FEBS Letters 582 (2008) 1197–1202

CDK5 activator p35 downregulates E-cadherin precursor independently of CDK5

Shuyong Lin[a], Jifeng Wang[a], Zhiyun Ye[a], Nancy Y. Ip[b], Sheng-Cai Lin[a,*]

[a] *Key Laboratory of Ministry of Education for Cell Biology and Tumor Cell Engineering, School of Life Sciences, Xiamen University, Fujian 361005, China*
[b] *Department of Biochemistry, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China*

Received 23 December 2007; accepted 12 February 2008

Available online 4 March 2008

Edited by Michael R. Bubb

Abstract **Dysfunction of E-cadherins often results in metastasis of cancerous cells. Here we show that p35, a critical regulator of cyclin-dependent kinase 5 (CDK5), specifically depletes the precursor form of E-cadherin, but not the mature form, by using a precursor-specific antibody. Most intriguingly, this downregulation of precursor E-cadherin by p35 is unequivocally independent of CDK5. Moreover, we found that p35 forms complexes with E-cadherin proteins. We also found that p35 co-expression can target E-cadherin to lysosomes and that p35-triggered disappearance of E-cadherin precursor can be blocked specifically by lysosomal protease inhibitors, indicating that p35 induces endocytosis and subsequent degradation of precursor E-cadherin.**

Structured summary:
MINT-6276674, MINT-6276685:
E-cadherin http://mint.bio.uniroma2.it/mint-curation/search/interactor.do?interactorAc=MINT-121804 & (uniprotkb: P09803) *physically interacts* (MI:0218) with *p35* (uniprotkb: P61809) by *coimmunoprecipitation* (MI:0019)
MINT-6276701, MINT-6276714:
Cdk5 (uniprotkb:P49615) *physically interacts* (MI:0218) with *p35* (uniprotkb:P61809) by *coimmunoprecipitation* (MI:0019)

Keywords: p35; E-cadherin; CDK5; Lysosomal degradation

1. Introduction

Cyclin-dependent kinase 5 (CDK5) shares sequence similarity with members of the superfamily of cyclin-dependent kinases [1]. A peculiar feature of CDK5 is that it does not exert canonical CDK functions that are related to cell cycle; instead, CDK5 seems to play critical roles in neuronal survival and paradoxically neuronal apoptosis, neuronal migration and axon guidance, as well as synaptic neurotransmission [2]. Like other cyclin-dependent kinases, CDK5 requires association of a regulatory activator, p35 or p39 that shares little sequence identity with cyclins [3–5]. p35 and p39 are targeted to the cell membrane by virtue of their N-terminal myristoylation signal [6,7]. They are proteolytically cleaved into p25 and p29, respectively, by calpain that is activated by elevated intracellular calcium levels upon stimulation by neurotoxic insults including ischemic and oxidative damages [7,8]. Whereas p35 is relatively short-lived, p25 has a long life-span and is believed to cause over-activity of CDK5 in neurons that exhibit pathological conditions. It is important to note that p35 may also act as an adaptor for various substrates to be phosphorylated by CDK5. The p35-interacting proteins include β-catenin, Pctaire1, mSds3 and so on [9–11].

CDK5/p35 plays important roles in the adhesion and migration of neuronal cells. Many known CDK5 substrates in neurons are cytoskeleton-associated proteins. In particular, CDK5 has been reported to interact with β-catenin through its regulatory subunit, p35, and regulates the affinity of β-catenin for cadherin by altering the phosphorylation levels of β-catenin in neurons [12,13]. In addition, it has also been shown that CDK5 plays a role in the regulation of cell adhesion and migration in lens epithelial cells, corneal epithelial cells and keratinocytes [14–16]. Cadherin–catenin complexes play pivotal roles in cell adhesion [17]. Mature E-cadherin requires correct proteolytic processing, which will remove the N-terminal region from the precursor of E-cadherin. Similar to mature E-cadherin, unprocessed E-cadherin can also be plasma membrane localized and has features in common with the mature protein, such as catenin binding and Ca^{2+}-dependent resistance to proteolytic degradation [18,19]. However, cells expressing the unprocessed form exhibit no E-cadherin dependent cell aggregation. Proteolytic removal of precursor segment results in the activation of adhesive properties, demonstrating that the presence of the precursor segment suppresses the cell adhesion function.

