The Cytosolic Loop of the γ-Secretase Component Presenilin Enhancer 2 Protects Zebrafish Embryos from Apoptosis

Henrik Zetterberg, William A. Campbell, Hong Wei Yang, and Weiming Xia

From the Center for Neurologic Diseases, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts 02115

The γ-secretase complex, composed of presenilin, presenilin enhancer 2 (Pen-2), nicastrin, and Aph-1, catalyzes the final cleavage of amyloid precursor protein to generate the toxic amyloid β protein, the major component of plaques in the brains of Alzheimer disease patients. To understand the in vivo function of Pen-2, we used morphant technology available in zebrafish and transiently knocked down the expression of endogenous Pen-2 by injecting the morpholino (MO) against Pen-2. Two truncated Pen-2 proteins lacking either the cytosolic or the C-terminal domain were expressed in MO-injected embryos. This deletion analysis demonstrated that the Pen-2 cytosolic loop is essential for protecting developing embryos from caspase-dependent apoptosis caused by the reduction of Pen-2. Twelve amino acids in the C terminus of Pen-2 were dispensable and could not rescue the Pen-2 knockdown-induced apoptotic phenotype. Surprisingly, double knockdown of Pen-2 and nuclear factor κB component p65 abrogated the single Pen-2 MO-induced caspase activation, indicating that a previously reported pro-apoptotic role of NF-κB in some cell types could be manifested in a whole animal and that knockdown of Pen-2 may trigger pro-apoptotic activation of NF-κB.

Alzheimer disease is characterized by the premature death of cholinergic neurons in the hippocampus and the frontal cortex of the human brain. The reason for the neuronal death is unknown, but biochemical, genetic, and pathological data suggest that increased production of β-amyloid (Aβ) plays an important and possibly pathogenic role in the disease process (1, 2). Aβ is generated from amyloid precursor protein (APP), a type I integral membrane protein with one transmembrane domain, by enzymatic digestion involving β- and γ-secretase activities (3). Cleavage of APP by β-secretase generates an ~100-kDa soluble N-terminal fragment and a 12-kDa C-terminal fragment (C99), which can be further cleaved by γ-secretase to yield the APP intracellular domain and 40- and 42-amino acid-long Aβ peptides (Aβ40 and Aβ42), the latter of which appears the most prone to aggregate (the amyloidogenic pathway) (4). γ-Secretase is a membrane-bound protease complex consisting of at least four essential components: the homologous presenilins 1 and 2, nicastrin, Aph-1, and Pen-2 (5–13). Besides its role in APP processing, γ-secretase can also modulate the proteolytic cleavage of the Notch receptor (6). The Notch signaling pathway mediates a wide range of developmental cell fate decisions. In response to ligand binding, Notch receptors undergo an extracellular cleavage at a site near the membrane, generating a membrane-tethered C-terminal domain. Subsequent cleavage by γ-secretase at a site within the transmembrane domain releases the Notch intracellular domain that translocates to the nucleus where it associates with RBP-Jκ and other transcription factors to regulate the promoters of specific target genes of the pathway (14–16). In addition to APP and Notch, γ-secretase also catalyzes the proteolytic release of the intracellular domains of numerous other type I transmembrane receptors, underscoring its complex role in cell signaling and development (17, 18).

Pen-2 is a small, hairpin-like membrane protein of 101 amino acids with two transmembrane domains and one cytosolic loop region (see Fig. 1A) (13). It is highly conserved, and 76% of its amino acids are identical when comparing zebrafish and human sequences (13). Pen-2 interacts with presenilin (12), and depletion of Pen-2 by RNA interference prevents endoproteolysis of presenilin and abrogates γ-secretase activity in both Drosophila and mammalian cells, including primary neurons (7, 12). When these cells were treated with an apoptotic stimulus, caspase-3 activity correlated with levels of full-length presenilin 1 (19). The C terminus of Pen-2 is critical for the functional γ-secretase complex in cultured cells and is essential for γ-secretase-mediated cleavage of Notch and APP (20–23).

