CNS Drug Reviews
Vol. 12, No. 2, pp. 113–122

Humanin and Colivelin: Neuronal-Death-Suppressing Peptides for Alzheimer's Disease and Amyotrophic Lateral Sclerosis

Masaaki Matsuoka[1], Yuichi Hashimoto[1], Sadakazu Aiso[2], and Ikuo Nishimoto[1]

Departments of [1]Pharmacology and [2]Anatomy, KEIO University School of Medicine, Tokyo, Japan

Keywords: Alzheimer's disease — Amyotrophic lateral sclerosis — β-Amyloid — Colivelin — Humanin.

ABSTRACT

Humanin (HN), a 24-amino-acid neuroprotective peptide, was originally found in the occipital lobe of an autopsied Alzheimer's disease (AD) patient. HN inhibits neuronal death by binding to its specific receptor on the cell membrane and triggering a Jak2/STAT3 prosurvival pathway. The activation of this pathway may represent a therapeutic approach to AD. HN also exhibits neuroprotective activity against toxicity by familial amyotrophic lateral sclerosis (ALS)-related mutant superoxide dismutase (SOD1). Recent investigations established that AGA-(C8R)-HNG17, a 17-amno-acid derivative of HN, is 10^5 times more potent as a neuroprotective than HN; at 10-picomolar and higher concentrations *in vitro* it completely suppresses neuronal death. Moreover, a 26-amino-acid peptide colivelin (CL), composed of activity-dependent neurotrophic factor (ADNF) C-terminally fused to AGA-(C8R)-HNG17, provides complete neuroprotection at 100-femtomolar or higher concentrations *in vitro*. A series of experiments using mouse AD and ALS models further established the efficacy of HN derivatives, including CL, against these diseases in vivo. HN and CL can be viewed as drug candidates for neuronal death suppression therapy in AD or ALS.

Address correspondence and reprint requests to: Dr. Masaaki Matsuoka, Department of Pharmacology, KEIO University School of Medicine, 35 Shnanomachi, Shijuku-ku, Tokyo 160-8582, Japan.
Tel.: +81 (3) 5363-3750, Fax: +81 (3) 5363-8428; E-mail: sakimatu@sc.itc.keio.ac.jp

INTRODUCTION

Neuronal Death as a Relevant Target for the Therapy of AD or ALS

Neuronal death is directly linked to the onset of irreversible manifestations of AD and ALS. In AD brains, irreversible progression of dementia and cognitive disorders are caused by neuronal death in the memory system of the association cortex (27). In ALS, motoneuronal death results in motor paralysis (2,7). Therefore, it is apparent that ideal therapy for these diseases would eliminate the primary neurotoxic insults that lead to the onset of neuronal death. However, the uncertainty regarding the neurotoxic mechanism underlying AD and ALS prevented the development of effective anti-AD and anti-ALS therapy. For example, although Aβ is now generally believed to be the primary factor causing neurotoxic insult in AD, the mechanism underlying AD pathogenesis is still controversial.

The importance of suppressing neuronal death in the treatment of these diseases justifies the use of alternative therapies that directly inhibit neuronal death, irrespective of the primary neurotoxic insults. It is anticipated that once neuronal death is inhibited, catastrophic deterioration in quality of life would be suppressed, even in the presence of functional disability caused by neurotoxic insults. Furthermore, it is highly likely that disorders originated from neuronal dysfunction could be managed by supportive therapy. For example, insufficiency in synaptic function of the cholinergic neuronal system is supposed to contribute to the development of clinical manifestation of AD. This could be at least partly recovered by conservative therapy that potentiates cholinergic neurotransmission, such as administration of cholinesterase inhibitors (29).

Neuronal-Death Mechanisms

It has been generally accepted that increased generation of Aβ is the primary event leading to all manifestations of AD including neuronal death (the Aβ cascade theory) (15). A number of studies have indicated that toxic Aβ at concentrations of 1–25 μM or higher causes neuronal cell death *in vitro* (9,10,17,18,22,30,38,41,52). This experimental finding has been considered to fundamentally support the Aβ cascade theory. However, since the concentrations of toxic Aβ capable of inducing neuronal death *in vitro* are about 100–1000 times higher than those actually observed in cerebrospinal fluids of AD brains, the relevance of this neuronal death model is still somewhat controversial.

