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Review

Going out of the brain: Non-nervous system physiological and pathological functions of Cdk5

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ABSTRACT

Cyclin-dependent kinase 5 (Cdk5) is a proline-directed serine/threonine kinase that is mostly active in the nervous system, where it regulates several processes such as neuronal migration, actin and microtubule dynamics, axonal guidance, and synaptic plasticity, among other processes. In addition to these known functions, in the past few years, novel roles for Cdk5 outside of the nervous system have been proposed. These include roles in gene transcription, vesicular transport, apoptosis, cell adhesion, and migration in many cell types and tissues such as pancreatic cells, muscle cells, neutrophils, and others. In this review, we will summarize the recently studied non-neuronal functions of Cdk5, with a thorough analysis of the biological consequences of these novel roles.

Contents

1. Introduction

Protein phosphorylation is mediated by protein kinases and is one of the most conserved mechanisms in the control of cell function. The importance of protein kinases is emphasized by the fact that the vast majority of known physiological processes are regulated by changes in the phosphorylation state of signaling proteins in the cell [1]. Cyclin-dependent kinases (Cdks) are protein kinases that specifically phosphorylate serine or threonine residues, as directed by proline residues, in

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several proteins that control the cell cycle. Cdk activation requires the binding of an activator, termed a cyclin, and phosphorylation at an activation site [2,3]. However, Cdk5 is a unique member of this family because despite sharing strong sequence identity with other Cdks, Cdk5 activation is mostly dependent on non-cyclin proteins and does not require phosphorylation at its activation site [4]. Moreover, unlike other Cdks whose primary function is to regulate the cell cycle, most Cdk5 substrates are not involved in cell cycle control and instead are part of the cytoskeleton or neuronal signaling molecules [5,6]. Therefore, the functions of Cdk5 have been described as controlling several neuronal processes such as neuronal migration, actin dynamics, stabilization and transport of microtubules, cell adhesion, axonal guidance, synaptic structure, endocytosis, memory and learning, apoptosis, and pain signaling [5,7–10]. Cdk5 phosphorylates proteins in the consensus motif (S/T)PX(K/H/R), but there are some sequence variations, such as the phosphorylated motif KSPXK found in the microtubule-associated protein 1B [5] and KSPXX found in the neurofilaments [11]. Activation of the kinase occurs upon binding of the non-cyclin activators p35 or p39 [12,13]. p35 is predicted to adopt a structural conformation that is quite similar to cyclins when it is bound to Cdk5 [14]. The high expression and complementary distribution of p35 and p39 are responsible for the prominent activity of Cdk5 in the nervous system [15], and the Cdk5–p35/p39 complex is fundamental to the development and function of the nervous system [16–18].

Over the last decade, several studies have shown that Cdk5 is active not only in neurons, but also in non-neuronal tissues [19]. Active Cdk5 also has been found in the ovary [20], pancreatic β cells [21], testis [22], corneal epithelial cells [23], and monocytes [24]. For example, Cdk5 regulates the differentiation of monocytes through phosphorylation of MEK1 [25], and Cdk5 regulates the development and regeneration of muscle cells in a nestin phosphorylation-dependent manner [26]. These two examples require the expression of p35 and the activation of Cdk5 outside neurons, suggesting that Cdk5 plays important roles in non-neuronal cells and tissues. In this review, we will summarize the latest findings about the participation of Cdk5 in several biological processes in non-neuronal cells such as aberrant proliferation, apoptosis, gene expression, activation of immune system cells, angiogenesis and cell migration. Moreover, we will discuss the existence of a novel splice variant of Cdk5 that is expressed in almost all tissues. Finally, we will discuss the function of Cdk5 in several pathological conditions that do not involve the nervous system such as cancer, diabetes, inflammation, and senescence.

2. Transcriptional and translational regulation by Cdk5

2.1. Cdk5 and transcriptional regulation

Cdk1, Cdk2, Cdk4, and Cdk6 are well known to be very important in the regulation of the cell cycle, and they provide a check-point system that controls cell cycle progression [2]. However, some other family members such as Cdk7, Cdk8, and Cdk9 indirectly control the cell cycle by modulating the function of several transcription factors [27–29]. Cdk7 and its activator cyclin H are part of the transcription factor II H complex, which is required for assembly of the initiation complex that allows transcription [27]. Cdk8 and its activator cyclin C are part of the Mediator Complex, which is a transcriptional coactivator that is required for the successful transcription of nearly all class II genes [28]. Finally, Cdk9 and its activator cyclin T are part of the positive transcription elongation factor [29]. All of these Cdk-cyclin complexes can phosphorylate the C-terminal domain of RNA polymerase II and thereby regulate expression of target genes and form part of a platform for RNA processing and chromatin regulation [30,31].

Because Cdk5 and p35 are found in the nucleus [32,33], and because some Cdk5 substrates are transcription factors, including p53 [34] and myocyte enhancer factor 2 [35], it is conceivable that Cdk5 may play a role in regulating gene expression. In this context, mSin3-associated protein (mSds3) has been reported to be an essential component of the broad, phylogenetically conserved, mSin3–histone deacetylase complex [36], and it can interact with p35 and be phosphorylated by Cdk5 at Ser228 (Table 1) [37]. This phosphorylation modulates the homodimerization of mSds3, repressing transcription in an mSin3-dependent manner (Fig. 1A). Interestingly, mSds3 is expressed at high levels mainly during development in brain and muscle [37]. Although the role of this complex in muscle is currently unclear, a co-repressor, termed Sin3B, is required for the proliferation and terminal differentiation of muscle cells [38] (Fig. 1A). In addition, histone deacetylase plays a key role in controlling the abnormal transcriptional activity underlying the development of muscular dystrophy [39]. Therefore, we speculate that an imbalance in levels of mSds3, mediated by Cdk5 activity, may be linked to muscular dystrophy.

On the other hand, one of the most abundant and ubiquitous chromatin-associated non-histone proteins is high mobility group box 1 (HMGB1) protein [40]. The function of this protein is to promote the assembly of nucleoprotein complexes in the nucleus [41]. HMGB1 can be post-translationally modified by acetylation, methylation, ADP-ribosylation, glycosylation, and phosphorylation [42]. Interestingly, Cdk5 can phosphorylate HMGB1 at Ser180 only if HMGB1 is previously acetylated at Lys2, which promotes binding to DNA (Table 1) [43] and leads to structural changes that elicit the recruitment of transcription factors [44]. Therefore, we propose that Cdk5 may facilitate the function of p53 and p73 transcriptional complexes *via* this indirect mechanism (Fig. 1B).

