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Wnt/β-catenin signal pathway stabilizes APP intracellular domain (AICD) and promotes its transcriptional activity

Fangfang Zhou [a,b,1], Kai Gong [a,1], Theo van Laar [b], Yandao Gong [a,*], Long Zhang [b,*]

[a] State Key laboratory of Biomembrane and Membrane Biotechnology, School of Life Sciences, Tsinghua University, Beijing 100084, China
[b] Leiden University Medical Center, Dept. of Molecular Cell Biology, Leiden, The Netherlands

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ABSTRACT

Amyloid precursor protein (APP), a key protein in pathogenesis of Alzheimer's disease (AD), is a type I transmembrane protein which can be cleaved by β- and γ-secretase to release the amyloidogenic β-amyloid peptides (Aβ) and the APP intracellular domain (AICD). While Aβ has been widely believed to initiate pathogenic cascades culminating AD, the physiological functions and regulations of AICD remain elusive. In present study, endogenous AICD was demonstrated to be increased by canonical Wnt signal. Instead of due to γ-secretase activity, enhanced AICD expression was found due to the increased protein stability by Wnt/β-catenin. β-Catenin was demonstrated to be an associating partner of AICD, capable of promoting AICD mediated transcriptional activity. Investigation by AICD mutants proved that Fe65, a previously identified AICD binding partner, is not involved in this regulation. Taken together, our results suggest that AICD is stabilized and the AICD mediated transcriptional activity is promoted by canonical Wnt/β-catenin signaling independent of Fe65.

1. Introduction

Amyloid precursor protein (APP), with the topology resembles of a membrane receptor protein which has a large extracellular portion, a single transmembrane segment, and a cytoplasmic tail domain that interacts with several proteins, is implicated in the pathogenesis of Alzheimer's disease [1]. APP can be cleaved by at least three "secretases": α-, β- and γ-secretase. When cleaved by β-secretase, APP releases the soluble βAPP and a membrane-linked C-terminal fragment (βCTFs) which is further cleaved by γ-secretase in membrane to release β-amyloid protein (Aβ) and intracellular domain (AICD). α-Secretase, cleaves within the Aβ region, yielding soluble αAPP and a membrane-linked C-terminal fragment (αCTFs) which is further processed by γ-secretase into P3 peptide and AICD [2].

Due to the receptor-like architecture of APP [1] and the similarity of AICD to NICD, an intracellular domain of Notch protein, AICD was considered as a key protein in Alzheimer disease [3]. It has been reported that AICD can exert a variety of physiological and pathological functions by interacting with different proteins such as X11, Shc, IRS-1, brain GTP-binding protein Go, APP-BP1, UV-DDB, JIP1 (JNK Interacting Protein 1) and Pin1 [4–10]. A well-characterized binding protein of AICD is Fe65 which was found by Yeast two-hybrid system. Fe65 stabilizes AICD and the Fe65-AICD complex translocates into the nucleus and activates the transcription of genes including KAI1, GSK3β, APP, BACE and Tip60 [3,11]. Noticeably, the ^{682}YENPTY^{687} (APP695 isoform numbering) motif of AICD is very important for the interaction. Deletion of this motif or mutation of the tyrosine residues in this motif will block the interaction [4]. Recently, phosphorylation of APP intracellular domain at Thr668 was also reported to be essential for the binding activity, stability, localization and functions of AICD [10,12,13]. For example, the neuron-specific c-Jun N-Terminal Kinase 3 can mediate the phosphorylation at Thr668, and this phosphorylation affects its binding to Fe65, its stability and its nuclear translocation, although it is still arguable whether phosphorylation plays a positive or negative role in these aspects [12,13]. Another study found that Pin1 could bind the phosphorylated Thr668-Pro motif and regulate the conformational change of the intracellular domain of APP and therefore APP processing [10]. In addition, AICD fragment C31, cleaved by caspase-8 and caspase-9, was reported to induce apoptosis in N_2a cells and in AD brains [14]. These findings suggest that AICD plays a pivotal role in the pathogenesis of AD.

Wnt signaling plays a crucial role in cell fate determination during animal development, and deregulation of this signaling leads to tumorigenesis [15]. Activation of canonical Wnt signaling prevents the phosphorylation, ubiquitination and proteasomal degradation of β-catenin, resulting its translocation into nucleus where it forms transcriptional complexes with TCF/LEF and regulates gene transcription [15–18]. Wnt signaling has been

* Corresponding authors.
E-mail addresses: gongyd@tsinghua.edu.cn (Y. Gong), L.zhang@lumc.nl (L. Zhang).
[1] These authors contribute equally to this work.

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implicated in AD progression by influencing the formation of hyperphosphorylated tau, another important risk factor in AD [19,20]. A recent study has demonstrated that GSK3β phosphorylation level is higher in AICD transgenic mouse than in wild-type mouse [21]. Moreover, AICD induces GSK3β expression and tau phosphorylation in NGF-treated PC12 cells and rat primary cortical neurons [12]. Overexpression of disheveled (Dvl), a key mediator of Wnt signaling, increases souble αAPP production via both JNK and PKC/MAP kinase [22]. Collectively, many Wnt signaling components, including Dvl, GSK3β, and β-catenin, were implicated in APP processing and AD progression. In this study, we report that the protein stability and transcriptional activity of AICD is Fe65 independently stabilized by Wnt/β-catenin signaling. First, increased endogenous AICD was found in Wnt1 expressing neuronal cells. Second, β-catenin, the essential mediator of canonical Wnt signaling component was showed to associate with AICD, causing increased AICD-dependent transactivation. Third, Wnt/β-catenin was proved to promote protein stability of AICD. In the end, we provide evidences that these regulations are independent of Fe65. Altogether, the present work implied a positive regulatory link of AICD by canonical Wnt.