There are several hypotheses as to how toxic Aβ causes neuronal cell death *in vitro* (27). One promising idea is that cell death is mediated by receptors for Aβ on the cell membrane. Several potential death-mediating receptors for toxic Aβ have been reported (27). In addition, p75NTR and/or PLAIDD, putative cell membrane receptors for Aβ, are able to cause neuronal cell death when ectopically overexpressed (18,44). We have recently demonstrated that $TGF_{\beta 2}$ is a natural ligand for amyloid-precursor protein (APP) that activates a neuronal cell death signal cascade (20). Based on of this finding and of the reported observation that $TGF_{\beta 2}$ expression is upregulated by toxic Aβ (21), we have put forward a new paradigm for neuronal cell death relevant to AD. Our proposal is that $TGF_{\beta 2}$, whose expression is upregulated in both glial and neuronal cells by toxic Aβ, paracrinally and autocrinally triggers APP-mediated cell death by binding to the extracellular domain of APP in neurons (Fig. 1A).

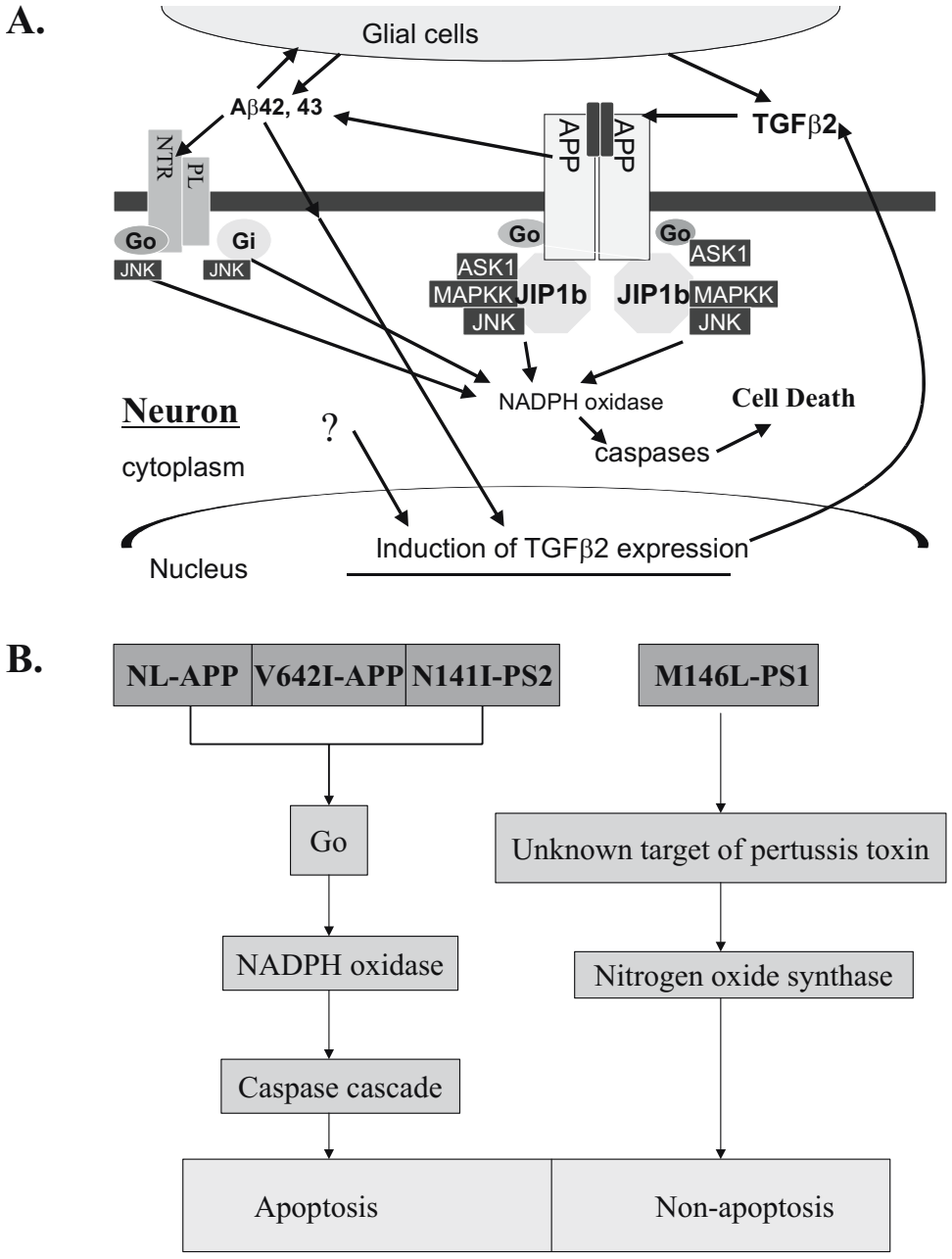

Fig. 1. A. $TGF_{\beta 2}$ theory. Upregulated production of toxic Aβ induces glial and neuronal expression of $TGF_{\beta 2}$. Upregulated $TGF_{\beta 2}$ autocrinally and paracrinally triggers a neuronal death pathway, mediated by APP/Go/JNK/NADPH oxidase/caspases, by binding to APP and inducing homodimerization of APP. Toxic Aβ may also induce neuronal death by binding to their specific death receptors, including p75NTR/PLAIDD.

B. Neuronal death pathways, triggered by ectopic expression of familial Alzheimer's disease genes (NL-APP, V642I-APP, N141I-PS2, and M146L-PS1).

NTR, P75NTR; PL, PLAIDD; ASK1, Apoptosis signal-regulating kinase 1; JIP1, JNK-interacting protein 1; Go, heterotimeric G protein Go; Gi, heterotimeric G protein Gi; APP, Amyloid-precursor protein; PS, Presenilin.

We and several other groups have also succeeded in developing another *in vitro* neuronal death model by expressing familial AD-related genes in cultured neuronal cells and primary cortical neurons (8,11,16,32,34,37,46,47,49,50,54). Increase in generation of toxic Aβ is not essential for the onset of this-type of neuronal death (41), supporting our notion that there may be an Aβ–independent pathway for the onset of AD-related neuronal death. A series of experiments has led us to the conclusion that there may be many intracellular signaling pathways for neuronal death relevant to AD, in addition to the one mediated by toxic Aβ (16,27,35) (Fig. 1B).