The tonicity-responsive enhancer binding protein (TonEBP/OREBP) is a transcription factor activated by hypertonicity resulting from high concentrations of slowly diffusible solutes [45]. Under hyperosmotic conditions, TonEBP/OREBP is related to the cellular osmoprotective response. TonEBP/OREBP can bind DNA in a phosphorylation-independent manner. However, the phosphorylation of TonEBP/OREBP by other kinases favors transcription by recruiting other coactivator proteins [46]. Interestingly, under hyperosmotic conditions, Cdk5 can phosphorylate TonEBP/OREBP at Thr135 (Table 1), promoting its accumulation and nuclear localization, which suggests that Cdk5-mediated phosphorylation of TonEBP/OREBP may contribute to increased transcription of osmoprotective genes (Fig. 1C) [47].

2.2. Cdk5 and cell cycle control

Cdk5 has been classified historically as an unusual member of the Cdk family because it is activated by other non-cyclin protein activators and does not require the phosphorylation of an activation loop to be active. More importantly, Cdk5 has been proposed to play no role in the control of the cell cycle [5,6]. However, this view has begun to change because it was reported recently that Cdk5 can interact with cyclin I, which in turn can activate the kinase [48]. Furthermore, it was reported previously that Cdk5 also can bind to other cyclins such as cyclin D1, D3, and E. However, binding to these cyclins does not seem to affect its kinase activity [49,50]. Interestingly, cyclin D2 also is able to bind Cdk5, but in this case, the activity of the kinase is not elicited. In fact, the presence of cyclin D2 can abrogate the activity of the Cdk5–p35 complex. A possible molecular explanation is related to the differential recognition of members of the Cip/Kip family to Cdk5 when it forms a complex with p35 or cyclin D2 [51].

The classical view that the expression of cyclins is controlled tightly during the different phases of the cell cycle has been changing since the discovery of cyclin I, which is not involved in cell proliferation and remains equally expressed during the entire cell cycle [52,53]. Interestingly, cyclin I is expressed primarily in terminally differentiated cells such as neurons, podocytes, and cardiomyocytes, and the absence of cyclin I makes these cells more prone to apoptosis [53,54]. The Cdk5–cyclin I complex was proposed to activate the MEK-ERK signaling pathway, leading to increased Bcl-2 and Bcl-X_L expression in postmitotic cells; these changes in anti-apoptotic proteins are dependent on ERK1/2 activity

Table 1
Novel substrates for Cdk5 function outside the nervous system. The table summarizes the most novel substrates reported to be phosphorylated by Cdk5, indicating when available the phosphorylation site in the aminoacid sequence for each protein. Also shown are the cell and tissue where the phosphorylation is found, the physiological process regulated by this phosphorylation and the possible pathology associated with abnormal Cdk5 phosphorylations.

Substrate	Cells/tissues	Phosphorylation site	Function	Possible related pathology	Reference
EPRS	Macrophage	Ser886	Translation	Inflammation	[66]
STAT3	Medullary thyroid carcinoma	Ser727	Transcription	Cancer	[106]
TonEBP/OREBP	HEK293 cells, rat renal inner medullas	Thr135	Transcription	n.d.	[47]
mSds3	COS7, NIH3T3, 293 T, and immortalized mouse embryonic fibroblasts	Ser228	Transcription	Muscular dystrophy	[37]
HMGB1	All nucleated eukaryotic cells	Ser180	Transcription	Inflammation	[43]
cMyc	NCI-H460, NCI-H1299 human non-small cell carcinoma and HCT116 cells	Ser62	Transcription	Cancer, inflammation	[57]
Titin	Muscle	C-terminal KSP repeats	Assembly of sarcomere	Muscular dystrophy	[90]
AATYK1A	Cos7, CHO-K1 cells	Ser34	Vesicular traffic	n.d.	[94]
Vps34	Hela cells	Thr159	Vesicular traffic	Cancer	[101]
E-syt1	3 T3-L1 adipocytes	Ser314	Glucose uptake	Diabetes	[124]
PLD2	Pancreatic β cells	Ser134	Insulin secretion	Diabetes	[125]
β2-syntrophin	Pancreatic b cells	Ser75	Insulin secretion	Diabetes	[127]
Vimentin	Neutrophils	Ser56	Secretion	Inflammation	[132]
Coronin 1a	T cells	Thr418	Actin dynamics	Inflammation	[133]
Androgen receptor	Prostate cancer cells	Ser81	Transcription	Cancer	[111]

[55]. Alternatively, the canonical Cdk5–p35 complex can phosphorylate Bcl-2 at Ser70, leading to Bcl-2 stabilization [56], which indicates that Cdk5 may indeed be able to regulate apoptosis through two different and probably independent mechanisms, which further suggests that Cdk5 may be a key protein controlling the survival of postmitotic cells.

In addition to the cyclins described above, Cdk5 also can interact with and be activated by cyclin G1, and in turn, it can phosphorylate c-Myc at Ser62 (Table 1); this effect has been observed mainly in lung cancer cells in which both cyclin G1 and Cdk5 are upregulated [57]. UV radiation-induced damage arrests cells in the G2 phase of the cell cycle, but cyclin G1 may overcome this effect in a cyclin B1-dependent manner. The phosphorylation of c-Myc, which is mediated by Cdk5–cyclin G1, induces its stabilization and promotes binding to the cyclin B1 promoter, leading to increased expression [57]. These results suggest that Cdk5 may regulate the cell cycle in lung cancer cells that overexpress cyclin G1, and thus, it will be interesting to study whether Cdk5 plays the same role in other types of cancer.