We have been interested in the biological functions and mechanisms of molecular components in the Wnt pathway. In an attempt to address how cadherin/β-catenin complex formation might regulate the cellular abundance of β-catenin, we tested several known or potential related regulators. As a result, we found that p35 drastically downregulated the precursor form of E-cadherin, and surprisingly that CDK5 kinase activity was not involved in such an effect. Addition of ammonium chloride or chloroquine but not proteasomal inhibitors significantly attenuated the downregulation, suggesting that the E-cadherin precursor is downregulated as a result of p35-enhanced endocytosis and subsequent lysosomal degradation. Consistently, based on a dextran colocalization experiment,

*Corresponding author. Fax: +86 592 2184687.
E-mail address: linsc@xmu.edu.cn (S.-C. Lin).

Abbreviations: CDK5, cyclin-dependent kinase 5; dn, dominant negative; PCR, polymerase chain reaction

0014-5793/$34.00
doi:10.1016/j.febslet.2008.02.053

we found that p35 coexpression results in colocalization of E-cadherin precursor in lysosomes.

2. Materials and methods

2.1. Plasmid constructions

Full length cDNA encoding mouse E-cadherin was subcloned into pCMV5 vector from EST clone. C-terminal HA tagged E-cadherin was cloned through fusing the EST clone with HA-tag using a polymerase chain reaction (PCR)-based approach. p35, p39, CDK5 and dominant negative (dn)CDK5 (D144N) were as described before [10]. p35dn1 (L151N/L152N), p35dn2 (D288A/L289A) were created using a site directed mutagenesis approach. C-terminally myc-tagged p35, HA-CDK5 and HA-dnCDK5 were constructed by PCR strategy.

2.2. Preparation of antibodies

Mouse anti-HA (F-7) and anti-Myc (9E10) were purchased from Santa Cruz Biotech. Rabbit anti-E-cad was raised against amino acids 752–850 of mouse E-cadherin. Rabbit anti-pre antibody was raised against the PstI–PvuII fragment (amino acids 31–164) of E-cadherin. Rabbit anti-p35 were purchased from Sigma, rabbit anti-p39 were as previously described [10].

2.3. Co-immunoprecipitation and Western blotting

Maintenance of HEK 293T cells, transient transfection using PEI were as previously described [20]. Transiently transfected HEK 293T cells in 60 mm dishes were lysed in a lysis buffer, sonicated and centrifuged at 13,200 rpm for 30 min at 4 °C. Proteins were immunoprecipitated from the cell lysate with various antibodies as indicated and Protein A/G Plus-agarose beads (Santa Cruz). Immunoprecipitates or TCLs were analyzed by Western blotting as previously described [20].

2.4. Immunofluorescent staining and lysosomal colocalization staining

At 24 h post-transfection, 293T cells were fixed in 4% paraformaldehyde for 20 min at room temperature. After washing with PBS, cells were permeabilized with 0.2% Tween-20 in PBS and blocked in 10% BSA. Rabbit anti-pre antibody (1:1000) was diluted in block solution. Rhodamine or FITC conjugated anti-rabbit secondary antibody was used. To determine if E-cadherin is targeted to lysosomes upon coexpression with p35, cells on coverslips were incubated with Texas Red-labeled dextran (Molecular Probes) (0.1 mg/ml) in serum-free DMEM with 1 mg/ml of BSA for 10 h before fixation. The fixed cells were then subjected to immunofluorescence staining with anti-precursor-specific antibody. The staining patterns were visualized with a confocal microscope (Leica).

3. Results

3.1. The precursor form of E-cadherin was specifically downregulated by p35

E-cadherin was co-transfected with p35 or p39 into 293T cells. It was detected as double bands with molecular weights of about 120 and 135 kDa. However, when co-expressed with p35, the upper band of E-cadherin was specifically downregulated, with the expression level of the lower one unchanged (Fig. 1A). Interestingly, p39, another CDK5 activator that shares 57% identity with p35 in protein sequence, did not exert any apparent effect on either form of E-cadherin. To investigate whether the p35-induced disappearance of E-cadherin precursor would ultimately lead to a decrease of the mature form of E-cadherin, we first transfected E-cadherin into 293T cells, and then p35 after 12, 18 or 24 h of E-cadherin expression. The levels of mature form of E-cadherin did not show any detectable decrease during the time course, indicating that the E-cadherin precursor that can be downregulated by p35 does not undergo the normal E-cadherin proteolytic processing to yield mature E-cadherin (Fig. 1B).

