Presenilin 1 and Cadherins: Stabilization of Cell-Cell Adhesion and Proteolysis-Dependent Regulation of Transcription

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Key Words

Alzheimer's disease · Amyloid precursor protein · Presenilin · Cadherin

Abstract

Presenilin-1 (PS1) has gained intensive attention in relation to Alzheimer's disease, since it has been shown that PS1 mutations are linked to familial Alzheimer's disease (FAD), and that PS1 is a member of the high molecular weight complex of γ -secretase, which generates the carboxyl end of β -amyloid peptide (γ -cleavage). A parallel line of evidence suggests that upon formation of cell-cell contacts, presenilin colocalizes with cadherins at the cell surface and stabilizes the cadherin-based adhesion complex. Under conditions stimulating cell-cell dissociation, cadherins are processed by a PS1/ γ -secretase activity, promoting disassembly of adherens junctions, and resulting in the increase of cytosolic β -catenin, which is an important regulator of the Wnt/Wingless signaling pathway. PS1 also controls the cleavage of a number of transmembrane proteins at the interface of their transmembrane and cytosolic domains (ϵ -cleavage), producing intracellular fragments with a putative transcriptional role. Remarkably, cleavage of N-cadherin by PS1 produces an intracellular fragment that downregulates CREB-mediated transcription, indicating a role of PS1 in gene expression. PS1 mutations associated with FAD abol-

ish production of the N-cadherin intracellular fragment and thus fail to suppress CREB-dependent transcription. These findings suggest an alternative explanation for FAD that is separate from the widely accepted 'amyloid hypothesis': dysfunction in transcription regulatory mechanisms.

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Alzheimer's disease (AD), originally described by Alois Alzheimer in 1907, is a neurodegenerative disorder resulting in dementia, characterized by progressive loss of cognitive abilities. The pathophysiology of AD is defined by a large number of fibrillar deposits called neuritic plaques, whose major proteinaceous component is β -amyloid peptide (A β), neurofibrillary tangles, oxidative neuronal damage and inflammatory cascades. The disease is inherited as a Mendelian trait in only 5% of all cases, and these are regarded as the familial form of AD (FAD) [for a review, see 1]. Mutations of three genes have been identified which are involved in the early-onset FAD: the amyloid precursor protein (APP) gene and the genes encoding the homologous presenilin (PS) proteins PS1 and PS2 [2]. Consistent with this idea, mutations in the APP and PS genes have been reported to be related with AD by altering the processing of APP [3–8]. The remaining cases of the disease are sporadic and affect individuals after the age of 65, in contrast with the FAD forms that

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occur earlier. Thus, it is easy to appreciate that AD is a multifaceted disease, and both environmental and genetic factors are responsible for its development.

The major constituent of amyloid plaques deposited in brain of AD patients is a short 39- to 43-amino acid peptide called A β . It is produced by the posttranslational proteolysis of a larger protein, the APP [9, 10]. APP gene locus was found on chromosome 21 and alternative exon splicing produces three major isoforms of 695, 751 or 770 amino acids [9]. APP is a type I transmembrane glycoprotein that through the course of its life span is processed by different proteases called secretases [11]. The first proteolytic cleavage identified takes place 12 amino acids NH₂-terminal to the transmembrane domain of APP and is catalyzed by the enzyme α -secretase [12]. Pharmacological studies reported that metalloprotease TACE (tumor necrosis factor- α -converting enzyme) [13] and ADAM 10 [14] are related with the α -secretase processing, which is considered nonamyloidogenic, as the enzyme cleaves within the A β region of APP. In the alternative pathway, two distinct proteolytic enzymes, β -secretase and γ -secretase, generate the amino-terminus and the carboxyl-terminus of A β , respectively [1].

Presenilin 1

PS1 gained great attention in relation to AD when gene mapping in patients with an autosomal dominant form of early onset AD led to the identification of human PS gene in 1995 [15]. According to the most widely accepted topological model, PS1 includes 8 transmembrane domains with the N- and C-domains as well as the large hydrophilic loop between transmembrane domain 6 and 7 being cytosolic [16, 17]. The PS holoprotein undergoes constitutive endoproteolysis resulting in the production of a stable heterodimer composed of an N-terminal fragment and a C-terminal fragment (CTF) [18, 19]. A number of studies support the idea first described by Wolfe et al. [20] that PS seems to be the catalytic component of γ -secretase [21–23]. However, the following observations show that the specific protein alone is not sufficient for protease activity, which is consistent with the notion that γ -secretase is a high-molecular-weight, multiprotein complex. Biochemical purification of the PS-containing high-molecular-weight complex led to the identification of the second member of the complex called nicastrin after the Italian village Nicastro, where genetic studies of FAD were performed [24]. Genetic screens designed to modify a PS-deficient phenotype in *Caenorhabditis elegans* yielded