In neurons, Cdk5 contributes to the suppression of the cell cycle through interaction with the transcription factor E2F1 to form a complex that includes p35. This interaction occurs in the nucleus and impairs the formation of the E2F1–DP1 complex whose function is to regulate the transcriptional activation of several genes. Despite the p35 requirement, the effect is not dependent on kinase activity, and p35 cannot be replaced by p25, a proteolytic fragment of p35, or by p39 [58]. Although this effect has not been observed in other terminally differentiated cell types such as podocytes and cardiomyocytes, it is still possible that a similar mechanism contributes to suppression of the cell cycle in other cell types. During cell cycle arrest, the CKI inhibitor p21 represses E2F activity by inhibiting the phosphorylation of Retinoblastoma protein (pRB) [59]. In this context, it also is worth mentioning that Cdk5 can phosphorylate and stabilize p53 [34], which increases the levels of p21 by activating its transcription [60]. Thus, it seems reasonable that Cdk5 may regulate both pathways to arrest cell cycle progression in highly differentiated cells.

Another interesting insight about the role of Cdk5 in the cell cycle is related to the localization of Cdk5, p35, and Cdk5rap2 at the centrosome of HeLa cells. This association is independent of microtubules because the localization persists even when the cells are treated with nocodazole, a microtubule destabilizing drug, suggesting that the complex may be at the core of the centrosome [61]. Cdk5rap2 is a protein involved in the regulation of microtubule dynamics, stabilization of the centrosome, and interaction with γ-TURC components. Mutations in Cdk5rap2 are linked to the development of primary microcephaly. The reduction in brain size is caused by defects in the asymmetric division of neuronal progenitors [62]. Of note, double knockout p35/p39 mice have a smaller body size compared with wild-type mice [63], suggesting the possibility that Cdk5 regulates cell division.

Interestingly, Cdk5rap2 is found in the midbody, a structure associated with the completion of cytokinesis. Defects in this structure have been linked to problems in cell division [64]. Similarly, Cdk5 also is found at the midbody in HeLa cells. In addition, a mutant Cdk5 form that lacks kinase activity, termed Cdk5–T33, also is located in the midbody, suggesting that Cdk5 kinase activity is not required for midbody localization [65]. Consequently, the role of Cdk5 in the control of cytokinesis may be independent of kinase activity. Moreover, Lee and colleagues described that fibroblasts derived from Cdk5 knockout mice display defects in cytokinesis that promote aneuploidy. Therefore, it will be interesting to ascertain whether terminally differentiated cells in Cdk5 knockout mice also exhibit aneuploidy.

2.3. Cdk5 and translational regulation

Cdk5 recently has been reported to regulate translation [66]. Thus, interferon-gamma-mediated activation of Cdk5 induces phosphorylation of glutamyl-prolyl tRNA synthetase (EPRS) at Ser886 (Table 1). EPRS becomes part of the interferon-gamma-activated inhibitor of translation complex, which suppresses the expression of proinflammatory genes in myeloid cells [67,68]. EPRS also can recognize and bind directly to target mRNAs transcribed in response to an inflammatory stimulus. Cdk5-dependent phosphorylation of EPRS releases it from the multi-synthetase complex, which enhances the binding of EPRS to the interferon-gamma-activated inhibitor of translation complex to inhibit translation of mRNAs associated with the inflammatory response [66]. Remarkably, this is the first report linking Cdk5 function with the control of translation (Fig. 1D).

3. Cdk5 isoform

A novel aspect of Cdk5 function is the discovery of a new isoform derived from alternative splicing, as recently shown by two independent groups. Cdk5-SV and Cdk5-V1 are different names for the same protein [69,70]. Li and colleagues reported that Cdk5-SV is a shorter version of the kinase that lacks 32 amino acids encoded by exon 7 and is found mainly in the testis, skeletal muscle, colon, bone marrow, and ovary [69]. In contrast, Kim and colleagues reported that Cdk5-V1 also is a shorter version expressed in brain, kidney, leukocytes, liver, lungs, placenta, prostate, small intestine, and thymus [70]. In this second paper, Cdk5-V1 was reported to originate from the absence of 32 amino