Activation of caspase-3, a member of the evolutionary conserved cysteine-aspartate-specific protease family, leads to caspase-dependent apoptosis (24, 25), and this also occurs in zebrafish (26). Under most circumstances, two transcription factors, p53 and nuclear factor κB (NF-κB) play opposite roles in promoting apoptosis. Activation of p53 enhances cell cycle arrest and apoptosis, and it is mutated in more than 50% of human tumors (27–29). Activation of NF-κB, on the other hand, usually leads to the expression of anti-apoptotic genes, like caspase-8/FADD-like interleukin-1β-converting enzyme inhibitor protein or inhibitors of apoptosis. In mammalian cells, depending on the specific cell type and the type of inducer, NF-κB can also promote apoptosis under certain conditions (30). The NF-κB transcription factor family includes several structurally related proteins, and in zebrafish, three NF-κB molecules, p65, c-Rel, and p100, have been identified (31).

Knocking down p65 and p100 caused defective posterior morphogenesis, resulting in mutant embryos with a short trunk and tail. Recent studies have demonstrated that the NF-κB subunit p65 can sequester p53 in an inactive complex, thereby inhibiting p53-stimulated apoptosis (32, 33). We have found that selective knockdown of Pen-2 in developing zebrafish embryos induced a strong p53-dependent apoptotic cascade throughout the whole animal (34). However, it was not clear whether NF-κB/p65 was involved in the Pen-2 knockdown-elicited apoptotic pathway.
Cytosolic Loop of Pen-2 Prevents Apoptosis

To dissect the molecular property of Pen-2 that plays an important and specific role in promoting cell survival and protecting cells from apoptosis in vivo, we generated constructs expressing truncated Pen-2 molecules lacking either the cytosolic or the C-terminal domain. We found that the cytosolic loop of Pen-2 harbors an anti-apoptotic activity and that the C-terminal 12 amino acids are dispensable for the anti-apoptotic activity of Pen-2. Importantly, knocking down Pen-2 appeared to trigger an unconventional pro-apoptotic activity of NF-κB in zebrafish, because double knockdown of Pen-2 and p65 suppressed caspase activation found in single Pen-2 knockout embryos.

MATERIALS AND METHODS

Fish Strains—Embryos were obtained from natural spawning of wild-type (Tübingen long fin strain) adults; they were raised and staged according to Kimmel et al. (35).

Cell Culture, Transient Transfections, and Western Blot—The standard biochemical procedures have been described previously (36).

Antisense Morpholino Injection—Pen-2 MO directed against the translation start codon of the Pen-2 mRNA (Gene Tools, Corvallis, OR) was injected into fertilized zebrafish eggs at the one-cell stage. The translation start codon of the Pen-2 mRNA (Gene Tools, Corvallis, OR) and subcloned into pCS2

RESULTS

We injected zebrafish embryos at the one-cell stage with an antisense modified oligonucleotide (morpholino) directed against the 5’ end of the first exon of the Pen-2 gene. The embryos were harvested at 24 h post fertilization (hpf) and lysed for Western blot with an antibody against zebrafish Pen-2. Compared with un.injected embryos or embryos injected with a control MO, Pen-2 MO clearly reduced the expression of Pen-2 (Fig. 1B). Injection of control MO against β-globin intron did not affect the expression level of endogenous Pen-2, which was identical to that from un.injected embryos.

We analyzed two phenotypic characteristics of Pen-2 MO-injected embryos, an oocyte region in the head and a curved tail (Table 1 and Fig. 2B). Previous studies have shown that γ-secretase inhibitor DAPT-treated zebrafish show defects in somitogenesis and defective anteroposterior polarity (40). Upon DAPT treatment, most posterior somites do not form normally, resulting in a short or curled tail. This phenotype strikingly resembles zebrafish Notch pathway mutants like beanter (bea), deadly-seven (des), after-eight (aet), and white-tail (wit), which
can be rescued by microinjection of notch intracellular domain. Therefore, the short or curved tail in Pen-2 MO-injected embryos was the phenotype related to impaired γ-secretase-mediated Notch signaling.