two novel genes APH-1 (anterior pharynx-defective phenotype) [25] and PEN-2 (PS enhancer) [26]. Coexpression of the four human proteins (PS1, Nct, Aph-1 and Pen-2) in cultured mammalian cells enhances γ -secretase activity [27–29]. Recent findings also suggest that these four proteins are sufficient to reconstitute the active γ -secretase complex in *Saccharomyces cerevisiae* that lacks endogenous γ -secretase activity [30]. There are at least two PS1/ γ -secretase-dependent cleavages namely the γ -cleavage and the ϵ -cleavage. The γ -cleavage takes place in the middle of the transmembrane domain of APP and other type I transmembrane proteins and is responsible for the generation of the C-terminus of A β or A β -like peptides [31, 32]. The ϵ -cleavage takes place close to the interface of the transmembrane and cytosolic domains of the proteins and is responsible for the release of their cytoplasmic domains to the cytosol [33–36].

Cadherins

E (epithelial)- and N (neuronal)-cadherin are the best-characterized of the classical cadherins that mediate cell adhesion, play a fundamental role in normal development and participate in the maintenance of proper cell-cell contacts. The cadherins are single pass transmembrane glycoproteins of about 700- to 750-amino acid residues that typically consist of five tandem repeated extracellular domains, a single membrane-spanning segment and a cytoplasmic region. The function of cadherins is Ca²⁺-dependent: removal of calcium abolishes adhesive activity and renders cadherins vulnerable to proteases. The large extracellular parts of cadherins of two opposing cells form calcium-dependent homophilic junctions, whereas a serine-rich region of the cytoplasmic domain has been shown to be associated with proteins termed catenins. Specifically, cadherins bind to β -catenin, which in turn associates with α -catenin, and α -actin, thus connecting the cadherin-catenin complex to the actin cytoskeleton, ensuring the integrity and stabilization of the adhesion.

Interaction of PS1 with Cadherins

Georgakopoulos et al. [37] presented data indicating that PS1 upon formation of cell-cell contact is recruited at sites of cell-cell adhesion, where it forms complexes with E-cadherin and β -catenin. Laser scanning confocal micrographs and immunogold electron microscopy of confluent MDCK cells showed a similar distribution of PS1 and

E-cadherin/ β -catenin at sites of cell-cell contacts on the lateral plasma membrane. This concentration of PS1 at the plasma membrane has given a satisfactory justification of the processing of Notch1 and APP at the plasma membrane, a fact that was inconsistent with the previously reported intracellular site of PS1 [38, 39]. In cells lacking cell-cell contacts, PS1 is predominantly localized in the ER/Golgi apparatus. To further support the initial data indicating cell surface expression of PS1, they performed coimmunoprecipitation experiments that show a physical interaction of PS1 with components of the cadherin-based adherens junction (CAJs). More specifically, it has been shown that E-cadherin binds directly to PS1 and the specific binding is mediated by 340- to 375-amino acid residues of PS1/CTF and by amino acid residues 604–615 of E-cadherin, which are also required for the binding of protein p120 [40]. Continuous adjustment and regulation of cadherins allow cells to control the critical events of cell adhesion, embryogenesis and tissue remodeling. The molecular mechanism underlying the modulation of cadherins remains elusive. A number of studies argue that p120 is an important regulator of cadherin function [41–44]. Using wild-type and PS1 knockout cells it has been shown that PS1 overexpression decreased significantly p120/E-cadherin binding, and conversely increasing levels of p120 suppressed PS1/E-cadherin interaction [40]. These observations suggest that PS1 competes with p120 for binding to E-cadherin, implying a key role for PS1 in the regulation of the cadherin-associated system. Besides PS1 localization at cell-cell contacts of epithelial tissue, it has been observed that it forms complexes with cadherins in brain, suggesting a functional role of PS1 in synaptic adhesions [37]. Moreover, double labeling in immunocytochemistry experiments of SH-SY5Y cells showed colocalization of PS1 and N-cadherin at the cell-cell contact sites. The reported colocalization is significantly reduced in dominant negative mutated PS (D385A)-transfected cells, indicating that overexpression of the mutant PS1 causes disassembly of the N-cadherin/PS1 complex.

WT PS1 but Not FAD Mutants Stabilizes the Cadherin-Based Junctions

In order to address a possible functional role of PS1 at cell-cell adhesion, HEK 293 cells were stably transfected with wild-type PS1 and PS1 mutant (D257A) [37]. They reported a statistically significant increase in cell-cell aggregation in PS1 stable transfectants in comparison with controls. Cells overexpressing PS1 mutant showed

no increased aggregation. These results indicate that PS1 stimulates cell-cell adhesion. This stimulation seems to be Ca^{2+} -dependent, since Ca^{2+} removal from the medium results in the loss of intercellular contacts, a finding that is in accordance with the experiments of Baki et al. [40]. In the presence of Ca^{2+} , PS1 $^{-/-}$ cells showed little aggregation in comparison with PS1 $^{+/+}$ cells and cell-cell aggregation was substantially increased in PS1 $^{-/-}$ cells by transfection with PS1. Restoration of effective cell-cell adhesion was not achieved by transfection of PS1 mutant lacking exon 9 (ΔE9). Interestingly, in PS $^{-/-}$ cells, E-cadherin/ α -, β -, γ -catenin interaction is significantly lower than in PS $^{+/+}$ cells, whereas the presence of PS1 increases cadherin/catenin cytoskeletal association. The above data demonstrate that PS1 promotes both E-cadherin interactions with other components of the adhesion complex and its cytoskeletal association, giving new insights into our understanding of the functional role of PS1 in cadherin-based adhesion (fig. 1, left).