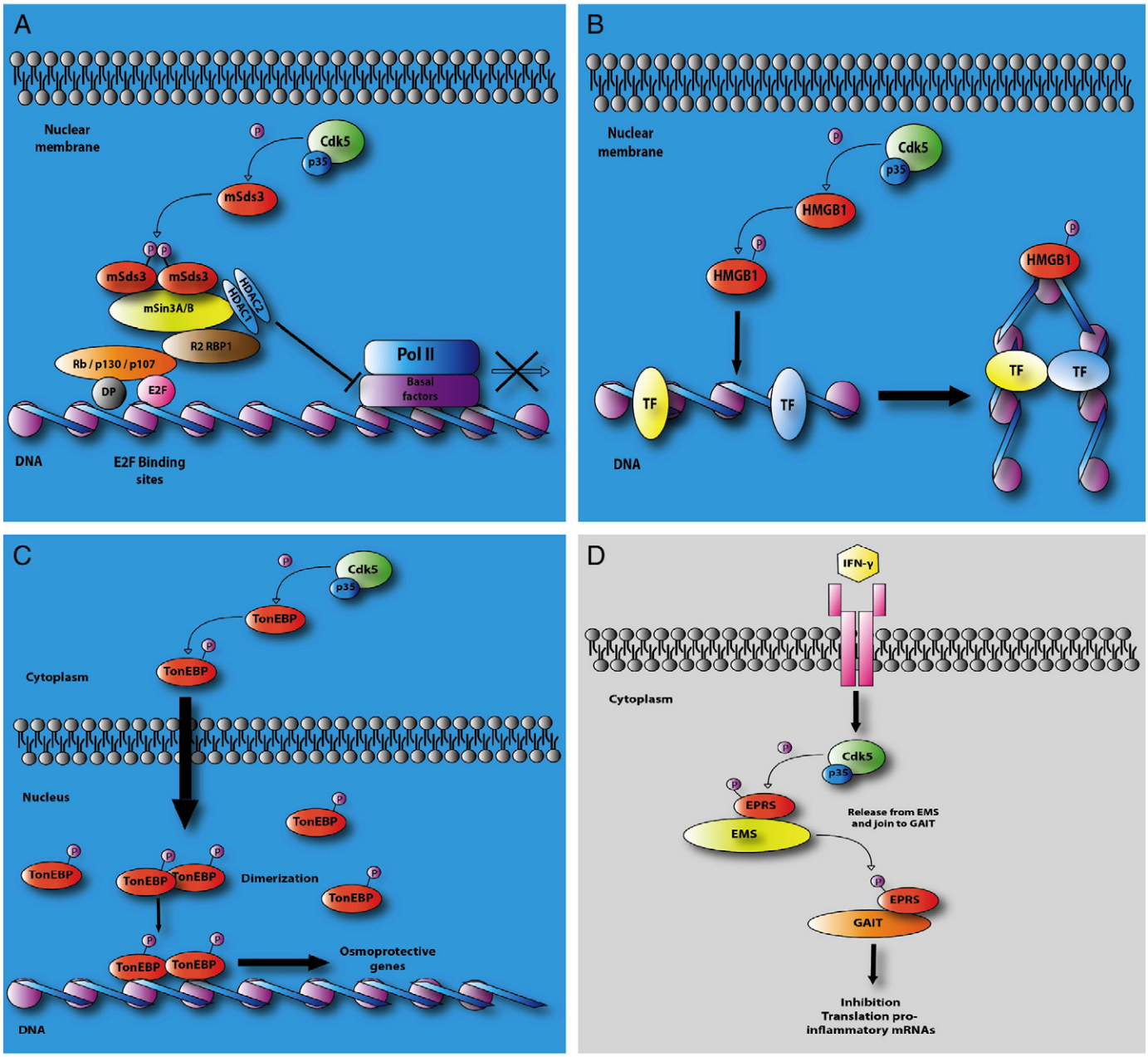

Fig. 1. The role of Cdk5 in controling transcription and translation. The figure depicts the molecular mechanisms involved in the participation of Cdk5 in the regulation of transcription (blue background panels) and translation (gray background panel). A) The active Cdk5/p35 complex may be localized in the nucleus where it phosphorylates mSds3 to regulate the function of RNA polymerase II. B) In another cellular context, Cdk5 can phosphorylate HMBG1 to promote transcription. C) Finally, Cdk5 can phosphorylate TonEBP in the cytoplasm. Phosphorylated TonEBP is transported to the nucleus where it can stimulate the transcription of osmoprotective genes. D) Finally, in response to interferon-gamma IFN-g Cdk5 can phosphorylate EPRS which then can bind the GAIT complex to downregulate the translation of mRNA encoding pro-inflammatory molecules.

acids encoded by exon 6, rather than exon 7. Discrepancies between these two studies may be related to different methodologies. Both studies reported that Cdk5-SV and Cdk5-V1 are enriched in the nucleus but expressed elsewhere as well, whereas the full-length Cdk5 has a cytoplasmic distribution. Previously, the presence of full-length Cdk5 in the nucleus was suggested by Ino and Chiba [32]. However, the description provided by Ino and Chiba was based on experiments using two different antibodies that recognize the N- or the C-terminal domain of Cdk5. Both Cdk5-SV and Cdk5-V1 lack 32 amino acids in the central region of the protein and therefore are recognized by the two antibodies. Interestingly, using HA-tagged Cdk5, Kim and colleagues demonstrated that in HeLa cells, the full-length protein is not present in the nucleus [70]. Despite the differences between these two studies, the observation of alternative isoforms of Cdk5 opens the possibility of differential roles for shorter isoforms in different cell compartments and cell types.

Cdk5-SV has been suggested to negatively regulate the Wnt signaling pathway by interacting with β-catenin [69], similar to full-length Cdk5 [71]. However, the authors of that study did not examine if this interaction occurs in the nucleus. On the other hand, Lee and colleagues demonstrated that the Cdk5-V1 isoform is located at the centrosome and in the perinuclear region [65]. Similarly, full-length Cdk5 was reported to be present at the centrosome [61]. However, the molecular description provided in the latter study was based on the use of antibodies that do not distinguish among the isoforms,

and therefore, it may not have been possible to distinguish between Cdk5 and its splice variant.

4. Cdk5 and cell migration, adhesion, and angiogenesis

Angiogenesis involves the formation of new capillaries and plays a physiological role in healing injured tissues and in the formation of the placenta. The balance between angiogenic and anti-angiogenic factors is fundamental in the development of cancer and has even been suggested to be involved in other diseases such as diabetic retinopathy, arthritis, and psoriasis [72,73]. Cdk5 has been proposed as a new target for anti-angiogenic therapies because inhibition or silencing of Cdk5 prevents angiogenesis [74,75]. Pharmacological inhibition of Cdk5 with roscovitine *in vitro* reduces endothelial cell migration in a dose-dependent manner. Indeed, roscovitine treatment *in vivo* also produces anti-angiogenic effects, and these effects are reproducible when Cdk5 is silenced using siRNA or shRNA, which discounts the possibility that the effect of pharmacological treatment was due to inhibition of Cdc2 or Cdk2. Moreover, Cdk5 kinase activity also is required because when the Cdk5 dominant negative mutant D145N is overexpressed, endothelial cell migration is reduced. Also, inhibition of Cdk5 produces a decrease in focal adhesion kinase phosphorylation at Ser732 without altering the formation of focal adhesions or changing microtubule dynamics. However, the overall structure of the actin cytoskeleton was differentially organized after inhibition of Cdk5, with subsequent increased RhoA and decreased Rac1 activities, suggesting a role for Cdk5 in controlling the actin cytoskeleton in endothelial cells that supports migration and angiogenesis [74].

Similarly, a correlation between Cdk5 activity and the function of small Rho GTPases has been established in lens epithelial cells [76,77]. Cdk5 suppresses Src activity, leading to decreased p190RhoGAP phosphorylation, which ultimately activates RhoA. The reversible inhibition of Cdk5 with olomoucine increases Src activity and reduces Rho-ROCK signaling, which is responsible for the phosphorylation of myosin and maintenance of the stress fibers that are necessary for cell adhesion. The fact that Cdk5 colocalizes with contractile stress fibers is consistent with local activation of Rho, which is mediated by inhibition of Src [76]. In agreement with the aforementioned results, olomoucine increases the expression of matrix metalloprotease-9, which is required for corneal epithelial cell migration, most likely through activation of Src to increase the migration of these cells [77].