The reduction of Pen-2 protein in Pen-2 MO-injected embryos also led to large opaque regions primarily located in the head of the fish (Fig. 2B, left panel), in contrast to control MO-injected embryos (Fig. 2A, left panel). These opaque regions were most likely due to massive cell death, accompanied by precipitation of proteins. To determine whether apoptosis contributed to these opaque regions, we used the vital dye, AO, to stain these live embryos. AO staining verified a significant degree of cell death throughout the embryos (Fig. 2B, center panel) and abundantly in the head (Fig. 2B, right panel). This is consistent with our previous finding that knockdown of Pen-2 results in extensive TUNEL-positive staining in the whole animal (34). Co-injection of in vitro transcribed mRNA encoding full-length Pen-2 along with Pen-2 MO rescued this phenotype (Fig. 2C), and over 85% of embryos showed normal phenotypes similar to those of control MO injected embryos, compared with only 4% of Pen-2 MO-injected embryos showing normal phenotypes (Table 1).

To determine which domain of Pen-2 harbors the anti-apoptotic activity, two deletion constructs were made, one containing a deletion of amino acids 40–54 in the cytosolic loop region of Pen-2 (Pen-2ΔL) and another containing a deletion of the C-terminal amino acids 90–101 (Pen-2ΔC) (Fig. 1A). The deletions did not change the topology of Pen-2, because the two-transmembrane domain structure of truncated Pen-2 still existed, according to the prediction using the dense alignment surface in silico method (41) (Fig. 3A). The truncated Pen-2 constructs were transiently transfected into HEK-293 cells, and the cells were lysed for Western blotting. Staining with antibody against the FLAG tag clearly showed different sizes of full-length and truncated Pen-2 molecules, in contrast to a lack of staining in untransfected cells (Fig. 3B, left panel). To determine whether the truncated Pen-2 could be stably expressed in zebrafish embryos, we injected capped RNA along with Pen-2 MO into embryos and lysed embryos at 24 hpf for Western blotting. Because the MO was designed to encompass the upstream of the ATG codon, as well as the codon itself, the FLAG-tagged Pen-2 RNA had no overlap with the MO. Thus, Pen-2 MO did not target injected Pen-2 RNA and affect the expression levels of Pen-2ΔL and Pen-2ΔC. Although Pen-2 MO significantly reduced endogenous Pen-2

![FIGURE 1. Construction of truncated zebrafish Pen-2 and knockdown of endogenous full-length Pen-2.](image)

**TABLE 1**

| MO/mRNA          | Total | Opaque region in head and curved tail | Curved tail only; no opaque region in head | Normal |
|------------------|-------|-------------------------------------|---------------------------------------------|--------|
| Uninjected       | 99    | 0 (0%)                              | 10 (10%)                                   | 89 (90%) |
| Control MO       | 107   | 0 (0%)                              | 8 (7%)                                     | 99 (93%) |
| Pen-2 MO         | 219   | 201 (92%)                           | 9 (4%)                                     | 9 (4%)  |
| Pen-2 MO/FL mRNA| 176   | 7 (4%)                              | 19 (11%)                                   | 150 (85%)|
| Pen-2 MO/ΔL mRNA| 205   | 174 (85%)                           | 17 (8%)                                    | 14 (7%) |
| Pen-2 MO/ΔC mRNA| 154   | 24 (16%)                            | 115 (75%)                                  | 15 (9%) |
Cytosolic Loop of Pen-2 Prevents Apoptosis

levels, compared with control MO-injected embryos, exogenous FLAG-tagged full-length Pen-2 and Pen-2ΔL were expressed at high levels (Fig. 3B, right panel). Even Pen-2ΔC was expressed at moderate levels, indicating that the truncated Pen-2 variants were stable and could be easily detected by Western blotting of lysates prepared from injected embryos (Fig. 3B).

We examined the Pen-2 MO/Pen-2 RNA co-injected embryos by AO staining. Co-injection of in vitro transcribed mRNA encoding Pen-2ΔL failed to rescue the apoptotic phenotype, resulting in a phenotype indistinguishable from that obtained by injection of Pen-2 MO alone (Fig. 2D). The majority of embryos (85%) showed opaque regions in the head and a curved tail, a phenotype represented by almost all Pen-2 MO-injected embryos (92%) (Table 1). A large number of apoptotic cells corresponded to the opaque areas in the brain region (Fig. 2D). On the contrary, co-injection of in vitro transcribed mRNA encoding Pen-2ΔC rescued the apoptotic phenotype. Only a small percentage of embryos still showed opaque regions in the head (16%), and almost no apoptotic cells were detected in Pen-2ΔC-injected embryos (Fig. 2E).