Selective motoneuronal death occurs in ALS. There is a genetic background for 5–10% of ALS cases. The most common type of familial ALS is caused by a point mutation in the SOD1 gene (2,7). Mutant SOD1 causes motoneuronal death by a gain of toxic function whose neurotoxic mechanism remains controversial. We have recently demonstrated that the second familial ALS (autosomal-recessive)-related gene, *ALS2* (13,51), causes motoneuronal death by loss of neuroprotective function (23,24).

HUMANIN AND OTHER NEUROPROTECTIVE PEPTIDES

Identification of Humanin as an AD-Related-Neuronal-Death-Suppressing Factor (Fig. 2)

To develop therapies capable of suppressing AD-relevant neuronal death, we began identifying endogenous bioactive factors that suppress the onset of AD. Our working hypothesis justifying this strategy was that endogenous defense factors may delay the onset of AD until middle age. We used the remaining occipital lobe of the brain of an autopsied AD patient as a source for a cDNA library because the neuronal-death-suppressing factor might be abundantly expressed there. Using disease-based death-trap screening, we identified a cDNA encoding HN from the remaining occipital lobe. HN inhibited death of F11 neurohybrid cells, induced by expression of V642I-APP, a familial AD-causative gene (17,36).

HN, a secreted peptide, exhibits suppressive activity against toxicity by any AD-relevant insult by binding to the putative receptor on the cell membrane (17). In addition, several other reports indicated that HN is also effective against serum-deprived apoptosis in PC12 cells (25) and a prion-derived toxic peptide-induced neuronal cell death (39). We have recently demonstrated that HN suppresses motoneuronal toxicity induced by mutant SOD1 (Chiba et al., unpublished observation). A rat homologue of HN named rattin has been demonstrated to exhibit similar effects against neurotoxicity related to AD (3). A secretion-defective HN mutant HNR (L9R-HN) did not show the HN activity when it was intracellularly expressed by transfection. Synthetic HNR had the HN activity only when it was added to the culture medium (48), indicating that HN exhibits its activity from the outside of the cells. This finding is in agreement with a binding experiment that demonstrated the presence of a HN receptor on the cell membrane (17). It was also reported that HN inhibited apoptosis mediated by Bax and some BH3-domain proteins by binding intracellularly to these proteins (12,31). It still remains to be determined, however, whether the latter mechanism is co-responsible for the neuronal death in AD and ALS.