5. Cdk5 and apoptosis

A link between Cdk5 and apoptosis was established primarily by Ahuja and colleagues [78]. Although most studies of the role of Cdk5 in cell death have focused on the central nervous system, evidence of a similar role for Cdk5 in non-neuronal cells is increasing [19]. Cdk5 is highly expressed in proliferating bovine aortic endothelial cells [79]. Pro-angiogenic growth factors such as basic fibroblast growth factor [80] and vascular endothelial growth factor [81] induce proliferation of endothelial cells (EC). In contrast, angiotensin inhibits this process [82]. Angiotensin is a potent inhibitor of angiogenesis, and it can both inhibit EC proliferation and stimulate EC apoptosis, with subsequent decreased Cdk5 expression. Indeed, stimulation of bovine aortic endothelial cells with basic fibroblast growth factor in the presence of roscovitine fails to promote proliferation and induces apoptosis instead [79].

Pharmacological and genetic inhibition of Cdk5 activity also is connected with increased susceptibility to cell death in several other cell types [83–85]. Retinal pigment epithelial cells treated with roscovitine display increased expression of pro-apoptotic Bax and decreased expression of anti-apoptotic Bcl-2, which may implicate Cdk5 [84].

Podocytes derived from p35 knockout mice have a normal morphology with no abnormalities in kidney function. However, when immortalized podocytes derived from p35 knockout mice are exposed to several stress conditions, including UV-C radiation, depletion of serum, puromycin aminonucleoside, or transforming growth factor β1, apoptosis is increased significantly. Moreover, following transfection with siRNA against p35, podocytes derived from wild-type mice show increased apoptosis. Finally, rescue experiments conducted to express p35 in podocytes derived from p35 knockout mice resulted in decreased apoptosis of these cells [83]. As mentioned in Section 2.2 on the cell cycle, the effects of Cdk5 on apoptosis in podocytes may be related to increased Bcl-2 expression under the control of the Cdk5–p35 complex or, alternatively, a stabilizing effect of Cdk5 phosphorylation on Bcl-2.

Cdk5 also has important functions in pancreatic β cells, which express both Cdk5 and p35. An increase in levels of extracellular glucose leads to increased p35 expression and subsequent Cdk5 activity, suggesting that the transcription [85] and secretion [86] of insulin may be dependent on the Cdk5–p35 complex. However, the activity of Cdk5 appears to be tightly controlled because overexpression of p35 can inhibit insulin secretion in pancreatic β cells. The normal physiology of the Cdk5–p35 complex seems to be modulated by glucose levels because when p35 is overexpressed under high glucose conditions, a calpain-dependent p25 fragment of p35 is detected, resulting in a decrease in both insulin secretion and apoptosis in these cells. Both effects can be reversed with the Cdk5 inhibitor, roscovitine [87].

6. Cdk5 and myogenesis

The participation of Cdk5 in myogenesis was proposed previously because Cdk5 controls the expression of MyoD and MFR4, two important transcription factors involved in myogenesis [88]. In addition, protein kinase C zeta (PKCzeta) inhibition affects myotube formation and nestin reorganization. PKCzeta can phosphorylate p35 at Ser33, enhancing its proteolysis by muscle-specific calpain 3 and resulting in increased Cdk5 activity. Indeed, PKCzeta concurrently activates calpain 3 by direct phosphorylation [89]. Thus, these experiments suggest that p35 proteolysis should not be considered merely a pathological process that activates Cdk5, as had been shown in the nervous system. In addition, Cdk5 not only promotes myogenesis but also regulates muscle function because it can phosphorylate connectin/titin [90], which is a giant elastic protein of muscle that contains several KSP motifs in the C-terminal region (Table 1) [91]. Cdk5-dependent phosphorylation of titin requires the participation of bridging integrator protein 1 as a scaffold protein to promote the proper assembly of myofiber and structure of the sarcomere [90].

7. Cdk5 and vesicular transport

The small monomeric GTPases of the Rab family are vesicle-associated proteins that are involved in the regulation of intracellular traffic. In particular, Rab11 regulates endosome recycling [92]. The tyrosine kinase associated with apoptosis, AATYK1A, is localized in Rab11A-positive pericentromal recycling endosomes in COS-7 cells [93], and it can be phosphorylated by Cdk5–p35 at Ser34 (Table 1), suggesting that phosphorylation of AATYK1A by Cdk5 may regulate the trafficking of recycling endosomes [94]. Phosphorylation of AATYK1A at Ser34 disrupts the formation of the endocytic recycling compartment (ERC), producing a diffuse distribution of Rab11A. Although a direct interaction between Rab11A and AATYK1A has not been detected, the amount of Rab11-GTP is decreased in the phosphomimetic AATYK1A-S34D mutant [95]. The activity of Rab11 in the ERC also is dependent on cholesterol metabolism. Increased cholesterol inhibits the binding of Rabs with GDP dissociation inhibitor, whereas decreased cholesterol acts in an opposite manner to inhibit ERC formation, similar to the effect of Cdk5 on AATYK1A function [96,97]. AATYK1A also can be phosphorylated at Tyr25 and Tyr46 by Src family kinases [93], and this phosphorylation is reduced when Cdk5 phosphorylates AATYK1A at Ser34. An analogous mechanism was proposed to explain the role of another member of this family, AATYK2/cprk, which is involved in the trafficking of early recycling endosomes [94].

Cdk5 also plays a role in vesicular transport in rat parotid acinar cells [98], which produce amylase. β-adrenergic agonists induce Cdk5 activity, resulting in enhanced amylase release. A rise in cAMP may be required to promote upregulation of Cdk5 in these cells because the Cdk5 promoter contains cAMP response elements. Interestingly, the classical activators for Cdk5, p35 and p39, are not detected in parotid acinar cells. Therefore, increased activity of Cdk5 is linked to cyclin I expression. The Cdk5–cyclin I complex may trigger Munc18 phosphorylation and a subsequent release of amylase [98].

Finally, vacuolar protein sorting 34 (Vsp34) is a protein kinase that phosphorylates phosphatidylinositol to produce phosphatidylinositol-3P [99]. This kinase forms protein complexes with Beclin 1, MTM1, and Rab5/7 and regulates processes such as autophagy and endocytic sorting [100]. Vsp34 can be phosphorylated by Cdk5 at Thr159 (Table 1), which impairs the interaction of Vsp34 with Beclin 1 and inhibits Vsp34 kinase activity, resulting in decreased PtdIns3P levels and autophagy. Concomitantly, downregulation of PtdIns3P may affect several different processes such as endocytosis, cell cycle progression, and the development of certain diseases such as cancer and neurodegeneration [101].