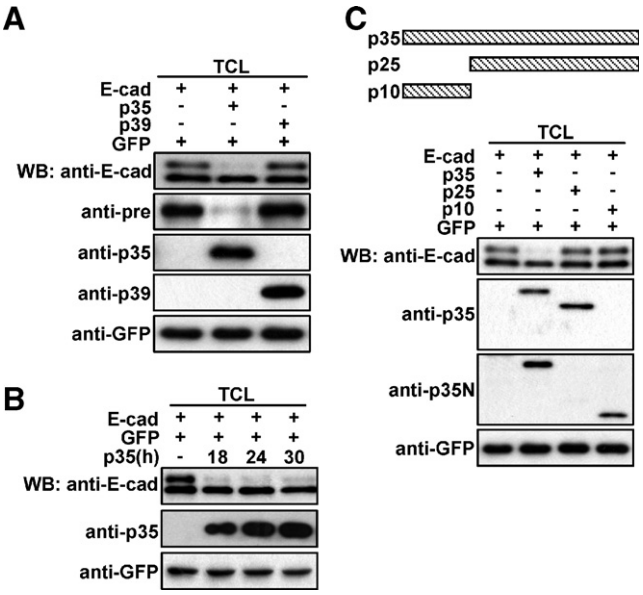

Fig. 1. E-cadherin precursor is associated and is downregulated by p35. (A) The precursor form of E-cadherin is specifically reduced by co-expression of p35. HEK 293T cells were transfected with E-cadherin, GFP, with p35 or p39. After 24 h of transfection, total cell lysates (TCL) were analyzed by Western blotting using various antibodies indicated. (B) Time course of the effect of p35 coexpression on the levels of mature E-cadherin. Cells were transfected with E-cadherin, and GFP as an internal control. The cells were then transfected at 12, 18 and 24 h after E-cadherin expression, and were harvested at 42 h post-transfection of E-cadherin, allowing for p35 expression for 30, 24 and 18 h, as indicated. TCLs were analyzed by Western blotting using various antibodies indicated. (C) Full length p35 is required for downregulation of E-cadherin precursor. Cells were transfected with E-cadherin, full length p35, p10, p25 or GFP as a control. TCL were analysed by Western blotting using various antibodies indicated.

It has been known that E-cadherin is firstly synthesized as a 135 kDa precursor polypeptide, and then processed to a 120 kDa mature polypeptide. To confirm that the upper band is the precursor form of E-cadherin, we raised a rabbit polyclonal antibody against the N-terminal PstI–PvuII fragment (amino acids 31–164) in the precursor region of E-cadherin. With this antibody (designated as anti-pre), a single species corresponding to the upper band was detected and found to be indeed downregulated by coexpression of p35 (Fig. 1A). Of note, no band corresponding to the precursor fragment was detected, further indicating that the disappearance of precursor was not a result of proteolytic processing. Neither the p25 fragment of p35, which is more stable than p35 and can lead to sustained activation of CDK5, nor the complementary N-terminal p10 fragment had the downregulating effect on the E-cadherin precursor as full length p35 (Fig. 1C). These results indicate that full length p35 seemed to be required for the downregulation of the precursor of E-cadherin.

As precursor and mature E-cadherin are differentially localized in the cell, we also carried out immunofluorescence study in 293T cells. E-cadherin and GFP vector (at the ratio of 5:1) were transfected with control vector or p35 into subconfluent 293T cells. Consistent with the results obtained by Western blotting analysis (Fig. 1A), the signal of the unprocessed polypeptide of E-cadherin detected by anti-pre antibody was greatly reduced when co-expressed with p35, while the number

