25 expression is not responsible for motor neuronal degeneration in this ALS mouse model [20]
Niemann–Pick type C model [18]	npc-1 mutation	p35 signal is several fold stronger than p25; Cdk5 activity is 1.5 to 2-fold increased	Hyperphosphorylation of NF, MAP2 and τ, elevated NF levels; neuronal cell loss	Extensive lipid storage accumulation, neuroaxonal dystrophy, neuronal cell loss	p25 formation also observed in human NPC patients; heterozygous npc-1 (+/−) show no cytoskeletal pathology
APP transgenic Tg2576 mice [16]	Human APP with Swedish mutations; mix of C57Bl/6 and SJL strain	p35 signal is several fold stronger than p25; Cdk5 activity is about 1.5-fold increased	Hyperphosphorylated τ; small-clustered core plaques in the hippocampus and cortex; dystrophic neurites and astrogliosis		Cdk5 colocalizes with plaques
Presenilin double mutant [17]	Conditional presenilin double KO mouse line; C57BL6/129 hybrid mice	p35 signal is several fold stronger than p25; Cdk5 activity not assessed	Age-dependent τ hyperphosphorylation and neurodegeneration	Memory impairment and altered synaptic plasticity	Presenilins are components of the γ-secretase protein complex and are thought to affect cellular levels of calcium
Rat ischemia model [7]	N/A	Ratio of p25/p35 is about 1:2 to 1:3; at different times after ischemia, Cdk5 activity was increased 2 to 4-fold	Apoptosis, neuronal cell loss		Study shows that production of p25 is mediated via Ca^{2+} influx through AMPA receptor channels in ischemic hippocampal CA1 neurons

Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; AMPA, α-amino 3-hydroxy-5-methylisoxazole-4-propionate; APP, amyloid precursor protein; Cdk5, cyclin-dependent kinase 5; KO, knockout; NF, neurofilament; NPC, Niemann–Pick type C disease; SOD, superoxide dismutase.

[27,28]. Thus, the activation of Cdk5 is thought to contribute to memory formation. As a result of the synaptic functions of Cdk5, and because p25 is found at synapses in p25 transgenic mice [22], p25-induced overactivation of Cdk5 was expected to impact on synaptic plasticity and memory formation. This was confirmed in a transgenic mouse line, expressing very low levels of p25, and not exhibiting signs of neurodegeneration [22,29]. In these mutants, p25 is expressed predominantly in the hippocampus [22], so that hippocampal memory formation and hippocampal plasticity could be analyzed. The hippocampus is affected in the early stages of Alzheimer's disease, and consequently these p25 transgenic mouse studies could be relevant for the early phase of the disease.

This study demonstrated that p25 expression impacts on spatial memory formation in two different genetic backgrounds [29]. The performance in the Morris water maze is improved in female but not in male transgenic mice. The female p25 mutant mice learn faster than wild-type controls. An additional penetrant function of p25 expression is to alter fear conditioning in female but not in male mice. When conditioned to a context and a tone, the female p25 mutant mice have a stronger conditioned response to the tone, and a weaker conditioned response to the context than wild-type mice [22,29]. This finding can be interpreted as an alteration in attention: the mutants would pay more attention to the tone than to the context [22]. Thus, p25 expression might be

beneficial for memory formation in a sex-dependent manner.

The expression of p25 does not affect basal glutamatergic synaptic transmission, or short-term synaptic plasticity [29]. Thus, p25-induced overactivation of Cdk5 does not impact on basal synaptic properties. Altered long-term potentiation, however, was observed in hippocampal CA1 synapses in a sex-dependent manner [29]. In female mice, low-level p25 expression enhances CA1 long-term potentiation, whereas it reduces CA1 long-term potentiation in male mice. While the reduction of long-term potentiation in male mice does not lead to impairments in memory formation, the increased long-term potentiation in female mice correlates with improved spatial learning. The molecular mechanisms by which low-level p25 expression influences long-term potentiation remain unknown, but it appears to act by mediating hyperphosphorylation of synaptic proteins [22]. Thus, it would be important to elucidate the synaptic processes that are regulated by p25 in order to get insights into mechanisms affected in the early stages of Alzheimer's disease.

p25 formation as a potential compensatory response for early learning deficits in Alzheimer's disease

Recent studies suggest that the early stages of Alzheimer's disease result from synaptic dysfunction even before the

formation of amyloid plaques and NFT [30]. Furthermore, it has been suggested that in the early stages of Alzheimer's disease, compensatory responses delay the onset of clinical symptoms. For example, functional magnetic resonance imaging studies showed an increased memory-related activation of the medial temporal lobe in patients with mild cognitive impairment, which is thought to be an early stage of Alzheimer's disease [31]. The increased activation of the medial temporal lobe in these patients may attenuate impairments in memory formation. Additionally, patients with mild cognitive impairment have an upregulation of choline acetyltransferase activity in the hippocampus and frontal cortex, suggesting a compensatory response in the cholinergic system in the early stage of Alzheimer's disease [32].

The improvements in reversal learning and the enhanced spatial memory formation in transgenic mice expressing low-level p25 have led to the hypothesis that formation of p25 is a compensatory response for early learning deficits in Alzheimer's disease [22,29]. This hypothesis assumes that formation of p25 occurs in Alzheimer's disease (see above), and it suggests that mouse models are adequate to predict mechanisms in human diseases. According to the hypothesis, p25 formation compensates for synaptic dysfunction, and delays the onset of cognitive impairment. A feature of the p25 compensation could be that it becomes detrimental over time, as high levels of p25 may contribute to neurodegeneration. Furthermore, the transgenic mouse studies imply that a p25 compensation could be more efficient in female than in male Alzheimer's disease patients. Further, this could explain why the predisposition for Alzheimer's disease is higher for females than males [29].

Conclusion

Human post-mortem studies of p25 accumulation in Alzheimer's disease remain inconclusive because of the confounding formation of p25. Many transgenic mouse studies, however, have been carried out to characterize the occurrence and physiological effects of p25 (Tables 1–3) [33–38]. Together, these studies suggest that p25 formation contributes to Alzheimer's disease. Low levels of p25 could present a compensatory response for early learning and memory deficits, whereas high levels of p25 may result in τ hyperphosphorylation and NFT formation. Furthermore, these studies have revealed a new role for Cdk5 in synaptic plasticity and memory formation. More research will be required to elucidate the diverse functions of Cdk5 and its regulator p25, and to understand their contribution to Alzheimer's disease.

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