8. Cdk5 and pathology

8.1. Cdk5 and cancer

Neuronal migration during the development of the nervous system and cancer cell migration during metastasis are mediated by quite similar cellular and molecular mechanisms [102]. Therefore, it is not surprising that Cdk5 may play a role in cancer cell migration. The Cdk5–p35 complex is active in pancreatic cancer cells. Inhibition of Cdk5 activity, using either a dominant negative Cdk5 (dn-Cdk5) or pharmacological inhibitors, decreases migration of both hTERT-HPNE and MIAPaCa-2 pancreatic carcinoma cells [103]. Cdk5 inactivation induces morphological changes in these cells that lead to a loss of cell polarity. Remarkably, Cdk5 regulates not only migration but also invasion by regulating the formation of invadopodia [104] Consistent with these effects, inhibition of Cdk5 reduces tumor growth and metastasis in pancreatic cancer [103]. Moreover, the monomeric GTPases RalA and RalB play a fundamental role in the development of pancreatic cancer cells [105]. Hence, Feldmann and colleagues analyzed the Ras-dependent signaling pathway and found a possible link with Cdk5. Using KRAS2 mutants, they found that the loss of function of RalA inhibits the tumorigenicity of pancreatic cancer cells. Interestingly, MIAPaCa-2 cells expressing dn-Cdk5 show decreased levels of RalA-GTP and RalB-GTP. Moreover, constitutively active forms of either RalA and RalB can overcome the effect of dn-Cdk5, suggesting that Ral proteins are downstream of Cdk5. Cdk5 may regulate the activation of RalA and RalB by controlling the function of RalGEFs or RalGAP, suggesting Cdk5 as a possible target for the treatment of pancreatic cancer [103].

Also related to the onset of cancer is the observation that Cdk5 can phosphorylate and regulate the transcriptional activity of STAT3 (Table 1) [24]. STAT3 can be phosphorylated at Ser727 in medullar thyroid carcinoma cells, resulting in increased cell proliferation and promotion of tumor formation. In addition, calcitonin aggregates derived from medullar thyroid carcinoma induce Cdk5 activity and thereby promote cell proliferation. In contrast, inhibition of Cdk5 in nude mice delays tumor growth in a STAT3 phosphorylation-dependent manner [106]. Retinoic acid also can modify Cdk5 activity in thyroid cancer cells through a mechanism involving sodium/iodide transporter, a plasma membrane glycoprotein that stimulates the uptake of iodide [107]. These observations open the possibility that Cdk5 may serve as a molecular target for the diagnosis and treatment of medullary thyroid cancer.

In breast cancer, Cdk5 may also play a role because MCF-7 and MDA-MB321 cells exhibit decreased proliferation rates in the presence of either roscovitine or siRNA against Cdk5. The use of carboplatin, a known chemotherapeutic drug against breast cancer, induces the activation of Cdk5 by increasing the activity of ERK in response to DNA damage [108]. Increased Cdk5 activity stimulates p53 stability, which can ultimately cause cell death in response to the DNA damage caused by carboplatin [108]. Therefore, tight regulation of Cdk5 activity may be important for normal cell physiology because local and temporal gain or loss of function may result in abnormal cell proliferation.

In cervical carcinoma caused by human papilloma virus (hPV), Cdk5 can phosphorylate p53 at Ser20 and Ser43, thereby inhibiting cell proliferation. However, hPV can induce p53 degradation. In fact, hPV-infected HeLa cells do not undergo apoptosis or cell cycle arrest even when p53 is over-expressed. However, modifying the phosphorylation state of p53, either by promoting the activity of protein kinases or inhibiting phosphatases, may induce apoptosis [109]. In this context, Cdk5-dependent phosphorylation of p53 should be a key event that leads to increased stability and transcriptional activation of p53 [34].

A previous report showed that Cdk5 activity is necessary to control cell motility and the metastatic potential of prostate cancer cells [110]. Interestingly, Cdk5–p35 can phosphorylate the androgen receptor at Ser81 and thereby regulate the stability of this protein, increasing the accumulation of androgen receptors in the nucleus and regulating the proliferation of cancer cells [111]. In addition, Hsu and colleagues observed that Cdk5 can increase both expression and secretion of prostate-specific antigen (PSA), perhaps by regulating exocytosis, which suggests that Cdk5 may play a critical role in the development of prostate cancer.

Recently, Cdk5 expression was suggested to be a risk factor for other types of cancer, although the molecular mechanisms explaining the relationship between cancer and Cdk5 remain elusive [112–114]. Thus, in some patients with non-small cell lung cancer, there is a significant correlation between the expression level of Cdk5–p35 and the degree of differentiation and metastasis to lymph nodes [112]. Another study reported that polymorphisms in the Cdk5 promoter increase the risk of lung cancer in a specific Korean population [113]. Moreover, Cdk5 overexpression induced by gene amplification in lung cells can synergize the epithelial growth factor receptor signaling pathway [114]. Finally, decreased methylation of the Cdk5 gene has been found in mantle cell lymphoma, a subtype of B cell lymphoma, and this is associated with an increase in Cdk5 mRNA levels [115]. Although the mechanism of generation of this cancer and the link with Cdk5 remain unknown, the possibility that Cdk5 can regulate DNA repair or cell cycle control appears to be an interesting scenario in which to study Cdk5 functions. Altogether, these studies suggest that Cdk5 may regulate the cell cycle *via* the canonical pathway that includes p35 binding and phosphorylation of key substrates. However, non-canonical mechanisms of Cdk5 function that involve the binding of cyclins such as cyclin I and cyclin G1 may also be important. Moreover, important effects unrelated to the kinase activity of Cdk5 may be involved, and in those contexts, protein–protein interactions with the kinase may contribute to regulation of the cell cycle, raising the possibility that Cdk5 acts as a scaffold protein (Fig. 2).