2. Materials and methods

2.1. Constructions and reagent

AICD (C57, the last 57 AA of APP695 isoform) was PCR from pCl-neo-APP695 plasmid and subcloned into pcDNA3.1/Myc-His with EcoRI and XhoI and into pcDNA3.1(+)-Gal4 construct with KpnI and EcoRI, respectively. The mutants T682A (AENPTY), 3A (AEAPTA) and T668A of AICD were generated by PCR-mediated mutagenesis using appropriate primers and subcloned into pcDNA3.1/Myc-His vector or into pcDNA3.1-Gal4 to form fusion protein. All of the sequences were verified by DNA sequencing. Wnt1 and β-cateninSA (S29A, S33A, S37A, T41A and S45A) and Notch 1delta E plasmids were as described previously [23–25]. APP C-terminal specific 369 antibody, 4G8 and 6E10 antibodies were as described previously [26]. Cycloheximide (CHX) and MG132 were obtained from Sigma (USA).

2.2. Cell lines and cell culture

HEK293 and HEK293T cells were cultured in Dulbecco's modified Eagle's medium (GIBCO) supplemented with 10% fetal bovine serum (Hyclone). N_2a cells were cultured in 50% Dulbecco's modified Eagle's medium and 50% OPTI-MEM (GIBCO) supplemented with 5% fetal bovine serum and 200 μg/ml G418 (GIBCO) for stable cell lines. All cells cultured in a 5% CO_2-containing atmosphere at 37 °C.

2.3. Transfection, Immunoprecipitation, and Immunoblotting

HEK293T cells were transiently transfected using the calcium phosphate method or Lipofectamine (Invitrogen, USA). N_2a cells were transiently transfected using Lipofectamine 2000 (Invitrogen, USA). Immunoprecipitation was performed as previously described [23,27,28]. Immunoblotting was performed with specific antibody and secondary anti-mouse or anti-rabbit antibodies that were conjugated to horseradish peroxidase (Amersham Biosciences). Immunodetection of Aβ was carried out as reported [29].

2.4. Luciferase reporter assays

As previously described [30]; HEK293 cells were transfected with various plasmids as indicated in the figures. Cells were harvested and luciferase activities were measured by a luminometer (Berthold Technologies). Reporter activity was normalized to the control *Renilla*. Experiments were repeated in triplicate.

3. Results

3.1. Wnt activation increases endogenous AICD

It has been reported that Dvl stimulates α-secretase activity [22]. To examine whether Wnt sigaling affects APP processing, Wnt-1 was expressed into mouse neuroblastoma N_2a cells stably expressing APP [31]. As shown in Fig. 1A and B, Wnt-1 had no effect on the total expression of APP but resulted in a threefold increase of sAPPα production, indicating that α-secretase activity

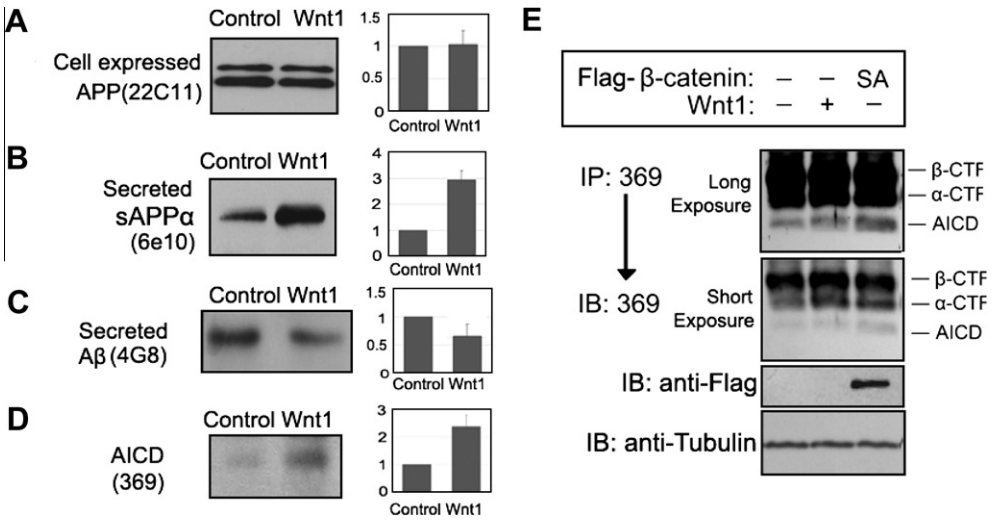

Fig. 1. Activation of Wnt signaling increases endogenous AICD protein level, (A–D) Examination of APP processing by Wnt-1 activation; Control and Wnt1 expressing N_2a APP cells were harvested and the total expression of APP was assessed by the N-terminal antibody 22C11 (A), the sAPPα produced by α-secretase was demonstrated by Western blots of concentrated media using 6E10 antibody (B), the Aβ was detected with concentrated media using 4G8 and 6E10 antibodies (C) and the AICD was probed by APP C-terminal 369 antibody (D and E), APP stably expressed N_2a cells were transfected with Wnt-1 and constitutive active β-cateninSA. At 36 h post-transfection, cells were harvested and the amount of cell lysates was used for immunoprecipitation with 369 antibody. Concentrated APP C-terminal splicing forms were immunoblotted with 369 antibody. The protein expression was confirmed by anti-FLAG immunoblotting with the total cell lysates (middle panel). Tubulin was immunoblotted as loading control (lower panel).