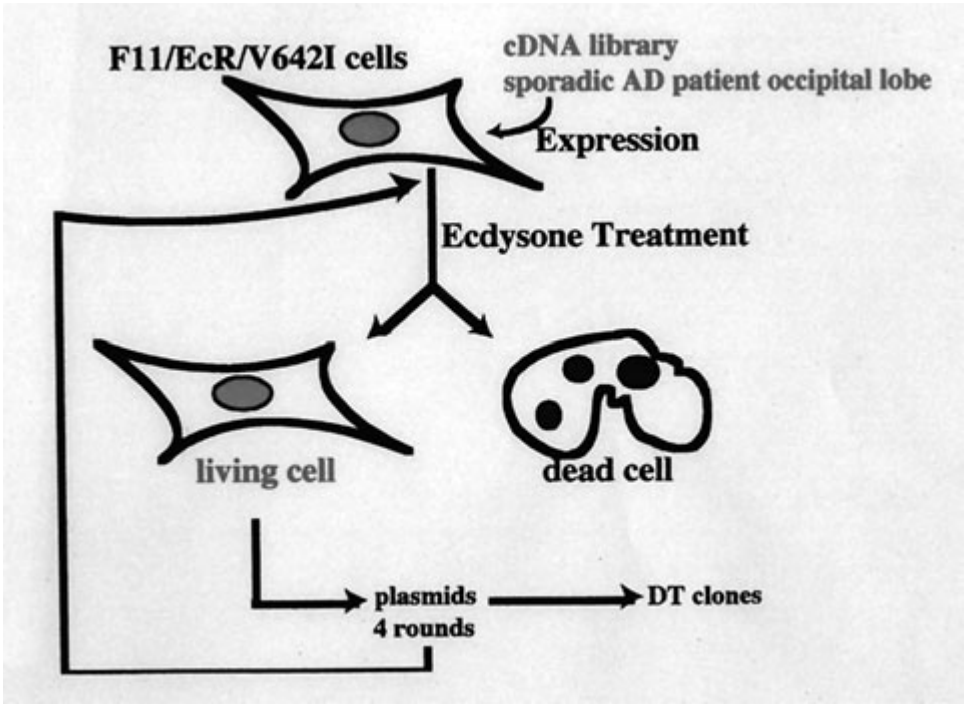

Fig. 2. Cloning of humanin-encoding cDNAs by disease-based death trap screening. A mammalian expression library was generated using an occipital lobe of a sporadic AD patient's brain. F11/EcD/V642I-APP cells were constructed by stably introducing a V642I-APP-encoding vector in which V642I-APP cDNA is located downstream of a promoter possessing an ecdysone response element, as well as the ecdysone receptor- and RXR-encoding vectors into F11 neurohybrid cells. The expression of V642I-APP was induced by treatment with ecdysone, an insect steroid. Induced expression of V642I-APP results in death in F11 cells. The cDNA library was transfected before treatment with ecdysone and cDNAs encoding proteins that suppress V642I-APP-induced death in F11 cells, were isolated from surviving F11 cells (DT clones). The humanin cDNAs were encoded in some of these DT clones.

Identification of a Humanin Receptor on the Cell Membrane

Ying et al. (2004) demonstrated that HN is bound to formylpeptide receptor-like-1 (FPRL-1), a G-protein coupled receptor that has been shown to be involved in chemotaxis in phagocytes (53). They also found that HN suppressed Aβ-induced death in PC12 rat pheochromocytoma cells by competing with Aβ for binding to FPRL-1. FPRL-2 has been also shown to associate with HN (14).

Independently, we have demonstrated that HN-mediated protection in F11 neurohybrid cells is completely blocked by expression of a dominant-negative STAT3 (19). We also recognized that an inhibitor to Jak2 kinase completely suppresses HN activity. These data suggest that there may be another HN receptor that mediates HN neuroprotection. We have recently found that such an HN receptor belongs to a cytokine receptor family. Based on this finding, we propose that endogenous HN or an HN-like protein may protect neurons from AD-related insults by binding to the receptor *in vivo*. If HN-mediated neuro-

protection occurs naturally *in vivo*, the clinical use of HN or its derivatives in the treatment of AD can be further justified.