8.2. Cdk5 and senescence

Cellular senescence is a phenomenon of cell growth arrest due to telomere shortening. However, this term also can be applied to the attrition process in response to cellular damage produced by endogenous or exogenous stimuli such as DNA damage, oxidative stress, or oncogene activation. In response to these noxious stimuli, cells enter a state of cell cycle arrest, which eventually results in senescence as an anti-proliferative mechanism [116]. pRB and p53 are important regulators of senescence in the p16INK4a/pRB and p14ARF/p53 signaling pathways. Thus, mutations that prevent activation of these pathways result in senescence [117,118]. The ectopic expression of pRB in SAOS-2 cells produces activation of Cdk5 during senescence, which promotes the phosphorylation of ezrin and the suppression of Rac activity, resulting in the acquisition of a pRB-induced senescent phenotype [119,120]. In addition, the expression of p35 is required to activate Cdk5 in SAOS-2 senescent cells. In contrast, decreasing p35 levels with shRNA produces

a decrease in the senescent phenotype in SAOS-2 cells. Of note, the direct relationship between pRB and increased p35 is still unclear because the p35 promoter seems to contain no regulatory elements responsive to pRB [121]. Thus, future studies will need to examine the relationship between senescence and the onset of diseases such as cancer because drugs used to treat cancer also can induce senescence. In this context, induction of senescence by anti-cancer drugs may be an unwanted side-effect (Fig. 2).

8.3. Cdk5 and diabetes

Type 2 diabetes mellitus (T2DM) results from the inability of cells to respond adequately to insulin produced by the pancreas. Food intake and environmental and genetic factors confer increased susceptibility to the development of the disease. There are several genetic polymorphisms that are correlated with an increased incidence of T2DM. *CNJ11*, which encodes a potassium rectifying channel, peroxisome proliferator-activated receptor gamma (*PPARγ*), hepatic transcription factor 2 (*TCF2*), and Wolfram syndrome 1 (*WFS1*) [122] are examples. In this context, a single nucleotide polymorphism recently was reported in intron 5 of the gene encoding Cdk5 regulatory subunit-associated protein 1-like 1 (Cdkal1) that increases susceptibility to T2DM. This polymorphic variant of Cdkal1 may cause decreased insulin secretion, which probably is due to abnormal regulation of Cdk5 activity [123].

On the other hand, Cdk5 is involved directly in the regulation of glucose transport in adipocytes [124]. Interestingly, membrane-associated Cdk5 is activated by insulin in 3 T3-L1 adipocytes, and this activation is dependent on PI3K. Cdk5 phosphorylates Extended synaptotagmin-1 (E-Syt1) at Ser314 (Table 1). Phosphorylated E-Syt1 can interact with GLUT4, and this interaction can be modulated with roscovitine. Notably, 3 T3-L1 adipocytes treated with roscovitine show decreased glucose uptake, an effect that is reproduced by silencing Cdk5, indicating the involvement of Cdk5 in the regulation of glucose transport and T2DM [124].

Similarly, the involvement of Cdk5 in insulin secretion also has been demonstrated in pancreatic β cells. Epidermal growth factor (EGF) can induce insulin secretion *via* the activation of phospholipase D2 (PLD2). Interestingly, roscovitine or dn-Cdk5 decreases EGF-dependent insulin secretion, suggesting that Cdk5 controls this process. In response to EGF, Cdk5 phosphorylates PLD2 at Ser134 (Table 1), which leads to the activation of PLD2 and prevents insulin secretion from pancreatic β cells, an effect that can be suppressed using a PLD2-S134A mutant. Notably, Cdk5 can be activated quickly after EGF treatment independent of increased p35 levels or Cdk5 tyrosine phosphorylation, raising the possibility of alternative mechanisms of regulation of Cdk5 activity [125]. In insulinoma INS-1 cells, treatment with high glucose and high potassium alters the electrophoretic pattern of β2-syntrophin due to phosphorylation [126]. β2-syntrophin has been reported to be a Cdk5 substrate that is phosphorylated at Ser75 (Table 1). Ser75 is adjacent to a PDZ domain, and phosphorylation may affect interaction with the granule protein ICA51, which is involved in granule mobility and insulin secretion. Overexpression of β2-syntrophin decreases basal insulin release, suggesting that phosphorylation of β2-syntrophin may be the key event in insulin secretion. This hypothesis was confirmed in cells that overexpress β2-syntrophin under high glucose and high potassium conditions [127].

In obese animals, adipocytes secrete abnormal amounts of cytokines and adipokines (cytokine-like molecules), which affect glucose homeostasis and deposition of free fatty acids (FFAs) in pancreatic β cells, thereby causing insulin resistance and T2DM [128]. Cdk5 activity may be activated by several cytokines [5], including tumor necrosis factor-alpha (TNF-α) [129], through the transcriptional activation of p35. Interestingly, TNF-α, interleukin (IL)-6, and FFAs activate Cdk5 to promote the phosphorylation of PPARγ at Ser273 in 3 T3-L1 adipocytes [130]. PPARs are a group of receptors that function as transcription factors and control the expression of several genes by acting as key regulators of systemic insulin secretion and adipogenesis [10,131]. Overall transcription levels were not altered in adipocytes with mutated S273A PPARγ, which cannot be phosphorylated by Cdk5; however, discrete changes in levels of the fatty acid transporter CD36, adiponectin, adipsin, and leptin were found. These changes may be dependent on the enhanced interaction of Ser273A PPARγ with other transcriptional co-regulators. In addition, adiponectin and adiposin are deregulated *in vivo* in obese animals and also are significantly reduced in mice fed a high-fat/high-sugar diet, which also show increased p25 levels and Cdk5 activity [130]. In addition, treatment with rosiglitazone, an anti-diabetic drug, inhibits Cdk5-dependent phosphorylation of PPARγ, suggesting that the development of anti-diabetic drugs that inhibit the phosphorylation of PPARγ by Cdk5 may lead to an effective treatment for T2DM (Fig. 2).