was stimulated by Wnt-1. Accordingly, Aβ secreted in the medium was decreased (Fig. 1C) while the cytosolic AICD level was obviously increased by Wnt-1 (Fig. 1D). Consistent with these data, Wnt-1 or β-catenin(SA), a constitutive active β-catenin mutant which can not be phosphorylated by GSK3β and thus is resistant to proteasome-mediated degradation [32], led to a higher level of αCTF and AICD (Fig. 1E). Together, these results suggested that Wnt signaling enhanced α-secretase activity and increased the AICD protein level.

3.2. Wnt/β-catenin promotes transcriptional activity of AICD via interaction

AICD was reported as a transcriptional factor and its transcriptional activity could be dramatically enhanced by Fe65. The above results suggest that Wnt signaling increases AICD thus may enhance the transcriptional activity of AICD. To further explore this possibility, Gal4-AICD was cotransfected with Gal4-Luci or Gal4-TK-Luci reporter with or without Wnt signaling activation. In Gal4-TK-Luci reporter system, Gal4-AICD showed a transactivation activity, and its transcriptional activity was obvious enhanced by Wnt-1 and β-cateninSA (Fig. 2A). In the Gal4-Luci system, Gal4-AICD itself exhibited little transcriptional activity, but its transcriptional activity was also enhanced by Wnt-1 and more obviously by β-cateninSA (Fig. 2B). As β-catenin is a crucial transcriptional activator of canonical Wnt signaling pathway, we tested whether β-catenin interact with AICD. Immunoprecipitation and immunoblotting analysis revealed that exogenously expressed AICD interacted with endogenous β-catenin (Fig. 2C). All these data indicated that Wnt/β-catenin induced AICD expression thus promoted its transcriptional activity.

3.3. AICD is stabilized by Wnt/β-catenin activation

Since exogenous AICD initiated transcriptional activity was promoted by Wnt/β-catenin. Increased endogenous AICD might not due to increased γ-secretase activity. We next extend to identify this hypothesis. To this end, Myc-tagged Notch1 delta N was overexpressed with or without Wnt-1 or β-catenin SA in HEK293T cells. As shown in Fig. 3A, NICD (Notch1 intracellular domain) that was cleaved by γ-secretase from Notch1 delta N was not increased with Wnt activation. This indicated that Wnt signaling could not enhance the activity of γ-secretase. AICD has been reported to be very unstable protein, and then we asked whether AICD was stabilized by Wnt/β-catenin due to association. If this hypothesis is true, tagged AICD should also be stabilized. Indeed, exogenous AICD was stabilized by Wnt-1 or β-cateninSA (Fig. 3B). To further confirm this, AICD with or without Wnt-1 were treated with the protein synthesis inhibitor cycloheximide (CHX). As shown in Fig. 3C, Wnt-1 slowed down the degradation of AICD. Statistical analysis of the degradation rate was shown in Fig. 3D. Similar results were obtained by using β-cateninSA (data not shown). Taken together, Wnt/β-catenin induced stabilization of AICD.

3.4. Wnt/β-catenin stabilizes AICD independent of Fe65

It has been reported that adaptor protein Fe65 interacts with AICD, stabilizes it and translocates it into nucleus to form Fe65-AICD-Tip60 complex and activates transcription of downstream genes [33]. Moreover, the YENPTY sequence of AICD is important for the AICD-Fe65 interaction. To explore whether Fe65 is required for the Wnt/β-catenin-mediated stabilization of AICD, several AICD mutants with residues replaced in this sequence were constructed (Fig. 4A). These AICD mutants were coexpressed with Fe65 and their ability to interact with Fe65 was detected by immunoprecipitation. Consistent with the previous reports, T668A greatly reduced the interaction between Fe65 and AICD, and AICD (1A) and AICD (3A) completely lost the ability to interact with Fe65 (Fig. 4B). To distinguish whether Fe65 was involved in the Wnt signaling stimulated-stabilization of AICD, wild-type (WT) and AICD mutants were coexpressed with Wnt-1 or β-cateninSA. Similar with wild-type, AICD mutants were still slightly enhanced by Wnt-1 (Fig. 4C), and more obviously by β-cateninSA (Fig. 4D). In Gal4-Luci reporter assay, Fe65 greatly promoted the transactivation activity of AICD (WT) while the promoting effect is partially impaired on AICD (T668) and AICD (1A), and completely lost on AICD (Fig. 4E). However, β-catenin SA enhanced the transcriptional activity of both wild-type AICD and mutants (Fig. 4E). Taken together, these results indicated that Wnt/β-catenin stabilized AICD in a Fe65-independent manner.