Development of More Potent Humanin Derivatives and Colivelin (Table 1)

The original HN showed full death-suppressing activity at a concentration of 10 μM. To enhance the neuroprotective activity of HN, we systematically modified the primary sequence of HN (5,17,43). We found that AGA-(C8R)-HNG17 is fully active at a concentration as low as 10 pM (43). In addition, we recently designed a hybrid peptide CL that is composed of ADNF (9 amino acids) fused to the N terminus of AGA-(C8R)-HNG17 (17 amino acids) (5). ADNF, originally purified from the conditioned media of primarily cultured astrocytes stimulated by vasoactive intestinal peptide, protected neurons *in vitro* from Aβ toxicity and from other neurotoxic insults, but only at low concentrations of 100 fM–10 pM (1). For unknown reasons, at higher concentrations ADNF loses its neuroprotective activity, while CL has the full neuroprotective activity at 100fM and higher concentrations. In contrast to ADNF, CL never loses its activity at higher concentrations. It has been reported that CL exerts activity by simultaneously triggering two neuroprotective pathways mediated by the HN receptor/STAT3 and by the ADNF receptor/Ca^{2+}/calmodulin-dependent protein kinase IV (CaMK IV) (5).

In Vivo Relevance of Humanin and Colivelin to AD-Related Dementia

Anti-AD activity of HN derivatives and CL has been demonstrated in AD-related mouse models (5,28,33,42). CL is very effective against mouse dementia induced by intracerebroventricular (i.c.v.) injection of Aβ and against neuronal death induced by direct injection of Aβ into the CA1 region of the hippocampus. Surprisingly, HN derivatives and CL are also very effective against mouse dementia induced by muscarinic receptor antagonists. This finding suggests that either HN derivatives or CL may also suppress demen-

TABLE 1. Humanin derivatives and colivelin

Name	Sequence	Effective concentration*
HN	MAPRGFSCLLLLTSEIDLPVKRRA	10 μM
HNG	MAPRGFSCLLLLT**G**EIDLPVKRRA	10 nM
HNA	MAPRGFS**A**LLLLT**S**EIDLPVKRRA	No effect
AGA-HNG	MA**AGA**FSCLLLLT**S**EIDLPVKRRA	100–300 pM
HN17	PRGFSCLLLLTSEIDLP	10 μM
HNG17	PRGFSCLLLLT**G**EIDLP	10 nM
AGA-C8R-HNG17	P**AGA**S**R**LLLLT**G**EIDLP	10 pM
Colivelin	**SALLRSIPA**P**AGA**S**R**LLLLT**G**EIDLP	100 fM

*Effective concentration against $A\beta_{1-43}$ *in vitro*.

tia induced by functional abnormality in the cholinergic system (5,33). The mechanism underlying this activity is unknown.

Probably due to its high potency, CL shows suppressive activity against AD-related dementia, also by i.p. administration, indicating that it passes the blood-brain barrier. It is, therefore, very likely that i.c.v. route is not essential for its clinical efficacy (5).

Efficacy of Colivelin in ALS-Related Motoneuronal Death *In Vitro* and in ALS Mouse Models

During our studies with HN, we found incidentally that *in vitro* ADNF at 100 fM and higher concentrations prevents mutant SOD1-induced motoneuronal death (4). It never loses its activity at higher concentrations. By i.c.v. administration, ADNF improves motor performance of ALS mice. Unfortunately, however, ADNF does not prolong survival of these mice. HN derivatives also show protective activity against mutant SOD1-induced motoneuronal death *in vitro* (Chiba et al., unpublished observation).

Since CL is a hybrid peptide composed of ADNF and AGA-(C8R)HNG17, we tested CL in ALS mice and found that it improves both motor performance and survival (6). Vascular endothelial growth factor (VEGF) and insulin-like growth factor-I (IGF-1) have been reported to prolong survival of ALS mice (26,40,45). In respect to survival, CL is at least as effective as these growth factors. Furthermore, it is noteworthy that CL acts via two neuroprotective pathways, involving the HN receptor/STAT3 and the ADNF receptor/CaMK IV. The neuroprotective mechanisms of VEGF or IGF-1 are completely different from those of HN or CL, VEGF and IGF-1 since they trigger the PI3 kinase and Akt pathways. It is therefore likely that the neuroprotective activity of HN or CL can be additive to that of either of these neurotrophic factors.

CONCLUSIONS

In the search for new drugs to treat neurodegenerative diseases major efforts have been focused on the elimination of the primary event of the neurotoxic cascade. Consequently, neuronal death has been considered to be a second-line target for the therapy. However, given the uncertainty of the initial neurotoxic insults and the complex nature of the neurotoxic mechanisms, a therapeutic strategy to directly suppress neuronal death appears to be attractive. The relevance of this strategy to the therapy of AD and ALS is strongly supported by the fact that the putatively physiological neuroprotective systems, involving HN and ADNF, suppress AD- or ALS-related neuronal death *in vivo*. Preclinical studies indicate that HN and CL suppress neuronal cell death and are promising drug candidates for the therapy of AD or ALS.

Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; HN, humanin; CL, colivelin; ADNF, activity-dependent neurotrophic factor; SOD1, Cu/Zn-superoxide dismutase; APP, amyloid-precursor protein; Aβ, β-Amyloid; FPRL-1, formylpeptide receptor-like-1; CaMK IV, Ca^{2+}/calmodulin-dependent protein kinase IV; VEGF, vascular endothelial growth factor; IGF-1, insulin-like growth factor-I; STAT3, signal transducer and activator of transcriptions; PLAIDD, p75-like apoptosis-inducing death domain; PI3, Phosphoinositide-3-kinase

Ackowledgments. We are indebted to Dr Yasuo Ikeda for essential help. We thank Dr. Mark C. Fishman, John T. Potts Jr., Etsuro Ogata and Mr. Yoshiomi & Mrs. Yumi Tamai for indispensable support; Ms. Takako Hiraki and Ms. Tomo Y-Nishimoto for essential cooperation; Dr. Dovie Wylie for expert assistance; and all members of the Departments of Pharmacology and Anatomy for essential cooperation. This work was supported in part by Takeda Science Foundation and Japan Society for the Promotion of Science.

REFERENCES

1. Brenneman DE, Gozes I. A femtomolar-acting neuroprotective peptide. *J Clin Invest* 1996;97:2299–2307.
2. Bruijn LI, Miller TM, Cleveland DW. Unraveling the mechanisms involved in motor neuron degeneration in ALS. *Annu Rev Neurosci* 2004;27:723–749.
3. Caricasole A, Bruno V, Cappuccio I, Melchiorri D, Copani A, Nicoletti F. A novel rat gene encoding a humanin-like peptide endowed with broad neuroprotective activity. *FASEB J* 2002;16:1331–1333.
4. Chiba T, Hashimoto Y, Tajima H et al. Neuroprotective effect of activity-dependent neurotrophic factor against toxicity from familial amyotrophic lateral sclerosis-linked mutant SOD1 *in vitro* and *in vivo*. *J Neurosci Res* 2004;78:542–552.
5. Chiba T, Yamada M, Hashimoto Y et al. Development of a femtomolar-acting Humanin derivative named Colivelin by attaching ADNF to its N terminus: Characterization of colivelin-mediated neuroprotection against Alzheimer's disease-relevant insults *in vitro* and *in vivo*. *J Neurosci* 2005;25:10252–10261.
6. Chiba T, Yamada M, Sasabe J, et al. Colivelin prolongs survival of an ALS model mouse. *Biochem Biophys Res Commun* 2006;343:793–798.
7. Cleveland DW, Rothstein JD. From Charcot to Lou Gehrig: Deciphering selective motor neuron death in ALS. *Nat Rev Neurosci* 2001;2:806–819.
8. Czech C, Lesort M, Tremp G, et al. Characterization of human presenilin 1 transgenic rats: Increased sensitivity to apoptosis in primary neuronal cultures. *Neuroscience* 1998;87:325–336.
9. Giovanni A, Wirtz-Brugger F, Keramaris E, Slack R, Park DS. Involvement of cell cycle elements, cyclin-dependent kinases, pRb, and E2F•DP, in β-amyloid-induced neuronal death. *J Biol Chem* 1999;274: 19011–19016.
10. Gschwind M, Huber G. Apoptotic cell death induced by β-amyloid 1–42 peptide is cell type dependent. *J Neurochem* 1995;65:292–300.
11. Guo Q, Fu W, Sopher BL, et al. Increased vulnerability of hippocampal neurons to excitotoxic necrosis in presenilin-1 mutant knock-in mice. *Nat Med* 1999;5:101–106.
12. Guo B, Zhai D, Cabezas E, et al. Humanin peptide suppresses apoptosis by interfering with Bax activation. *Nature* 2003;423:456–461.
13. Hadano S, Hand CK, Osuga H, et al. A gene encoding a putative GTPase regulator is mutated in familial amyotrophic lateral sclerosis 2. *Nat Genet* 2001;29:166–173.
14. Harada M, Habata Y, Hosoya M, et al. N-Formylated humanin activates both formyl peptide receptor-like 1 and 2. *Biochem Biophys Res Commun* 2004;324:255–261.
15. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science* 2002;297:353–356.
16. Hashimoto Y, Niikura T, Ito Y, Nishimoto I. Multiple mechanisms underlie neurotoxicity by different types of Alzheimer's disease mutations of amyloid precursor protein. *J Biol Chem* 2000;275:34541–34551.
17. Hashimoto Y, Niikura T, Tajima H, et al. A rescue factor abolishing neuronal cell death by a wide spectrum of familial Alzheimer's disease genes and Aβ. *Proc Natl Acad Sci USA* 2001;98:6336–6341.
18. Hashimoto Y, Kaneko Y, Tsukamoto E, et al. Molecular characterization of neurohybrid cell death induced by Alzheimer's amyloid-beta peptides via p75NTR/PLAIDD. *J Neurochem* 2004;90:549–558.
19. Hashimoto Y, Suzuki H, Aiso S, Niikura T, Nishimoto I, Matsuoka M. Involvement of tyrosine kinases and STAT3 in Humanin-mediated neuroprotection. *Life Sci* 2005;77:3092–3104
20. Hashimoto Y, Chiba T, Yamada M, et al. Transforming growth factor beta2 is a neuronal death-inducing ligand for amyloid-beta precursor protein. *Mol Cell Biol* 2005;25:9304–9317.
21. Hashimoto Y, Nawa M, Chiba T, Aiso S, Nishimoto I, Matsuoka M. Transforming growth factor beta2 autocrinally mediates neuronal cell death induced by amyloid-beta. *J Neurosci Res* 2006;83:1039–1047.

