Molecular Approaches to the Treatment, Prophylaxis, and Diagnosis of Alzheimer’s Disease:
Possible Involvement of HRD1, a Novel Molecule Related to Endoplasmic Reticulum Stress, in Alzheimer’s Disease

Masayuki Kaneko1,*, Yasunobu Okuma1, and Yasuyuki Nomura2

1Department of Pharmacology, Faculty of Pharmaceutical Sciences, Chiba Institute of Science, 15-8 Shiomi-cho, Choshi 288-0025, Japan
2Laboratory of Pharmacotherapeutics, Yokohama College of Pharmacy, 601 Matano-cho, Totsuka-ku, Yokohama 245-0066, Japan

Received October 18, 2011; Accepted November 21, 2011

Abstract. Endoplasmic reticulum (ER)-associated degradation (ERAD) is a protective mechanism against ER stress in which unfolded proteins accumulated in the ER are selectively transported to the cytosol for degradation by the ubiquitin–proteasome system. We cloned the novel ubiquitin ligase HRD1, which is involved in ERAD, and showed that HRD1 promoted amyloid precursor protein (APP) ubiquitination and degradation, resulting in decreased generation of amyloid β (Aβ). In addition, suppression of HRD1 expression caused APP accumulation and promoted Aβ generation associated with ER stress and apoptosis. Interestingly, HRD1 levels were significantly decreased in the cerebral cortex of patients with Alzheimer’s disease (AD), and the brains of these patients experienced ER stress. Our recent study revealed that this decrease in HRD1 was due to its insolubilization; however, controversy persists about whether the decrease in HRD1 protein promotes Aβ generation or whether Aβ neurotoxicity causes the decrease in HRD1 protein levels. Here, we review current findings on the mechanism of HRD1 protein loss in the AD brain and the involvement of HRD1 in the pathogenesis of AD. Furthermore, we propose that HRD1 may be a target for novel AD therapeutics.

Keywords: Alzheimer’s disease, amyloid β, endoplasmic reticulum–associated degradation, HRD1, neurodegenerative disease

1. Introduction

The endoplasmic reticulum (ER) is an organelle involved in the maturation of membrane and secretory proteins, including folding, glycosylation, and disulfide bond formation, and calcium homeostasis. When ER functions are perturbed by various stressors, mutant proteins are generated or wild-type proteins are overloaded, resulting in the accumulation of unfolded proteins in the ER. This situation is termed ER stress. To prevent further accumulation of unfolded proteins and subsequent apoptosis, cells can initiate a series of protective responses known as the unfolded protein response (UPR), including suppression of protein synthesis, induction of ER chaperone expression, and protein degradation by the ubiquitin–proteasome system. The last response is termed ER-associated degradation (ERAD) (1, 2). In the ERAD system, unfolded proteins accumulated in the ER are transported back to the cytosol through translocons; polyubiquitinated by ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), ubiquitin ligase (E3), and other components; and degraded by the 26S proteasome. E3 plays an important role in the recognition and ubiquitination of unfolded proteins. Specifically, the RING finger domain of E3 mediates the transfer of ubiquitin from E2 to substrates (Fig. 1) (3, 4).

A large number of ER stress–related diseases and conditions, such as Alzheimer’s disease (AD), Parkin-
son’s disease (PD), polyglutamine disease, amyotrophic lateral sclerosis, ischemia, bipolar disorder, diabetes, arteriosclerosis, and inflammation, have been reported (5). Because ER stress is linked to neuronal death in a large portion of neurodegenerative diseases, further elucidation of the UPR could improve the understanding of pathogenic mechanisms and speed the development of new drugs for neurodegenerative diseases that currently have no treatment. In particular, if novel human genes involved in ERAD could be identified, considerable progress in the understanding of neurodegenerative disease could result, because human ERAD genes have been rarely identified.

In this review, we describe the involvement of ER stress in neurodegenerative disease and highlight a novel relationship between the ubiquitin ligase HRD1 involved in ERAD and AD.

2. ER stress and neurodegenerative disease

Over the past decade, the possible involvement of ER stress in pathogenic mechanisms of neurodegenerative diseases has been reported (6). The first neurodegenerative disease in which ER stress was demonstrated was AD (7). AD is the most common neurodegenerative disease and is characterized by senile plaques, neurofibrillary tangles, and dementia. Senile plaques are composed of amyloid β (Aβ) peptides derived from the amyloid precursor protein (APP) (8). The Aβ peptides, including Aβ40 and the more fibrilligenic Aβ42, are generated by a two-step cleavage of APP by β- and γ-secretases (9). Although the toxicity of Aβ in neurons is well established, the intervening mechanisms from Aβ production to neurodegeneration are controversial (for details, see the article by Shoji in this Forum Minireview series: Ref. 10). Neurofibrillary tangles in AD are formed from hyperphosphorylated tau proteins. Tau proteins interact with tubulin to stabilize microtubules and promote tubulin assembly into microtubules. Hyperphosphorylated tau proteins can assemble into tangles of paired helical filaments, which are associated with AD pathogenesis (for details, see the article by Takata and Kitamura in this Forum Minireview series: Ref. 11) (12, 13).