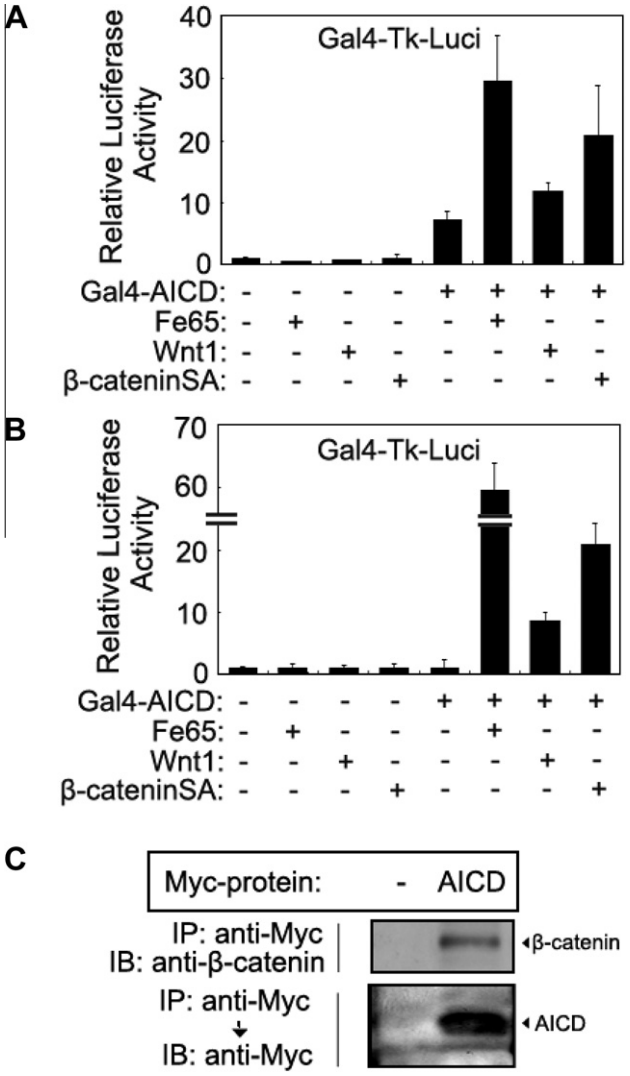

Fig. 2. Wnt/β-catenin signaling enhances the transcriptional activity of AICD, (A and B) HEK293T cells were transiently transfected with Gal4-TK-Luci (A) or Gal4-Luci reporter (B), *Renilla*, and the indicated Gal4-AICD, Fe65, Wnt-1 and β-cateninSA constructs. At 36 h post-transfection, the transfected cells were harvested for luciferase analysis. Fe65 was used as a positive control in both experiments, (C) Interaction between AICD and β-catenin. HEK293T were transiently transfected with Myc-AICD or control pcDNA vector (negative) plasmids. At 36 h post-transfection, cells were harvested for immunoprecipitation with anti-Myc antibody. AICD-associated endogenous β-catenin was revealed by anti-β-catenin immunoblotting (upper panel). The immunoprecipitated AICD was confirmed by anti-Myc immunoblotting (lower panels).