The morphology of rescued embryos was different from that of wild-type embryos (Fig. 2E). Consistent with previous findings that Pen-2ΔC is not functional in the γ-secretase complex (20–23), embryos expressing Pen-2ΔC had a curved tail, a phenotype similar to that seen in zebrafish embryos treated with the γ-secretase inhibitor DAPT (40). Apparently, Pen-2ΔC could not rescue the phenotype caused by the lack of a functional γ-secretase complex in Pen-2 knockdown embryos, and 75% of the embryos carried curved tails (Table 1). However, expression of Pen-2ΔC prevented embryos from undergoing apoptosis, because the opaque regions were no longer observed in 84% of the embryos injected with Pen-2 MO and Pen-2ΔC mRNA (Table 1).

To verify that the opaque regions in the head represented excessive apoptosis, we established a biochemical measurement of apoptosis in whole animals by optimizing a method to determine caspase-3 activity in zebrafish embryos (Fig. 4). Treatment of embryos with staurosporine, a potent inducer of apoptosis (38), induced a significant caspase-3 activation compared with embryos treated with an equal volume of the carrier (Me2SO). Injection of embryos with Pen-2 MO alone resulted in an even stronger activation of caspase-3. This activation could be completely abrogated by co-injecting the Pen-2 MO along with capped mRNA encoding full-length Pen-2. However, co-injection of Pen-2ΔL mRNA could not inhibit the activation of caspase-3, consistent with the earlier finding that it failed to rescue the apoptotic phenotype induced by Pen-2 knockdown (Fig. 2D). Co-injection of Pen-2ΔC mRNA completely suppressed the caspase-3 activation. Although the 12 amino acids deleted in Pen-2ΔC have been shown to be critical for the stabilization of presenilin fragments and restoration of γ-secretase activity (20–23), this region was not critical for protecting embryos from undergoing apoptosis (Fig. 4).

We further examined the effect of reduced γ-secretase activity on inducing apoptosis by analyzing the embryos for TUNEL-positive cells. In 1% Me2SO-treated embryos at 24 hpf, we observed a few scattered red dots that represent the end labeling of DNA occurring in apoptotic cells (Fig. 5A). In contrast, embryos injected with Pen-2 MO had a massive increase in the number of TUNEL-positive cells, consistent with the AO staining pattern (Fig. 2B).

Next, we used the previously reported γ-secretase inhibitor DAPT to treat embryos. We found that blocking γ-secretase activity with 100 μM DAPT did not result in extensive apoptosis in treated embryos (Fig. 5C). Compared with Me2SO-treated embryos, only a few more TUNEL-positive cells were found in DAPT-treated embryos, which was significantly less than what was observed in embryos injected with Pen-2 MO.

Because our recent studies demonstrated a p53-dependent apoptotic cascade elicited by Pen-2 knockdown (34), we further tested the potential involvement of NF-κB signaling in this cascade. This was based on recent studies showing that the NF-κB subunit p65 can sequester p53 in an inactive complex, thereby inhibiting p53-stimulated apoptosis (32, 33). Because the Pen-2-induced apoptotic cascade was dependent on p53 (34), it is possible that apoptosis caused by Pen-2 knockdown may relate to p65. We used a published MO sequence against p65 and reproduced the previously described phenotypic characteristics of zebrafish lacking p65 (31), including disturbed posterior body formation with a
Cytosolic Loop of Pen-2 Prevents Apoptosis

DISCUSSION

Taking advantage of zebrafish as an excellent model system to study the neuronal function of complex proteins (42), we have expressed truncated mutants of the γ-secretase component Pen-2 in zebrafish embryos knocked down of endogenous Pen-2 and defined the functional, anti-apoptotic domain of Pen-2. We show that the anti-apoptotic activity of Pen-2 resides within 15 amino acids in the cytosolic loop of Pen-2. The C terminus of Pen-2, although essential for γ-secretase activity, was dispensable for suppressing apoptosis. Embryos expressing Pen-2ΔC displayed the characteristics of Notch signaling deficiency (e.g. curved tail), similar to embryos treated with the potent γ-secretase inhibitor DAPT (40). However, the Pen-2ΔC mutant could rescue the apoptotic phenotype of Pen-2 knockdown embryos and completely inhibit caspase-3 activation. Almost no opaque regions were found in Pen-2ΔC-expressing embryos. Taken together, these data suggest that the anti-apoptotic activity represents a novel function of Pen-2 in addition to its well documented function as an essential, stabilizing component of the γ-secretase complex to cleave APP and Notch.