In addition to the hallmark plaques and tangles, several other pathways leading to ER stress have been reported in AD. For example, gene mutation of presenilin, which is a component of the γ-secretase complex and is associated with familial AD, results in an attenuation of ER chaperone expression during ER stress (7). Mutations in presenilin inhibit activation of IRE1, ATF6, and PERK, all of which act as signal transducers of ER stress in the ER membrane (for details, see the article by Hosoi and Ozawa in this Forum Minireview series: Ref. 14), thereby making neurons vulnerable to ER stress (15). In addition, nitric oxide–induced S-nitrosylation of ER chaperone protein disulfide isomerase (PDI) inhibits its enzymatic activity, leading to ER stress–induced neuronal death. Interestingly, S-nitrosylation of PDI has been observed in the brain of sporadic AD and PD patients (16). ER stress can also cause AD onset. For example, eIF2α phosphorylation, which is induced by eIF2α kinase PERK, elevates BACE1 (β-secretase) levels and causes increased Aβ production (17). Furthermore, Aβ has been reported to induce ER stress, leading to activation of ER stress–specific initiator caspases, including mouse caspase-12 and human caspase-4 (18–20). In conclusion, because postmortem AD patient brain samples exhibit activated UPR markers, including phosphorylated PERK, eIF2α, and IRE1, ER stress probably plays a key role in AD pathogenesis as a cause or consequence (21).

PD is the second most common neurodegenerative disease and is characterized by the loss of dopaminergic neurons of the substantia nigra pars compacta and move-
ment disorders. A number of genes linked to familial PD have been identified (22). One of them is Parkin (*PARK2*), a ubiquitin ligase involved in ERAD, whose expression is induced by ER stress and which suppresses ER stress–induced cell death (23, 24). A substrate of Parkin is the Parkin-associated endothelin-like receptor (Pael-R). The lack of Parkin enzymatic activity caused by gene mutations can lead to the accumulation of Pael-R in the ER, resulting in ER stress–induced apoptosis (25). Another role of ER stress in PD involves neurotoxins, including 6-hydroxydopamine, 1-methyl-4-phenylpyridinium (MPP⁺), and rotenone, which are used as inducers of PD symptoms in animal models. These compounds cause ER stress and trigger the UPR in vitro and in vivo (26, 27).

### 3. HRD1 and neurodegenerative disease

We have identified human HRD1 as a homolog of yeast Hrd1p/Der3p using human genome databases (25). In yeast, Hrd1p, a component of the ERAD complex in the ER membrane, acts as a ubiquitin ligase (29–31). One of its substrates is yeast HMG-CoA reductase (Hmg2p), and thus the acronym “HRD” is derived from “HMG-CoA reductase degradation.” We have also identified human SEL1L as a homolog of yeast Hrd3p that is the stabilizer of HRD1 and is involved in substrate recognition (32, 33). Expression of HRD1 and SEL1L is induced in response to ER stress, consistent with the situation in yeast, and their expression is mediated by the IRE1–XBP1 and ATF6 pathways, respectively (for details, see the article by Hosoi and Ozawa in this Forum Minireview series: Ref. 14) (34, 35). Furthermore, we demonstrated that HRD1 protects against ER stress–induced apoptosis and this protective effect depends on its RING finger domain-mediated E3 activity (28). In addition, we have found that the five transmembrane domains of HRD1 act on the transport of its substrate Pael-R to the cytoplasm similar to a translocon (36). In mouse and human brains, HRD1 is selectively expressed in neurons but not in glia, especially in most neurons of the cerebral cortex, pyramidal cells of the hippocampus, dopaminergic neurons of the substantia nigra, and Purkinje cells of the cerebellum, which are closely related to neurodegenerative diseases (37). Therefore, HRD1 could be associated with several neurodegenerative diseases.