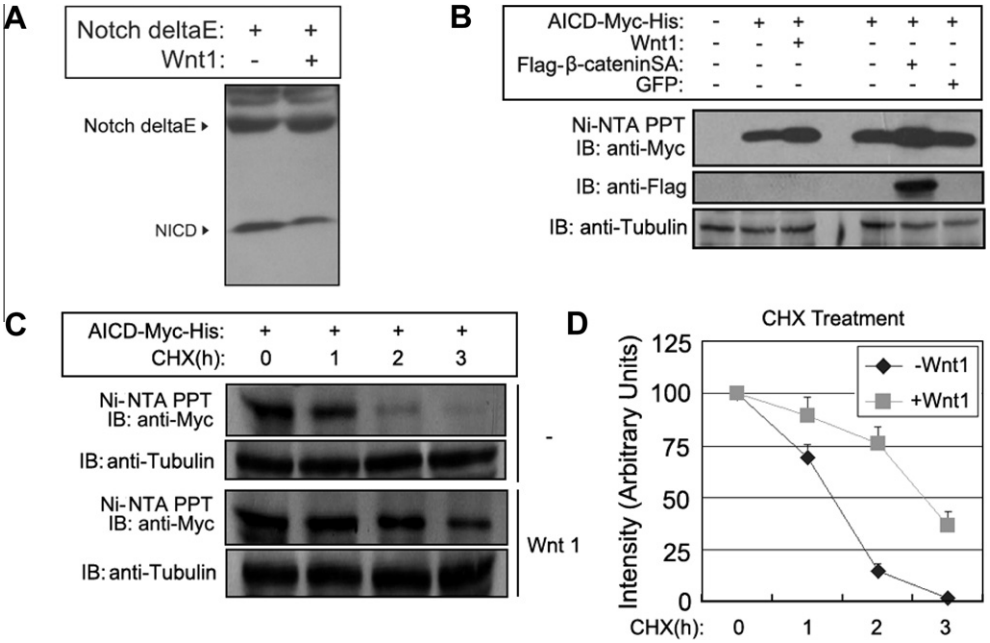

Fig. 3. Wnt/β-catenin stabilizes AICD by increasing the protein half-life, (A) HEK293T cells were cotransfected with C-terminal Myc-tagged mouse Notch1 delta N plasmid with or without Wnt-1. At 36 h post-transfection, cells were harvested for immunoblotting by anti-Myc antibody, (B) HEK293T cells were cotransfected with Myc-His-AICD, Wnt-1, FLAG-β-cateninSA or GFP constructs as indicated. At 36 h post-transfection, cells were harvested for Nickel pull-down by Ni-NTA beads and anti-Myc immunoblotting. The protein expression was confirmed by anti-FLAG from total cell lysates. Tubulin was immunoblotted as loading control (lower panel), (C and D) Myc-His-AICD overexpressed HEK293T cells were seeded in two batches of 60-mm dishes with or without Wnt1 stimulation. 12 h after stimulation, cells were treated with CHX (20 μg/ml) for 0–3 h as indicated. The same amount of cell lysis were harvested for Nickel pull-down and followed with immunoblotting with anti-Myc antibody. Protein intensity was quantitated by BCA kit and the total protein for loading was equal shown by anti-tubulin immunoblotting. The data shown are representative of three such experiments. The protein level at time 0 was set as 100% (D).

4. Discussion

AICD is being studied only in latest decade due to its great susceptibility to degradation. However, as a byproduct of APP that cleaved by γ-secretase, AICD increases along with Aβ in AD patients. AICD has been disputing since the day it was discovered. While some findings support a biologic role of AICD in regulating gene expression and cell signaling [34,35], others think it is unusable [36]. Our results here bring a new perspective to the field of AICD research, suggesting that there is interplay between AICD and Wnt/β-catenin signaling.

APP shares similar proteolysis with Notch, a key protein in Notch signal transduction pathway that controls cell fates in metazoans, which raise the intriguing possibility that APP is a cell surface receptor that also traduces signals by releasing and translocating AICD into the nucleus. Several studies have demonstrated that AICD translocated from cytoplasm to nucleus, promoted gene transcription and induced apoptosis [3,12,35]. In this study, we found that Wnt signaling leads to the accumulation of endogenous AICD in neuronal cells. Increased AICD level appears not due to γ-secretase cleavage since the Notch intracellular domain (NICD), which proved also the product of γ-secretase cleavage, was not affected. The results that exogenous AICD was also stabilized by associated Wnt/β-catenin promoted us to check the protein stability of AICD. Due to the property of very unstable, detection of AICD was proved to be difficult even in over-expressed state. In the presence of Wnt, We indeed observed increased half-life of AICD.

The fact that transcriptional factor Fe65 was identified as an AICD interacting protein suggests AICD is involved in transcriptional regulation. Recent study discovered by microarray in neuroblastoma cells that AICD up-regulates genes such as IGFBP3, TAGLN related to cytoskeletal dynamics which is critical to cell differentiation, also confirmed on neuronal cell differentiation [37]. These findings lead us to check regulation of AICD-mediated transcription. Promising evidences come from reporter gene essay that AICD promoted transactivation is enhanced by Wnt/β-catenin.

The mechanism that Wnt/β-catenin stabilized AICD may independent of Fe65. The AICD mutants lacking the Fe65 binding ability were still found to be stabilized by Wnt/β-catenin. This opens a new field of study how AICD stability is regulated besides Fe65 in cells. As AICD degradation through the lysosome pathway has been reported [38], we found similar with endogenous, exogenous AICD was accumulated in the presence of either lysosome inhibitors or proteasome inhibitors (our unpublished result). Several lysine residues in AICD appear to be involved in its great speed of degradation, awaiting further investigations.

AICD was also reported to associate with tumor suppressor gene p53 and triggers apoptosis through the p53-dependent mechanisms [39]. A recent study reported that endogenous AICD directly binds to EGFR promoter and mediates transcriptional regulation of *EGFR*, indicating an important role of PS/γ-secretase-generated APP metabolite AICD in gene transcription and in EGFR-mediated tumorigenesis [40]. Here we afford a new clue that AICD is regulated and involved in the tumorigenesis-related Wnt signaling, highlight the possible mechanism of AICD accumulation by Wnt activation and the subsequent effects on neural cell proliferation and differentiation, indicating the existence of a well-balanced AICD is important in vivo. Regulated by Wnt/β-catenin, AICD seems also modulates Wnt/β-catenin signaling as a negative regulator (our unpublished results). Thus a Wnt triggered, APP involved negative feedback loop may exist in neuronal cells, awaiting our further investigations.