Although our results demonstrated that reduction of γ-secretase activity was not the sole cause for the apoptotic phenotype associated with Pen-2 knockdown embryos, we cannot rule out the possibility that the involvement of Pen-2 in an apoptotic pathway may be associated with Pen-2 knockdown embryos. It is possible that reduced γ-secretase-mediated cleavage of these substrates may affect their involvement in inducing/preventing apoptosis, thereby causing the apoptotic phenotype in zebrafish lacking endogenous Pen-2. Because Nicastrin has been shown to play a critical role in substrate recognition (43), it is possible that the cytosolic loop of Pen-2 plays a similar role and interacts with certain γ-secretase substrate(s) with an anti-apoptotic function.

Analysis of Pen-2ΔL/ΔC-expressing embryos by Western blot indicated that the levels of Pen-2ΔL were usually higher than the levels of Pen-2ΔC. Nevertheless, large amounts of Pen-2ΔL failed to suppress the apoptosis phenotype in zebrafish lacking endogenous Pen-2. Because Pen-2ΔL is observed to be active as a component of γ-secretase (40), it is possible that the cytosolic loop of Pen-2 prevents apoptosis by interacting with certain substrates, thereby affecting their involvement in inducing/preventing apoptosis. It is possible that the cytosolic loop of Pen-2 prevents apoptosis by interacting with certain substrates, thereby affecting their involvement in inducing/preventing apoptosis.

shorter tail (Fig. 6A). Knockdown of p65 did not affect Pen-2 expression (data not shown), and embryos co-injected with Pen-2 and p65 MO demonstrated a phenotype similar to that observed in embryos injected with single p65 MO (Fig. 6A). We further examined the levels of caspase-3 activity in embryos injected with p65 MO. Although injection of Pen-2 MO activated caspase-3, co-injection of p65 MO suppressed its activation to almost basal levels (Fig. 6B). Our longitudinal measurements of cleaved caspase-3 substrate accumulation excluded the risk that the apparent reduction of caspase-3 activity was due to the consumption of substrate. Taken together, these results indicate that knockdown of the NF-κB subunit p65 could rescue the apoptotic cascade elicited by knockdown of Pen-2 expression.

Taking advantage of zebrafish as an excellent model system to study the neuronal function of complex proteins (42), we have expressed truncated mutants of the γ-secretase component Pen-2 in zebrafish embryos knocked down of endogenous Pen-2 and defined the functional, anti-apoptotic domain of Pen-2. We show that the anti-apoptotic activity of Pen-2 resides within 15 amino acids in the cytosolic loop of Pen-2. The C terminus of Pen-2, although essential for γ-secretase activity, was dispensable for suppressing apoptosis. Embryos expressing Pen-2ΔC displayed the characteristics of Notch signaling deficiency (e.g. curved tail), similar to embryos treated with the potent γ-secretase inhibitor DAPT (40). However, the Pen-2ΔC mutant could rescue the apoptotic phenotype of Pen-2 knockdown embryos and completely inhibit caspase-3 activation. Almost no opaque regions were found in Pen-2ΔC-expressing embryos. Taken together, these data suggest that the anti-apoptotic activity represents a novel function of Pen-2 in addition to its well documented function as an essential, stabilizing component of the γ-secretase complex to cleave APP and Notch.

Although our results demonstrated that reduction of γ-secretase activity was not the sole cause for the apoptotic phenotype associated with Pen-2 knockdown embryos, we cannot rule out the possibility that the involvement of Pen-2 in an apoptotic pathway may be associated with γ-secretase-mediated cleavage of certain substrates, especially in light of the fact that new γ-secretase substrates are continuously being identified. Among all known γ-secretase substrates, some directly contribute to apoptotic pathways, and others are related to the transcriptional activation of genes involved in apoptotic pathways. It is possible that reduced γ-secretase-mediated cleavage of these substrates may affect their involvement in inducing/preventing apoptosis, thereby causing the apoptotic phenotype in zebrafish lacking endogenous Pen-2. Because Nicastrin has been shown to play a critical role in substrate recognition (43), it is possible that the cytosolic loop of Pen-2 plays a similar role and interacts with certain γ-secretase substrate(s) with an anti-apoptotic function.