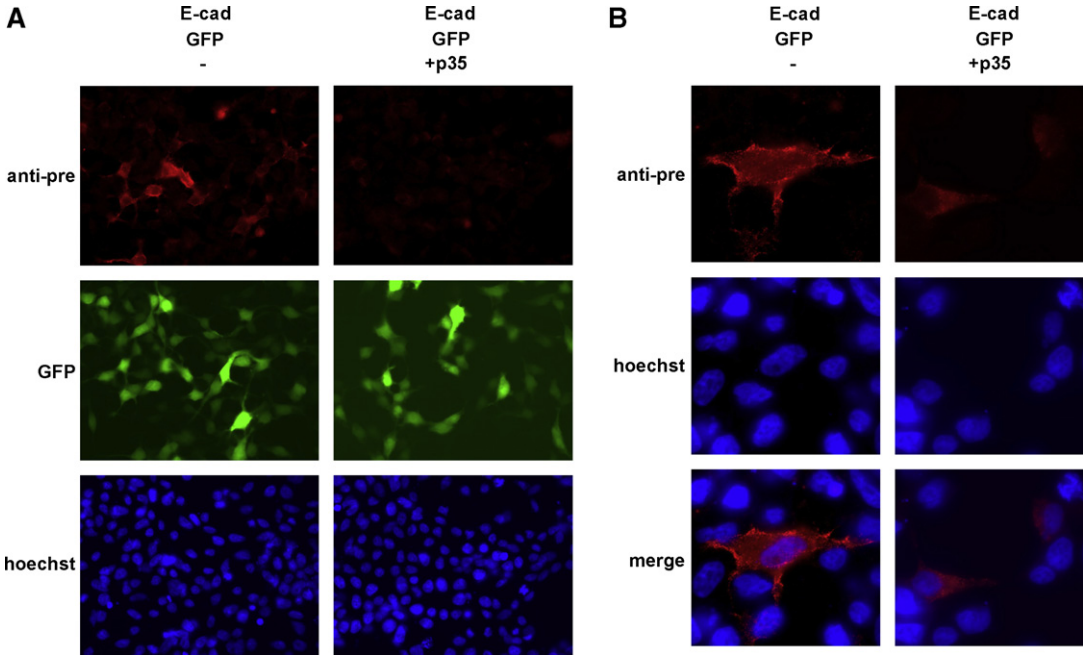

Fig. 2. Plasma membrane associated E-cadherin precursor is preferentially reduced by p35 co-expression. (A) 293T cells were transfected with E-cadherin, GFP or p35. At 24 h post-transfection, cells were stained with anti-pre antibody and hoechst33342. Staining patterns were visualized by a fluorescent microscope (Olympus). (B) Cells were transfected with E-cadherin, or together with p35, followed by immunofluorescent staining with anti-pre antibody and hoechst as mentioned above.

of GFP positive cells or GFP signal intensity did not decrease, showing that the reduced signal of the precursor peptide is not due to a lower transfection efficiency (Fig. 2A). When single cells were examined, the ectopically expressed precursor form of E-cadherin was mainly localized close to the plasma membrane with relatively weaker signal in the cytosol (Fig. 2B). The portion of precursor close to the plasma membrane disappeared when co-transfected with p35, leaving the cytosolic portion virtually unaffected (Fig. 2B).

3.2. Both the precursor and mature form of E-cadherin associated with p35

We then tested if p35 might exert its downregulation of precursor E-cadherin by forming a complex with E-cadherin. We performed reciprocal co-immunoprecipitation assays using 293T cell lysates that contained ectopically expressed untagged p35 and E-cadherin with antibodies against C-terminal of E-cadherin and p35, respectively. Considering the precursor form of E-cadherin can be downregulated by p35, we pretreated cells with NH_4Cl to inhibit its lysosomal degradation. Both the precursor and mature form of E-cadherin were detected in the immunoprecipitate of p35, and p35 detected in the E-cadherin precipitate (Fig. 3A). These results are consistent with a previous report showing that E-cadherin was detected in the p35 immunoprecipitates from human keratinocytes [16].

We next tried to determine the region of p35 required for E-cadherin association. C-terminally myc-tagged p35, its N-terminal p10, and C-terminal p25 fragment, along with p39 as a control, were separately co-transfected with E-cadherin. It was found that p25 fragment but not p10 fragment or p39 was detected in the E-cadherin precipitate (Fig. 3B); indicating that the p25 fragment was required and sufficient to form a complex with E-cadherin.

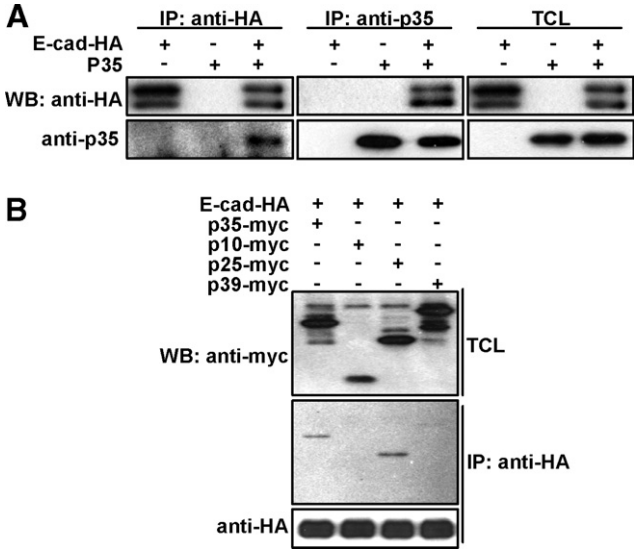

Fig. 3. Complex formation between p35 and E-cadherin. (A) Both the precursor and mature forms of E-cadherin are associated with p35. Cells were transfected with C-terminally HA-tagged E-cadherin and p35 alone or in combination. E-cadherin-HA and p35 were separately immunoprecipitated from TCLs using anti-HA or anti-p35 antibody. (B) The p25 fragment of p35 is sufficient to interact with E-cadherin. Cells were transfected with C-terminally HA-tagged E-cadherin and c-terminally myc-tagged p35, p10, p25 or p39. E-cadherin was immunoprecipitated by anti-HA antibody.