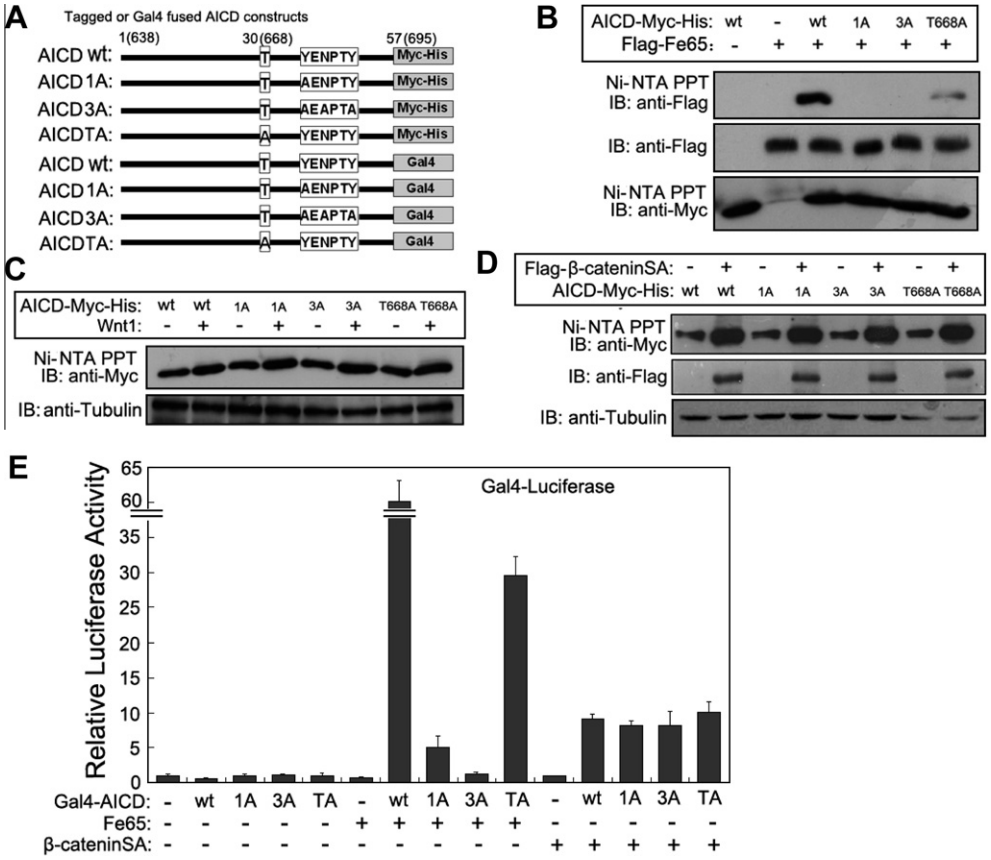

Fig. 4. Wnt/β-catenin stabilizes AICD independent of Fe65, (A) Schematic representative of AICD mutants. AICD contains a Fe65 interacting motif (YENPTY) and a critical phosphorylation site (T) in position 668 (APP695 isoform numbering), (B) Association between Fe65 and AICD mutants. HEK293T cells were transfected with FLAG-tag Fe65 and wild-type or truncation Myc-His-tag AICD mutants as indicated. At 40 h post-transfection, the cells were harvested for pull-down by Ni–NTA beads. AICD associated Fe65 was revealed by anti-FLAG immunoblotting (upper panel). The protein expression was confirmed by anti-Myc or anti-FLAG immunoblotting (middle and lower panels), (C and D), AICD mutants were stabilized by Wnt-1(C) or β-catenin (D). HEK293T cells were co-transfected with Myc-His-AICD, Wnt-1, and FLAG-β-cateninSA as indicated. At 36 h post-transfection, cells were harvested and AICD were detected by Ni–NTA beads pull-down and anti-Myc immunoblotting. The β-catenin expression was confirmed by anti-FLAG from total cell lysates. Tubulin was immunoblotted as loading control (lower panel), (E) HEK293 cells were transiently transfected with Gal4-Luci reporter, *Renilla*, and the indicated Fe65, Wnt1, β-cateninSA and Gal4-AICD mutant constructs. At 36 h post-transfection, the transfected cells were harvested for luciferase analysis.

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References

[1] J. Kang, H.G. Lemaire, A. Unterbeck, J.M. Salbaum, C.L. Masters, K.H. Grzeschik, G. Multhaup, K. Beyreuther, B. Muller-Hill, The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor, Nature 325 (1987) 733–736.

[2] W.P. Esler, M.S. Wolfe, A portrait of Alzheimer secretases–new features and familiar faces, Science 293 (2001) 1449–1454.

[3] W.T. Kimberly, J.B. Zheng, S.Y. Guenette, D.J. Selkoe, The intracellular domain of the beta-amyloid precursor protein is stabilized by Fe65 and translocates to the nucleus in a notch-like manner, Journal of Biological Chemistry 276 (2001) 40288–40292.

[4] J.P. Borg, J. Ooi, E. Levy, B. Margolis, The phosphotyrosine interaction domains of X11 and FE65 bind to distinct sites on the YENPTY motif of amyloid precursor protein, Molecular and Cellular Biology 16 (1996) 6229–6241.

[5] F. Fiore, N. Zambrano, G. Minopoli, V. Donini, A. Duilio, T. Russo, The regions of the Fe65 protein homologous to the phosphotyrosine interaction/phosphotyrosine binding domain of Shc bind the intracellular domain of the Alzheimer's amyloid precursor protein, Journal of Biological Chemistry 270 (1995) 30853–30856.

[6] T. Watanabe, J. Sukegawa, I. Sukegawa, S. Tomita, K. Iijima, S. Oguchi, T. Suzuki, A.C. Nairn, P. Greengard, A 127-kDa protein (UV-DDB) binds to the cytoplasmic domain of the Alzheimer's amyloid precursor protein, Journal of Neurochemistry 72 (1999) 549–556.