Analysis of Pen-2ΔL/ΔC-expressing embryos by Western blot indicated that the levels of Pen-2ΔL were usually higher than the levels of Pen-2ΔC. Nevertheless, large amounts of Pen-2ΔL failed to suppress the apoptosis phenotype in zebrafish lacking endogenous Pen-2. Because Pen-2ΔL is observed to be active as a component of γ-secretase (40), it is possible that the cytosolic loop of Pen-2 prevents apoptosis by interacting with certain substrates, thereby affecting their involvement in inducing/preventing apoptosis. It is possible that the cytosolic loop of Pen-2 prevents apoptosis by interacting with certain substrates, thereby affecting their involvement in inducing/preventing apoptosis.
the activation of caspase-3, whereas small amounts of Pen-Δ2C were capable of preventing cells from undergoing apoptosis. Therefore, the reduced Pen-2ΔL activity in preventing cell death was not due to the expression levels of the truncated Pen-2 protein; instead, it was related to the independent function associated with two different domains in Pen-2, i.e. the loop region and the C terminus.

We have found that knockdown of Pen-2 induced a strong p53-dependent apoptotic cascade throughout the whole animal (34). Because p53-dependent apoptosis can be suppressed by NF-κB/p65 through sequestering of p53 in an inactive complex (32, 33), we examined the involvement of NF-κB/p65 in the Pen-2 knockdown-induced apoptotic pathway. We found that co-knockdown of p65 and Pen-2 rescued the apoptotic phenotype and inhibited caspase-3 activation, suggesting a pro-apoptotic activation of NF-κB by Pen-2 knockdown. This result is consistent with findings from a number of studies showing a pro-apoptotic role of activated NF-κB. First, two NF-κB-binding sites have been identified in the promoter of Fas ligand (FasL) (44), and expression of FasL critically depends on these two NF-κB-binding sites (45). Because FasL delivers a signal that induces apoptosis once it binds to Fas, NF-κB may promote apoptosis by directly binding to the promoter of FasL and up-regulating FasL gene expression. Indeed, mutations in the NF-κB-binding sites block the response of FasL promoter to DNA damage, and activation of NF-κB leads to the expression of FasL and apoptosis induced by etoposide, teniposide, and UV irradiation (46). Second, reovirus infection of HeLa cells causes activation and nuclear translocation of NF-κB and apoptosis, and mutant cell lines deficient in the p65 subunit of NF-κB are resistant to reovirus-induced apoptosis (47). Third, overexpression of p65 increases levels of the receptor of tumor necrosis factor-related apoptosis inducing ligand (TRAIL) and promotes TRAIL-induced apoptosis. Blockage of NF-κB activation eliminates the sensitivity of cells to TRAIL or etoposide-induced apoptosis (48). Fourth, activation of the NF-κB signaling pathway is required for T-cell activation and apoptosis through TRAIL expression, which is induced by Tax oncoprotein (encoded by human T-cell leukemia virus) (49). Therefore, results from these studies and our analysis in whole animals support a pro-apoptotic role for activated NF-κB.

Many studies have also demonstrated a major anti-apoptotic role of activated NF-κB (30). At least in cultured cells, the dual roles of NF-κB could be in part explained by competing roles of its two subunits, p65/RelA and c-Rel. In TRAIL signaling, the c-Rel subunit inhibits expression of the receptors for TRAIL, but activation of the p65/RelA subunit increases expression of the apoptosis inhibitor, Bcl-XL (50). Depletion of c-Rel in mouse embryonic fibroblasts blocks cytokine-induced apoptosis, whereas depletion of p65/RelA increases apoptosis (51). In p65 knockdown zebrafish embryos (31), it is not clear whether there is an alteration in cell survival. Therefore, it is interesting to determine whether zebrafish exhibit dual roles of NF-κB upon activation.