3.3. p35 downregulates E-cadherin precursor independently of CDK5 kinase activity

Considering the function of p35 is always associated with CDK5 and CDK5 is quite abundant in 293T cells (data not shown), we wondered whether the downregulation of the

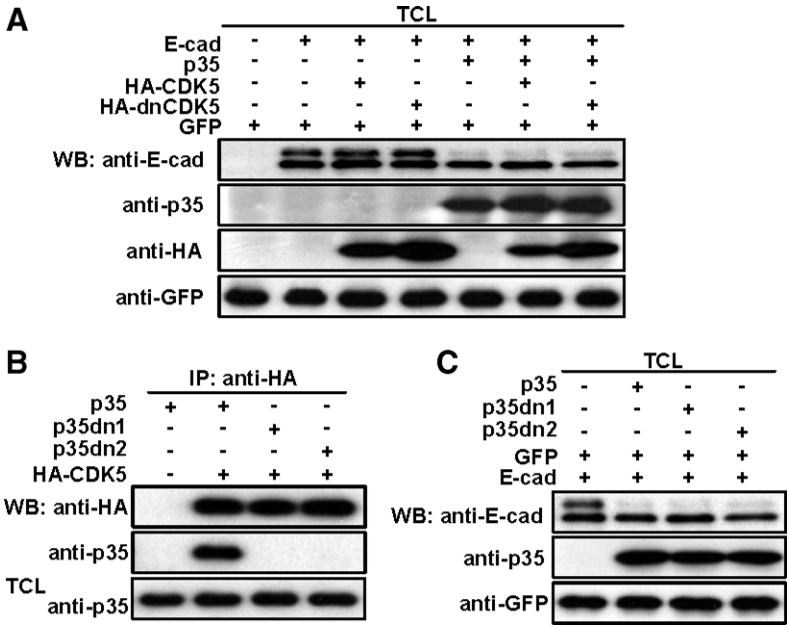

Fig. 4. CDK5 kinase activity is not required in p35 mediated E-cadherin precursor degradation. (A) Dominant negative mutant CDK5 (HA-dnCDK5) did not block p35 mediated E-cadherin precursor degradation. Cells were transfected with E-cadherin, GFP, HA-CDK5, HA-dnCDK5 or p35 in different combination indicated. Total cell lysates were analyzed by Western blotting. (B) p35 mutant L151N/L152N (p35dn1) and D288A/L289A mutant (p35dn2) did not interact with CDK5. Immunoprecipitation was performed using anti-HA antibody. (C) p35dn1 and p35dn2 retain full ability to downregulate E-cadherin precursor. Cells were transfected with E-cadherin, GFP with or without p35, p35dn1 or p35dn2. Total cell lysates were analyzed by Western blotting.

precursor of E-cadherin by p35 overexpression was a consequence of activation of endogenous CDK5 kinase activity. To address this point, we included dominant negative CDK5 and p35 into our study. Transfection of wild-type or dominant negative CDK5 (D144N) alone did not change E-cadherin protein level. Transfection of kinase dead form of CDK5 cannot block or even diminish the downregulation of unprocessed E-cadherin by p35 (Fig. 4A). We also created dominant negative p35 mutants, p35dn1 (altering leucine 151/152 to asparagines, L151/152N), and p35dn2 for D288A/L289A as previously described [21]. Although p35dn1 and p35dn2 failed to interact with CDK5 (Fig. 4B), they still downregulated the E-cadherin precursor to a similar extend as wild-type p35 (Fig. 4C). These data strongly suggested that p35 downregulation of E-cadherin is not a consequence of CDK5 activation, but through a CDK5 independent mechanism, in accordance with the observation that p39 or p25 fragment of p35, both of which can activate CDK5, has no effect on E-cadherin precursor degradation.

3.4. E-cadherin precursor is degraded via a lysosomal pathway

It's reported that mature E-cadherin can undergo proteasomal degradation and is trafficked to and from the cell surface by exocytic and multiple endocytic pathways. To investigate the possible contribution of these two major degradation pathways in the process of downregulation of unprocessed E-cadherin by p35, various lysosomal and proteasomal inhibitors were tested in the precursor degradation assay. We found that treating cells with lysosomal inhibitors NH_4Cl and chloroquine prevented the disappearance of E-cadherin precursor triggered by p35. In contrast, proteasomal inhibitors MG132 and ALLN did not have such an effect (Fig. 5A-left), although they did stabilize Arkadia, a protein known to undergo proteasomal degradation [22] (Fig. 5A-right). Levels of p35 remained unchanged under treatment of the lysosomal inhibitors, indicating that p35 is not co-endocytosed or degraded with the precursor (Fig. 5A-right). These results indicate that the downregulation of E-cadherin precursor by p35 is through lysosomal pathway but not proteasomal degradation.