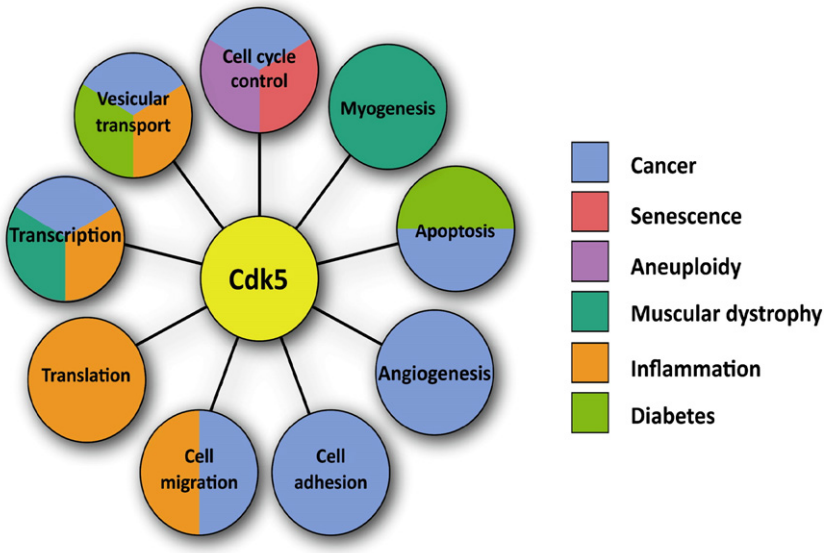

Fig. 2. Physiological processes regulated by Cdk5 and their involvement in the development of pathological conditions. The figure shows nine physiological processes that are believed to be regulated by Cdk5 (yellow circle). Under pathological conditions, these processes are affected by changes in the activity of Cdk5 that results in specific phosphorylation on key substrate molecules. The color code for each pathology is as follows: Cancer (blue), Senescence (red); Aneuplody (purple); Muscular Dystrophy (dark green); Inflammation (orange) and Diabetes (light green). Each of the physiological processes regulated by Cdk5 is divided into colored regions related to the pathological conditions.

8.4. Cdk5 and immune system function and inflammation

The immune system has emerged as a new field where Cdk5 may have a regulatory function and may be involved in the control of the inflammatory response and cellular activation. In this context, phosphorylation of the intermediate filament protein vimentin has been reported to play an important role in regulating its intracellular distribution and dynamics. Interestingly, Cdk5 can phosphorylate vimentin at Ser56 (Table 1), and this phosphorylation plays an important role in the GTP-dependent secretion of proinflammatory molecules by neutrophils. Phosphorylation of vimentin at Ser56 can be detected after stimulation of neutrophils with GTP for 1 to 3 min, but there is no apparent colocalization of Cdk5 and vimentin, and thus, this phosphorylation may be catalyzed by another kinase. After a longer stimulation period, increased phosphorylation of vimentin and colocalization with Cdk5 are observed. Interestingly, consistent with increased Cdk5-dependent vimentin phosphorylation is increased secretion of β-hexosaminidase, lactoferrin, and matrix metalloprotease-9. This effect is inhibited largely, but not abrogated, with roscovitine or Cdk5 siRNA. Thus, other protein kinases may be involved in the secretion of these molecules by neutrophils. Cdk5 therefore is an interesting target to control neutrophil-mediated inflammatory responses by inhibiting the secretion of proinflammatory molecules [132].

In another aspect of inflammation control, p35 expression triggers the activation of Cdk5, which is required for T cell receptor-dependent activation of T cells. Activation of T cells induces the proliferation and migration of lymphocytes. Pharmacological or genetic inhibition of Cdk5 affects proliferation of T cells in response to CD3/CD28 stimulation. During T cell activation, the actin modulator coronin1a is phosphorylated at Thr418 (Table 1). Coronin 1a and F-actin are polarized abnormally in lymphocytes derived from Cdk5 knockout mice, suggesting that the phosphorylation of coronin 1a, which is mediated by Cdk5, is important for regulation of actin dynamics in T cells. Actin dynamics may be essential for promoting T cell activation and CCL19-dependent lymphocyte migration, which are involved in the pathophysiology of experimental autoimmune encephalomyelitis. Although this autoimmune model involves the CNS, this novel role for Cdk5 in the regulation of T cells opens new opportunities for the study of therapies to treat inflammatory diseases [133].

Other roles for Cdk5 in the inflammatory response may be inferred from two recent studies. Du and colleagues showed that roscovitine inhibits the production of $^{\bullet}$NO by concurrently decreasing lipopolysaccharide-induced activation of the IKK-IκB–NFκB pathway and suppressing the GTP cyclohydrolase-1-mediated production of Tetrahydrobiopterin [134]. In a second study, Berberich and colleagues showed that pharmacological inhibition of Cdk5 inhibits the transmigration of leukocytes by blocking NFκB-dependent gene expression. These effects were recapitulated by genetic inactivation of both Cdk5 and Cdk9 using siRNAs [135] (Fig. 2).

9. Conclusions/summary

Year after year, novel functions and substrates are proposed for Cdk5 in both neuronal and non-neuronal cells. In the past, this important and atypical member of the Cdk family was believed to be unrelated to the cell cycle. However, new antecedents indicate the participation of Cdk5 in this process, most likely through non-canonical mechanisms involving the binding of cyclins such as cyclin I and cyclin G1. In addition, cumulative evidence supports a role for Cdk5 in the regulation of gene transcription through the canonical pathway involving p35 binding and the phosphorylation of key substrates. Furthermore, Cdk5 recently was shown to regulate protein translation, suggesting that novel substrates and mechanisms remain to be discovered. Currently, with the emergence of a novel spliced isoform of the kinase, novel regulatory mechanisms based not only on Cdk5 kinase activity may be envisioned. It is tempting to propose that protein–protein interactions with the kinase may serve as a complementary signaling mechanism, which could result in Cdk5 acting as a scaffold protein. The emergence of novel substrates and mechanisms in which Cdk5 is involved confirms that the complexity and fine regulation of this kinase will be important to understand its participation in normal cellular physiology. It may be possible in the future to elucidate a new scenario in which pathways leading to deregulation of Cdk5 kinase activity are related to the differential binding of co-activators (p35, p39, or cyclins), the differential expression of shorter Cdk5 isoforms, or the gain of toxic functions due to unregulated protein-protein interactions. All of these processes, if unregulated, may be linked to the development of pathological conditions, including cancer and diabetes.

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