22. Kaneko I, Yamada N, Sakuraba Y, Kamenosono M, Tutumi S. Suppression of mitochondrial succinate dehydrogenase, a primary target of β-amyloid, and its derivative racemized at Ser residue. *J Neurochem* 1995;65:2585–2593.
23. Kanekura K, Hashimoto Y, Kita Y, et al. A Rac1/phosphatidylinositol 3-kinase/Akt3 anti-apoptotic pathway, triggered by AlsinLF, the product of the ALS2 gene, antagonizes Cu/Zn-superoxide dismutase (SOD1) mutant-induced motoneuronal cell death. *J Biol Chem* 2005;280:4532–4543.
24. Kanekura K, Hashimoto Y, Niikura T, Aiso S, Matsuoka M, Nishimoto I. Alsin, the product of ALS2 gene, suppresses SOD1 mutant neurotoxicity through RhoGEF domain by interacting with SOD1 mutants. *J Biol Chem* 2004;279:19247–19256.
25. Kariya S, Takahashi N, Ooba N, Kawahara M, Nakayama H, Ueno S. Humanin inhibits cell death of serum-deprived PC12h cells. *Neuroreport* 2002;13(6):903–907.
26. Kaspar BK, Llado J, Sherkat N, Rothstein JD, Gage FH. Retrograde viral delivery of IGF-1 prolongs survival in a mouse ALS model. *Science* 2003;301:839–842.
27. Kawasumi M, Hashimoto Y, Chiba T, et al. Molecular mechanisms for neuronal cell death by Alzheimer's amyloid precursor protein-relevant insults. *NeuroSignals* 2002;11:236–250.
28. Krejcova G, Patocka J, Slaninova J. Effect of humanin analogues on experimentally induced impairment of spatial memory in rats. *J Pept Sci* 2004;10:636–639.
29. Lane RM, Kivipelto M, Greig NH. Acetylcholinesterase and its inhibition in Alzheimer disease. *Clin Neuropharmacol* 2004;27:141–149.
30. Loo DT, Copani A, Pike CJ, Whittemore ER, Walencewicz AJ, Cotman CW. Apoptosis is induced by β-amyloid in cultured central nervous system neurons. *Proc Natl Acad Sci USA* 1993;90:7951–7955.
31. Luciano F, Zhai D, Zhu X, et al. Cytoprotective peptide humanin binds and inhibits proapoptotic Bcl-2/Bax family protein BimEL. *J Biol Chem* 2005 280:15825–15835.
32. Luo JJ, Wallace W, Riccioni T, et al. Death of PC12 cells and hippocampal neurons induced by adenoviral-mediated FAD human amyloid precursor protein gene expression. *J Neurosci Res* 1999;55:629–642.
33. Mamiya T, Ukai M. [Gly(14)]-Humanin improved the learning and memory impairment induced by scopolamine *in vivo*. *Br J Pharmacol* 2001;134:1597–1599.
34. Niikura T, Yamada M, Chiba T, Aiso S, Matsuoka M, Nishimoto I. Characterization of V642I-A6421PP-induced cytotoxicity in primary neurons. *J Neurosci Res* 2004;77:54–62.
35. Niikura T, Chiba T, Aiso S, Matsuoka M, Nishimoto I. Humanin: After the discovery. *Mol Neurobiol* 2004; 30:327–340
36. Nishimoto I, Matsuoka M, Niikura T. Unravelling the role of humanin. *Trends Mol Med* 2004;10:102–105.
37. Nishimura I, Uetsuki T, Dani SU, et al. Degeneration *in vivo* of rat hippocampal neurons by wild-type Alzheimer amyloid precursor protein overexpressed by adenovirus-mediated gene transfer. *J Neurosci* 1998;18: 2387–2398.
38. Pike CJ, Ramezan-Arab N, Cotman CW. Aβ-amyloid neurotoxicity *in vitro*: Evidence of oxidative stress but not protection by antioxidants. *J Neurochem* 1997;69:1601–1611.
39. Sponne I, Fifre A, Koziel V, Kriem B, Oster T, Pillot T. Humanin rescues cortical neurons from prion-peptide-induced apoptosis. *Mol Cell Neurosci* 2004;25:95–102.
40. Storkebaum ED, Lambrechts M, Dewerchin MP, et al. Treatment of motoneuron degeneration by intracerebroventricular delivery of VEGF in a rat model of ALS. *Nat Neurosci* 2005;8:85–92.
41. Sudo H, Hashimoto Y, Niikura T, et al. Secreted Aβ does not mediate neurotoxicity by antibody-stimulated amyloid precursor protein. *Biochem Biophys Res Commun* 2001;282:548–556.
42. Tajima H, Kawasumi M, Chiba T, et al. A humanin derivative, S14G-HN, prevents amyloid-beta-induced memory impairment in mice. *J Neurosci Res* 2005;79:714–723.
43. Terashita K, Hashimoto Y, Niikura T, et al. Two serine residues distinctly regulate the rescue function of humanin, an inhibiting factor of Alzheimer's disease-related neurotoxicity: Functional potentiation by isomerization and dimerization. *J Neurochem* 2003;85:1521–1538.
44. Tsukamoto E, Hashimoto Y, Kanekura K, Niikura T, Aiso S, Nishimoto I. Characterization of the toxic mechanism triggered by Alzheimer's amyloid-β peptides via p75 neurotrophin receptor in neuronal hybrid cells. *J Neurosci Res* 2003;73:627–636.
45. Vande Velde C, Cleveland DW. VEGF: Multitasking in ALS. *Nat Neurosci* 2005;8:5–7.
46. Weihl CC, Ghadge GD, Kennedy SG, Hay N, Miller RJ, Roos RP. Mutant presenilin-1 induces apoptosis and downregulates Akt/PKB. *J Neurosci* 1999;19:5360–5369.
47. Wolozin B, Iwasaki K, Vito P, et al. Participation of presenilin 2 in apoptosis: Enhanced basal activity conferred by an Alzheimer mutation. *Science* 1996;274:1710–1713.
48. Yamagishi Y, Hashimoto Y, Niikura T, Nishimoto I. Identification of essential amino acids in Humanin, a neuroprotective factor against Alzheimer's disease-relevant insults. *Peptides* 2003;24:585–595.