[7] N. Zambrano, J.D. Buxbaum, G. Minopoli, F. Fiore, P. De Candia, S. De Renzis, R. Faraonio, S. Sabo, J. Cheetham, M. Sudol, T. Russo, Interaction of the phosphotyrosine interaction/phosphotyrosine binding-related domains of Fe65 with wild-type and mutant Alzheimer's beta-amyloid precursor proteins, Journal of Biological Chemistry 272 (1997) 6399–6405.

[8] N. Chow, J.R. Korenberg, X.N. Chen, R.L. Neve, APP-BP1, a novel protein that binds to the carboxyl-terminal region of the amyloid precursor protein, Journal of Biological Chemistry 271 (1996) 11339–11346.

[9] M.H. Scheinfeld, R. Roncarati, P. Vito, P.A. Lopez, M. Abdallah, L. D'Adamio, Jun NH2-terminal kinase (JNK) interacting protein 1 (JIP1) binds the cytoplasmic domain of the Alzheimer's beta-amyloid precursor protein (APP), Journal of Biological Chemistry 277 (2002) 3767–3775.

[10] L. Pastorino, A. Sun, P.J. Lu, X.Z. Zhou, M. Balastik, G. Finn, G. Wulf, J. Lim, S.H. Li, X. Li, W. Xia, L.K. Nicholson, K.P. Lu, The prolyl isomerase Pin1 regulates amyloid precursor protein processing and amyloid-beta production, Nature 440 (2006) 528–534.

[11] R.C. von Rotz, B.M. Kohli, J. Bosset, M. Meier, T. Suzuki, R.M. Nitsch, U. Konietzko, The APP intracellular domain forms nuclear multiprotein complexes and regulates the transcription of its own precursor, Journal of Cell Science 117 (2004) 4435–4448.

[12] K.A. Chang, H.S. Kim, T.Y. Ha, J.W. Ha, K.Y. Shin, Y.H. Jeong, J.P. Lee, C.H. Park, S. Kim, T.K. Baik, Y.H. Suh, Phosphorylation of amyloid precursor protein (APP) at Thr668 regulates the nuclear translocation of the APP intracellular domain and induces neurodegeneration, Molecular and Cellular Biology 26 (2006) 4327–4338.

[13] W.T. Kimberly, J.B. Zheng, T. Town, R.A. Flavell, D.J. Selkoe, Physiological regulation of the beta-amyloid precursor protein signaling domain by c-Jun N-terminal kinase JNK3 during neuronal differentiation, Journal of Neuroscience 25 (2005) 5533–5543.

[14] D.C. Lu, S. Rabizadeh, S. Chandra, R.F. Shayya, L.M. Ellerby, X. Ye, G.S. Salvesen, E.H. Koo, D.E. Bredesen, A second cytotoxic proteolytic peptide derived from amyloid beta-protein precursor, Nature Medicine 6 (2000) 397–404.

[15] T. Reya, H. Clevers, Wnt signalling in stem cells and cancer, Nature 434 (2005) 843–850.