In conclusion, the cytosolic loop of Pen-2 is essential for rescuing the apoptotic phenotype in Pen-2 knockdown zebrafish. This finding is critical for us to understand the anti-apoptotic function of Pen-2 at the molecular level during embryonic development. Its significance will be illustrated in future studies of apoptosis relevant γ-secretase substrates that are potentially involved in the neurodegenerative process associated with Alzheimer disease.

REFERENCES

1. Selkoe, D. J. (2001) Physiol. Rev. 81, 742–761
2. Xia, W. (2003) Curr. Opin. Investig. Drugs 4, 55–59
3. Xia, W. (2001) Curr. Neurol. Neurosci. Rep. 1, 422–427
4. Lorenzo, A., and Yankner, B. A. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 12243–12247
5. De Strooper, B., Saftig, P., Craessaerts, K., Vanderstichele, H., Gundula, G., Annaert, W., Von Figura, K., and Van Leuven, F. (1998) Nature 391, 387–390
6. De Strooper, B., Annaert, W., Cupers, P., Saftig, P., Craessaerts, K., Mumm, J. S., Schroeter, E. H., Schrijvers, V., Wolfe, M. S., Ray, W. J., Goate, A., and Kops, R. (1999) Nature 398, 518–522
7. Takasugi, N., Tomita, T., Hayashi, I., Tsuruoka, M., Niimura, M., Takahashi, Y., Thnakanar, G., and Iwatsubo, T. (2003) Nature 422, 438–441
8. Edsbauer, D., Winkler, E., Regula, J. T., Pesold, B., Steiner, H., and Haass, C. (2003) Nat. Cell Biol. 5, 486–488
9. Kimberly, W., LaVoie, M., and Ostaszewski, B. L. Y., Wolfe, M. S., and Selkoe, D. J. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 6382–6387
10. Baulac, S., LaVoie, M. J., Kimberly, W. T., Strahle, J., Wolfe, M. S., Selkoe, D. J., and Xia, W. (2003) Neurobiol. Dis. 14, 194–204
11. Hu, Y., and Fortini, M. (2003) J. Cell Biol. 161, 685–690
12. Luo, W., Wang, H., Li, H., Kim, B. S., Shah, S., Lee, H. J., Thnakanar, G., Kim, T. W., Yu, G., and Xu, H. (2003) J. Biol. Chem. 278, 7850–7854
13. Francis, R., McGrath, G., Zhang, J., Ruddy, D., Sym, M., Apfeld, J., Nicoll, M., Maxwell, M., Hais, B., Ellis, M. C., Parks, A. L., Xu, W., Li, J., Gurney, M., Myers, R. L., Himes, C. S., Heibsch, R., Ruble, C., Nye, J. S., and Curtis, D. (2002) Dev. Cell 3, 85–97
14. Artavanis-Tsakonas, S., Rand, M. D., and Lake, R. J. (1999) Science 284, 770–776
15. Fortini, M. E. (2001) Curr. Opin. Cell Biol. 13, 627–634
16. Kopan, R., and Goate, A. (2000) Genes Dev. 14, 2799–2806
17. De Strooper, B. (2003) Neuron 38, 9–12
18. Xia, W., and Wolfe, M. (2003) J. Cell Sci. 116, 2839–2844
19. Xie, Z., Romano, D. M., Kovacs, D. M., and Tanzi, R. E. (2004) J. Biol. Chem. 279, 34130–34137
20. Hasegawa, H., Sanjo, N., Chen, F., Gu, Y. J., Shier, C., Petit, A., Kawarai, T., Katayama, T., Hasegawa, H., Sanjo, N., Chen, F., Gu, Y. J., Shier, C., Petit, A., Kawarai, T., Katayama, H., Schmidt, S. D., Mathews, P. M., Schmitt-Ulms, G., Fraser, P. E., and St George-Hyslop, P. (2006) J. Biol. Chem. 281, 46455–46463
21. Kim, S. H., and Sisodia, S. S. (2005) J. Biol. Chem. 280, 1992–2001
22. Prokop, S., Haas, C., and Steiner, H. (2005) J. Neurochem. 94, 57–62