It has previously been reported that dextrans are internalized in cells and delivered through endosomal compartments to the lysosomes. HEK 293T cells were treated with Texas Red-labeled dextran for 10 h to label the endocytic-lysosomal compartments. When cotransfected with p35, the E-cadherin precursor exhibited partial colocalization with Texas Red-dextran positive vacuoles (yellow), conforming to the notion that the disappearance of the precursor was mediated by p35 through lysosomal degradation pathway (Fig. 5B).

4. Discussion

It is important to note that although p35 is mostly expressed in the nervous system, it is also detected in systemic cell types [14–16,23], consistent with a potential role in controlling the abundance of precursor E-cadherin in non-neuronal cells. Alternatively, p35 may control cadherin-related cell adhesion in the nervous system as some studies have demonstrated that E-cadherin is also locally and transiently expressed in the developing nervous system, and that anti-E-cadherin antibody affected the overall morphology of developing brains in vitro [24]. The processing of E-cadherin into mature form takes place before it reaches the plasma membrane [19]. The finding that p35 did not affect the levels of mature form of E-cadherin, even after their prolonged coexpression as assessed by expression time course, indicates that the degraded portion of E-cadherin precursor would not be later processed into mature E-cadherin.

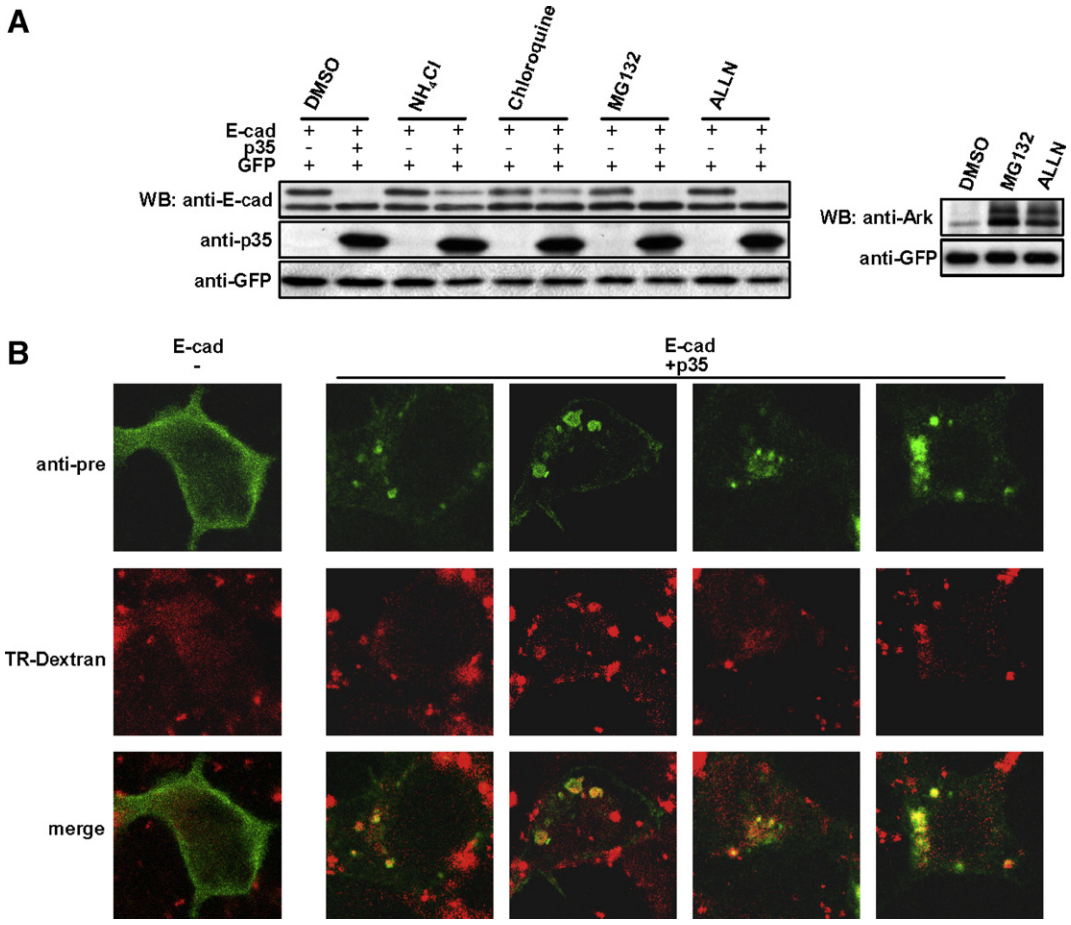

Fig. 5. E-cadherin precursor degradation mediated by p35 is through a lysosomal pathway. (A) Left, p35 mediated E-cadherin degradation was blocked by lysosomal inhibitors but not proteasomal inhibitors. About 40 mM NH_4Cl, 200 μM chloroquine, 10 μM MG132 or 40 μM ALLN was added into growth medium 12 h post-transfection and cells were harvested at 24 h post-transfection. Right, proteasomal inhibitors can stabilize Arkadia, which serves as a positive control for the efficacy of the inhibitors. (B) Colocalization of E-cadherin precursor with TR-dextran in 293T cells. 293T cells were transfected with E-cadherin alone or with p35. At 18 h post-transfection, cells were incubated with Texas Red-labeled dextran (0.1 mg/ml) diluted in serum free DMEM with BSA (1 mg/ml). Approximately 10 h after incubation, cells were fixed and stained using anti-precursor-specific (anti-pre) antibody and FITC conjugated secondary antibody. Staining patterns were visualized by a confocal microscope (Leica).