[16] G.W. Yardy, S.F. Brewster, Wnt signalling and prostate cancer, Prostate Cancer Prostatic Disease 8 (2005) 119–126.
[17] H. Clevers, Wnt/beta-catenin signaling in development and disease, Cell 127 (2006) 469–480.
[18] W. Ching, R. Nusse, A dedicated Wnt secretion factor, Cell 125 (2006) 432–433.
[19] W.H. Stoothoff, C.D. Bailey, K. Mi, S.C. Lin, G.V. Johnson, Axin negatively affects tau phosphorylation by glycogen synthase kinase 3beta, Journal of Neurochemistry 83 (2002) 904–913.
[20] G.R. Jackson, M. Wiedau-Pazos, T.K. Sang, N. Wagle, C.A. Brown, S. Massachi, D.H. Geschwind, Human wild-type tau interacts with wingless pathway components and produces neurofibrillary pathology in Drosophila, Neuron 34 (2002) 509–519.
[21] K.A. Ryan, S.W. Pimplikar, Activation of GSK-3 and phosphorylation of CRMP2 in transgenic mice expressing APP intracellular domain, Journal of Cell Biology 171 (2005) 327–335.
[22] A. Mudher, S. Chapman, J. Richardson, A. Asuni, G. Gibb, C. Pollard, R. Killick, T. Iqbal, L. Raymond, I. Varndell, P. Sheppard, A. Makoff, E. Gower, P.E. Soden, P. Lewis, M. Murphy, T.E. Golde, H.T. Rupniak, B.H. Anderton, S. Lovestone, Dishevelled regulates the metabolism of amyloid precursor protein via protein kinase C/mitogen-activated protein kinase and c-Jun terminal kinase, Journal of Neuroscience 21 (2001) 4987–4995.
[23] L. Zhang, X. Gao, J. Wen, Y. Ning, Y.G. Chen, Dapper 1 antagonizes Wnt signaling by promoting dishevelled degradation, Journal of Biological Chemistry 281 (2006) 8607–8612.
[24] W. Huang, H.Y. Chang, T. Fei, H. Wu, Y.G. Chen, GSK3 beta mediates suppression of cyclin D2 expression by tumor suppressor PTEN, Oncogene 26 (2007) 2471–2482.
[25] E.H. Schroeter, J.A. Kisslinger, R. Kopan, Notch-1 signalling requires ligand-induced proteolytic release of intracellular domain, Nature 393 (1998) 382–386.
[26] J.D. Buxbaum, S.E. Gandy, P. Cicchetti, M.E. Ehrlich, A.J. Czernik, R.P. Fracasso, T.V. Ramabhadran, A.J. Unterbeck, P. Greengard, Processing of Alzheimer beta/A4 amyloid precursor prote: modulation by agents that regulate protein phosphorylation, Proceedings of the National Academy of Sciences of the United States of America 87 (1990) 6003–6006.
[27] L. Zhang, H. Zhou, Y. Su, Z. Sun, H. Zhang, Y. Zhang, Y. Ning, Y.G. Chen, A. Meng, Zebrafish Dpr2 inhibits mesoderm induction by promoting degradation of nodal receptors, Science 306 (2004) 114–117.
[28] L. Zhang, F. Zhou, T. van Laar, J. Zhang, H. van Dam, P. Ten Dijke, Fas-associated factor 1 Antagonizes Wnt Signaling by Promoting {beta}-catenin Degradation, Mol Biol Cell 22 (2011) 1617–1624.
[29] H. Xu, G.K. Gouras, J.P. Greenfield, B. Vincent, J. Naslund, L. Mazzarelli, G. Fried, J.N. Jovanovic, M. Seeger, N.R. Relkin, F. Liao, F. Checler, J.D. Buxbaum, B.T. Chait, G. Thinakaran, S.S. Sisodia, R. Wang, P. Greengard, S. Gandy, Estrogen reduces neuronal generation of Alzheimer beta-amyloid peptides, Nature Medicine 4 (1998) 447–451.
[30] F. Zhou, L. Zhang, A. Wang, B. Song, K. Gong, M. Hu, X. Zhang, N. Zhao, Y. Gong, The association of GSK3 beta with E2F1 facilitates nerve growth factor-induced neural cell differentiation, Journal of Biological Chemistry 283 (2008) 14506–14515.
[31] G.K. Gouras, H. Xu, R.S. Gross, J.P. Greenfield, B. Hai, R. Wang, P. Greengard, Testosterone reduces neuronal secretion of Alzheimer's beta-amyloid peptides, Proceedings of the National Academy of Sciences of the United States of America 97 (2000) 1202–1205.
[32] M. Kitagawa, S. Hatakeyama, M. Shirane, M. Matsumoto, N. Ishida, K. Hattori, I. Nakamichi, A. Kikuchi, K. Nakayama, K. Nakayama, An F-box protein, FWD1, mediates ubiquitin-dependent proteolysis of beta-catenin, EMBO Journal 18 (1999) 2401–2410.
[33] X. Cao, T.C. Sudhof, A transcriptionally [correction of transcriptively] active complex of APP with Fe65 and histone acetyltransferase Tip60, Science 293 (2001) 115–120.
[34] S.H. Baek, K.A. Ohgi, D.W. Rose, E.H. Koo, C.K. Glass, M.G. Rosenfeld, Exchange of N-CoR corepressor and Tip60 coactivator complexes links gene expression by NF-kappaB and beta-amyloid precursor protein, Cell 110 (2002) 55–67.
[35] H.S. Kim, E.M. Kim, J.P. Lee, C.H. Park, S. Kim, J.H. Seo, K.A. Chang, E. Yu, S.J. Jeong, Y.H. Chong, Y.H. Suh, C-terminal fragments of amyloid precursor protein exert neurotoxicity by inducing glycogen synthase kinase-3beta expression, FASEB Journal 17 (2003) 1951–1953.
[36] S.S. Hebert, L. Serneels, A. Tolia, K. Craessaerts, C. Derks, M.A. Filippov, U. Muller, B. De Strooper, Regulated intramembrane proteolysis of amyloid precursor protein and regulation of expression of putative target genes, EMBO Reports 7 (2006) 739–745.
[37] T. Muller, C.G. Concannon, M.W. Ward, C.M. Walsh, A.L. Tirniceriu, F. Tribl, D. Kogel, J.H. Prehn, R. Egensperger, Modulation of gene expression and cytoskeletal dynamics by the amyloid precursor protein intracellular domain (AICD), Molecular Biology of the Cell 18 (2007) 201–210.
[38] V. Vingtdeux, M. Hamdane, S. Begard, A. Loyens, A. Delacourte, J.C. Beauvillain, L. Buee, P. Marambaud, N. Sergeant, Intracellular pH regulates amyloid precursor protein intracellular domain accumulation, Neurobiology Diseases 25 (2007) 686–696.
[39] T. Ozaki, Y. Li, H. Kikuchi, T. Tomita, T. Iwatsubo, A. Nakagawara, The intracellular domain of the amyloid precursor protein (AICD) enhances the p53-mediated apoptosis, Biochemical and Biophysical Research Communications 351 (2006) 57–63.
[40] Y.W. Zhang, R. Wang, Q. Liu, H. Zhang, F.F. Liao, H. Xu, Presenilin/gamma-secretase-dependent processing of beta-amyloid precursor protein regulates EGF receptor expression, Proceedings of the National Academy of Sciences of the United States of America 104 (2007) 10613–10618.