23. Prokop, S., Shirotani, K., Edsbauer, D., Haas, C., and Steiner, H. (2004) J. Biol. Chem. 279, 23255–23261
24. Robertson, G. S., Crocker, S. J., Nicholson, D. W., and Schulz, J. B. (2000) Brain Pathol. 10, 283–292
25. Steller, H. (1995) Science 267, 1445–1449
26. Negrón, J. F., and Lockshin, R. A. (2004) Dev. Dyn. 231, 161–170
27. Lane, D. P. (1992) Nature 358, 15–16
28. Bates, S., and Voussden, K. H. (1996) Curr. Opin. Genet. Dev. 6, 12–18
29. Hollstein, M., Sidransky, D., Vogelstein, B., and Harris, C. C. (1991) Science 253, 49–53.
30. Barkett, M., and Gilmore, T. D. (1999) Oncogene 18, 6910–6924.
31. Correa, R. G., Tergaonkar, V., Ng, J. K., Dubova, I., Izpisua-Belmonte, J. C., and Verma, I. M. (2004) Mol. Cell. Biol. 24, 5257–5268.
32. Jeong, S. J., Pise-Masison, C. A., Radonovich, M. F., Park, H. U., and Brady, J. N. (2005) J. Biol. Chem. 280, 10326–10332.
33. Jeong, S. J., Radonovich, M., Brady, J. N., and Pise-Masison, C. A. (2004) Blood 104, 1490–1497.
34. Campbell, W. A., Yang, H. W., Zetterberg, H., Baulac, S., Sears, J. A., Liu, T., Wong, S. T. C., Zhong, T. P., and Xia, W. (2006) J. Neurochem. 96, 1423–1440.
35. Xia, W., and Xu, H. (2004) Amyloid Precursoe Protein: A Practical Approach, pp. 9–10, CRC Press, Boca Raton, FL.
36. Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B., and Schilling, T. F. (1995) Dev. Dyn. 203, 253–310.
37. Westerfield, M. (1994) The Zebrafish Book, University of Oregon Press, Eugene, OR.
38. Bertrand, R., Solary, E., O’Connor, P., Kohn, K. W., and Pommier, Y. (1994) Exp. Cell Res. 211, 314–321.
39. Tamasoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M., and Tomita, F. (1986) Biochem. Biophys. Res. Commun. 135, 397–402.
40. Geling, A., Steiner, H., Willem, M., Bally-Cuif, L., and Haass, C. (2002) EMBO Rep. 3, 688–694.
41. Cserzo, M., Wallin, E., Simon, I., von Heijne, G., and Elofsson, A. (1997) Protein Eng. 10, 673–676.
42. Key, B., and Devine, C. A. (2003) Methods Cell Sci. 25, 1–6.
43. Shah, S., Lee, S. F., Tabuchi, K., Hao, Y. H., Yu, C., LaPlant, Q., Ball, H., Dann, C. E., III, Sudhof, T., and Yu, G. (2005) Cell 122, 435–447.
44. Matsui, K., Fine, A., Zhu, B., Marshak-Rothstein, A., and Ju, S. T. (1998) J. Immunol. 161, 3469–3473.
45. Zheng, Y., Ouaaz, F., Bruzzo, P., Singh, V., Gerondakis, S., and Beg, A. A. (2001) J. Immunol. 166, 4949–4957.
46. Kasibhatla, S., Brunner, T., Genestier, L., Echeverri, F., Mahboubi, A., and Green, D. R. (1998) Mol. Cell 1, 543–551.
47. Connolly, J. L., Rodgers, S. E., Clarke, P., Ballard, D. W., Kerr, L. D., Tyler, K. L., and Dermon, T. S. (2000) J. Virol. 74, 2981–2989.
48. Shetty, S., Gladden, J. B., Henson, E. S., Hu, X., Villanaevo, J., Haney, N., and Gibson, S. B. (2002) Apoptosis 7, 413–420.
49. Rivera-Walsh, I., Waterfield, M., Xiao, G., Fong, A., and Sun, S. C. (2001) J. Biol. Chem. 276, 40385–40388.
50. Ravi, R., Bedi, G. C., Engstrom, L. W., Zeng, Q., Mookerjee, B., Gelinas, C., Fuchs, E. J., and Bedi, A. (2001) Nat. Cell Biol. 3, 409–416.
51. Chen, X., Kandalsamy, K., and Srivastava, R. K. (2003) Cancer Res. 63, 1059–1066.