Up to now, p35 has been regarded exclusively as a CDK5 regulator. However, Cyclin D1 has been shown to activate estrogen receptor independently of CDK, suggesting that the cyclins may carry out functions independently of their cognate CDKs [25]. Here, we have unequivocally demonstrated that p35 can regulate the abundance of E-cadherin precursor with the involvement of CDK5. First of all, we showed that the kinase-dead form of CDK5 did not attenuate the downregulating effect of p35 on E-cadherin. Second, the p35 mutants that cannot interact with and are unable to activate CDK5 can effectively reduce the abundance of E-cadherin precursor. Moreover, p25, the activating form of p35 for CDK5, on the contrary fails to exert the same effect as p35. This brings to another finding of our current study that an intact p35, but not p10 or p25, is required for degradation of E-cadherin. Consistently, tagging of p35, N-terminally or C-terminally abolished its function, although it did not affect its interaction with E-cadherin (data not shown). How exactly p35 specifically regulates the precursor of E-cadherin remains unclear. It is feasible that p35 may target E-cadherin to degradation by cooperating with some unknown factors that can discriminate the precursor form from the mature form. It is therefore of great interest to test what signals may upregulate p35 levels in the cell.

Acknowledgements: This work was supported by Grants from the Cultivation Fund of the Key Scientific and Technical Innovation Project, Ministry of Education of China (#705030), the Foreign Outstanding Young Co-Investigator Award (#30528014), the Natural Science Foundation of Fujian Province of China (#C0520001), the Xiamen Council of Science and Technology (#3502Z20055004), the "111" Project (#B06016) sponsored by the Bureau of Foreign experts and MOE, and from the "863" Program (#2006AA02A303).

References

[1] Lew, J., Beaudette, K., Litwin, C.M. and Wang, J.H. (1992) Purification and characterization of a novel proline-directed protein kinase from bovine brain. J. Biol. Chem. 267, 13383–13390.
[2] Dhavan, R. and Tsai, L.H. (2001) A decade of CDK5. Nat. Rev. Mol. Cell Biol. 2, 749–759.
[3] Tsai, L.H., Delalle, I., Caviness Jr., V.S., Chae, T. and Harlow, E. (1994) p35 is a neural-specific regulatory subunit of cyclin-dependent kinase 5. Nature 371, 419–423.