We have identified Pael-R as a substrate of HRD1. HRD1 promotes ubiquitination and degradation of Pael-R by recognizing its unfolded state. Suppression of HRD1 expression results in accumulation of unfolded Pael-R and ER stress–mediated apoptosis (38). These results suggest that functional loss of other E3s, including HRD1, is implicated in the dysfunctional Parkin-induced neurodegeneration in autosomal recessive juvenile parkinsonism. Furthermore, the involvement of HRD1 was reported in Huntington’s disease and prion disease. HRD1 enhances the degradation of polyglutamine-containing Huntington protein and prevents its induction of cell death in an E3 activity–dependent manner (39). Moreover, the unglycosylated form of human prion protein is degraded by the Hrd1p–Hrd3p pathway in yeast (40). These findings suggest that selective expression of HRD1 in neurons plays a critical role in many types of protein degradation and that its functional loss likely affects the degradation of proteins linked to neurodegeneration either directly or indirectly.

### 4. HRD1 and AD

While the metabolism of APP to Aβs has been intensively investigated, the degradation of APP holoprotein has not been fully elucidated. Because APP, a transmembrane protein, matures by folding and glycosylation in the ER, it has long been speculated that APP is degraded by the ERAD pathway (41–43). We found that APP is a substrate of HRD1. HRD1 is coexpressed with APP in brain neurons and interacts specifically with unfolded APP in neuronal cells. Furthermore, HRD1 promotes ubiquitination and degradation of APP, resulting in decreased Aβ production. In contrast, suppression of HRD1 expression causes APP and Aβ accumulation accompanied by ER stress and apoptosis (44). These results suggest that HRD1 promotes unfolded APP degradation through ERAD, resulting in the prevention of Aβ generation.

We demonstrated that HRD1 protein levels were significantly decreased in the NP-40 detergent–soluble fraction of the cerebral cortex of AD patients, compared with non-AD controls, whereas HRD1 mRNA levels were increased (44). In addition, HRD1 protein levels in the NP-40 detergent–soluble fraction were negatively correlated with Aβ levels (45). To determine what causes the decrease in HRD1 protein levels in the NP-40 detergent–soluble fraction, we investigated protein levels in the detergent-insoluble fraction. HRD1 protein levels were decreased in the soluble fraction, but increased in the insoluble fraction in AD patients in the biochemical experiment, indicating its protein insolubilization (46). Furthermore, SEL1L protein levels significantly increased in the insoluble fraction, suggesting that HRD1 coinsolubilizes with SEL1L in the AD brain (46). Based on these findings, we propose that the functional loss of HRD1 caused by its protein insolubilization is possibly linked to increased Aβ production, leading to AD onset (Fig. 2); however, it remains unclear how HRD1 protein is insolubilized. A report indicating that Parkin forms aggregates in response to oxidative stress may provide a
It is controversial whether a decrease in HRD1 protein causes increased Aβ generation (Fig. 3, Model 1) or whether increased Aβ generation causes the decrease in HRD1 protein (Fig. 3, Model 2). Because high levels of Aβ were observed in the non-AD patients with normal levels of HRD1, the decrease in HRD1 protein could contribute to neurodegeneration as AD progresses after increased generation of Aβ (45). To address these two possibilities, we are investigating Aβ levels in HRD1-deficient mice and the effect of Aβ on HRD1 insolubilization. Taken together, our findings indicate that compounds that prevent the insolubilization of HRD1 could become novel drugs with a unique mechanism of action for the treatment of AD.

5. Conclusions

The pathological importance of Aβ as the initiator of AD is well defined; however, there is a significant minority of people with high Aβ levels who are hardly affected by AD (45). This observation suggests that a critical turning point exists in the onset of AD after increased generation of Aβ. As discussed in this Forum issue, many novel factors, such as TEK/Tie2 (48), WAVE (49, 50), CRMP2 (49, 50), and HRD1, could play crucial roles in the onset or progression of AD. To clarify their roles, there needs to further investigations on postmortem brains that are classified into detailed categories by pathological stage, including mild cognitive impairment, and clinical diagnosis (for details, see the article by Shoji in this Forum Minireview series: Ref. 10). Clarifying these roles could lead to the development of novel PET/SPECT imaging probes for in vivo detection of such molecules (for details, see the article by Ono and Saji in this Forum Minireview series: Ref. 51) (52). In conclusion, not only is the understanding of pathogenic mechanisms by which such novel molecules mediate disease onset and progression important but also the development of diagnostics using novel probes is essential for...
the establishment of treatments utilizing new drugs for neurodegenerative diseases including AD.

Acknowledgments

We thank Dr. Yoshihisa Kitamura and Dr. Kazuyuki Takata (Kyoto Pharmaceutical University, Kyoto) for helpful discussions. Our research described in this review was supported by Grants-in-Aid for Science Research (KAKENHI) 21790089, 21300142, and 20659013 from the Ministry of Education, Culture, Sports, Science and Technology, Japan and by the Research Foundation for Pharmaceutical Sciences.

References