49. Yamatsuji T, Matsui T, Okamoto T, et al. G protein-mediated neuronal DNA fragmentation induced by familial Alzheimer's disease-associated mutants of APP. *Science* 1996;272:1349–1352.
50. Yamatsuji T, Okamoto T, Takeda S, Murayama Y, Tanaka N, Nishimoto I. Expression of V642 APP mutant causes cellular apoptosis as Alzheimer trait-linked phenotype. *EMBO J* 1996;15:498–509.
51. Yang Y, Hentati A, Deng HX, et al. The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. *Nat Genet* 2001;29: 160–165.
52. Yankner BA, Dawes LR, Fisher S, Villa-Komaroff L, Oster-Granite ML Neve RL. Neurotoxicity of a fragment of the amyloid precursor associated with Alzheimer's disease. *Science* 1989;245:417–420.
53. Ying G, Iribarren P, Zhou Y, et al. Humanin, a newly identified neuroprotective factor, uses the G protein-coupled formylpeptide receptor-like-1 as a functional receptor. *J Immunol* 2004;172:7078–7085.
54. Zhao B, Chrest FJ, Horton Jr WE, Sisodia SS, Kusiak JW. Expression of mutant amyloid precursor proteins induces apoptosis in PC12 cells. *J Neurosci Res* 1997;47:253–263.