[4] Lew, J., Huang, Q.Q., Qi, Z., Winkfein, R.J., Aebersold, R., Hunt, T. and Wang, J.H. (1994) A brain-specific activator of cyclin-dependent kinase 5. Nature 371, 423–426.
[5] Tang, D., Yeung, J., Lee, K.Y., Matsushita, M., Matsui, H., Tomizawa, K., Hatase, O. and Wang, J.H. (1995) An isoform of the neuronal cyclin-dependent kinase 5 (CDK5) activator. J. Biol. Chem. 270, 26897–26903.
[6] Patrick, G.N., Zhou, P., Kwon, Y.T., Howley, P.M. and Tsai, L.H. (1998) p35, the neuronal-specific activator of cyclin-dependent kinase 5 (CDK5) is degraded by the ubiquitin-proteasome pathway. J. Biol. Chem. 273, 24057–24064.
[7] Patzke, H. and Tsai, L.H. (2002) Calpain-mediated cleavage of the cyclin-dependent kinase-5 activator p39 to p29. J. Biol. Chem. 277, 8054–8060.
[8] Lee, M.S., Kwon, Y.T., Li, M., Peng, J., Friedlander, R.M. and Tsai, L.H. (2000) Neurotoxicity induces cleavage of p35 to p25 by calpain. Nature 405, 360–364.
[9] Kesavapany, S., Lau, K.F., McLoughlin, D.M., Brownlees, J., Ackerley, S., Leigh, P.N., Shaw, C.E. and Miller, C.C. (2001) p35/CDK5 binds and phosphorylates beta-catenin and regulates beta-catenin/presenilin-1 interaction. Eur. J. Neurosci. 13, 241–247.
[10] Cheng, K., Li, Z., Fu, W.Y., Wang, J.H., Fu, A.K. and Ip, N.Y. (2002) Pctaire1 interacts with p35 and is a novel substrate for CDK5/p35. J. Biol. Chem. 277, 31988–31993.
[11] Li, Z., David, G., Hung, K.W., DePinho, R.A., Fu, A.K. and Ip, N.Y. (2004) CDK5/p35 phosphorylates mSds3 and regulates mSds3-mediated repression of transcription. J. Biol. Chem. 279, 54438–54444.
[12] Schuman, E.M. and Murase, S. (2003) Cadherins and synaptic plasticity: activity-dependent cyclin-dependent kinase 5 regulation of synaptic beta-catenin–cadherin interactions. Philos. Trans. R. Soc. Lond. B: Biol. Sci. 358, 749–756.
[13] Kwon, Y.T., Gupta, A., Zhou, Y., Nikolic, M. and Tsai, L.H. (2000) Regulation of N-cadherin-mediated adhesion by the p35–CDK5 kinase. Curr. Biol. 10, 363–372.
[14] Negash, S., Wang, H.S., Gao, C., Ledee, D. and Zelenka, P. (2002) CDK5 regulates cell–matrix and cell–cell adhesion in lens epithelial cells. J. Cell Sci. 115, 2109–2117.
[15] Gao, C., Negash, S., Guo, H.T., Ledee, D., Wang, H.S. and Zelenka, P. (2002) CDK5 regulates cell adhesion and migration in corneal epithelial cells. Mol. Cancer Res. 1, 12–24.
[16] Nakano, N., Nakao, A., Ishidoh, K., Tsuboi, R., Kominami, E., Okumura, K. and Ogawa, H. (2005) CDK5 regulates cell–cell and cell–matrix adhesion in human keratinocytes. Br. J. Dermatol. 153, 37–45.
[17] Jamora, C. and Fuchs, E. (2002) Intercellular adhesion, signalling and the cytoskeleton. Nat. Cell Biol. 4, E101–E108.
[18] Shore, E.M. and Nelson, W.J. (1991) Biosynthesis of the cell adhesion molecule uvomorulin (E-cadherin) in Madin–Darby canine kidney epithelial cells. J. Biol. Chem. 266, 19672–19680.
[19] Ozawa, M. and Kemler, R. (1990) Correct proteolytic cleavage is required for the cell adhesive function of uvomorulin. J. Cell Biol. 111, 1645–1650.
[20] Rui, Y., Xu, Z., Xiong, B., Cao, Y., Lin, S., Zhang, M., Chan, S.C., Luo, W., Han, Y., Lu, Z., Ye, Z., Zhou, H.M., Han, J., Meng, A. and Lin, S.C. (2007) A beta-catenin-independent dorsalization pathway activated by Axin/JNK signaling and antagonized by aida. Dev. Cell 13, 268–282.
[21] Tang, D., Chun, A.C., Zhang, M. and Wang, J.H. (1997) Cyclin-dependent kinase 5 (CDK5) activation domain of neuronal CDK5 activator. Evidence of the existence of cyclin fold in neuronal CDK5a activator. J. Biol. Chem. 272, 12318–12327.
[22] Koinuma, D., Shinozaki, M., Komuro, A., Goto, K., Saitoh, M., Hanyu, A., Ebina, M., Nukiwa, T., Miyazawa, K., Imamura, T. and Miyazono, K. (2003) Arkadia amplifies TGF-beta superfamily signalling through degradation of Smad7. EMBO J. 22, 6458–6470.
[23] Chen, F. and Studzinski, G.P. (2001) Expression of the neuronal cyclin-dependent kinase 5 activator p35Nck5a in human monocytic cells is associated with differentiation. Blood 97, 3763–3767.
[24] Shimamura, K. and Takeichi, M. (1992) Local and transient expression of E-cadherin involved in mouse embryonic brain morphogenesis. Development 116, 1011–1019.
[25] Zwijsen, R.M., Wientjens, E., Klompmaker, R., van der, S.J., Bernards, R. and Michalides, R.J. (1997) CDK-independent activation of estrogen receptor by cyclin D1. Cell 88, 405–415.