Indication for a Role of PS1 in the Trafficking of Cadherins

In order to explain the mode by which PS1 modulates cell-cell adhesion, it has been suggested that it influences the trafficking of the components of the adhesion to the cell surface [37, 45]. This explanation is in accordance with previous studies that attribute a role in protein trafficking to PS1 [46, 47]. Consistent with this idea, Uemura et al. [45] noticed that N-cadherin expression is reduced in cells expressing mutant PS1 (D385A) and the protein has an abnormally higher molecular weight compared to that in control cell lines. This finding indicates an impaired maturation and trafficking of N-cadherin. Newly synthesized N-cadherin is proteolytically processed into the mature form before its transport to the plasma membrane [48]. Provided that the N-cadherin-dependent cell-cell adhesion requires the recruitment of an intracellular N-cadherin pool to the plasma membrane [49], it is easy to appreciate that proper trafficking of N-cadherin is of crucial importance for the efficient function of the synaptic adhesions assuring the interneuronal communication and the processing of the information. It is tempting to speculate that PS1 FAD mutations may interfere with the trafficking of N-cadherin, resulting in the impairment of the synaptic activity of neurons [45]. This theory is of great importance if one considers that a pathological feature of AD is extensive synaptic loss, which is strongly correlated with the degree of dementia [50].

Fig. 1. PS1 has two distinct roles in relation to CAJs. PS1 is recruited at sites of cell-cell adhesion and stabilizes the E-cadherin/catenin complex (left of the vertical dashed line). Under conditions stimulating apoptosis, E-cadherin is processed in a PS1/ γ -secretase dependent manner, inducing disassembly of the CAJs. This cleavage produces E-cad/CTF2, which is released to the cytosol in complex with β -catenin, a key modulator of the Wnt/Wingless pathway, and might indirectly be implicated in transcriptional regulation (right of the vertical dashed line).

E-Cadherin Is Processed by the PS1/ γ -Secretase Activity

Loss of cell-cell contact is one of the numerous morphological changes observed in the absence of Ca^{2+} or after induction of apoptosis. Under both conditions, the cadherins, who represent the major adhesive system in vertebrates, undergo a conformational change and are processed by specific enzymes, resulting in disassembly of CAJs [51, 52].

Steinhusen et al. [51] have presented data showing that after induction of apoptosis E-cadherin is cleaved by two distinct proteolytic activities, generating three fragments with molecular masses of 24, 29 and 84 kD. More specifically, they suggested that E-cadherin is cleaved by a metalloprotease resulting in the shedding of extracellular domain from the cell surface and the production of a transmembrane carboxyl-terminal fragment, which is further processed to a smaller fragment (24 kD). Production of this peptide is abolished by a specific caspase-3 inhibitor, indicating the involvement of caspase-3. Marambaud et al. [35] extended the study of Steinhusen et al. [51] and they reported a novel PS1/ γ -secretase activity implicated in the proteolysis of E-cadherin after induction of apoptosis. Staurosporine treatment of A431 cells yielded three CTFs of E-cadherin (E-cad/CTF1, E-cad/CTF2, and

E-cad/CTF3). The E-cad/CTF1 is produced by the action of a matrix metalloproteinase after Pro700 residue at the extracellular domain of E-cadherin (fig. 1, right). Moreover, this MMP-mediated cleavage of the protein results in the release of an extracellular N-terminal fragment. E-cad/CTF1 is processed to the E-cad/CTF2 in a PS1/ γ -secretase-dependent manner, since the specific γ -secretase inhibitor L-685,458 completely blocked its production (fig. 1, right). Sequencing of the amino terminus of E-cad/CTF2 revealed that the cleavage takes place between amino acids 731–732 at the interface of the transmembrane and cytoplasmic domains of E-cadherin and corresponds to the ϵ -cleavage by PS1/ γ -secretase. Multiple lines of experimental evidence suggest a potent role of PS1/ γ secretase cleavage of E-cadherin in the disassembly of CAJs. Ionomycin induces a decrease of cytoskeletal associated (ionic detergent insoluble) E-cadherin, α - and β -catenin, and a subsequent increase of soluble cytosolic E-cad/CTF2, α - and β -catenin. Treatment with the γ -secretase inhibitor blocks the production of soluble cytosolic E-cad/CTF2 and inhibits the solubilization of the catenins. This data indicates that the PS1/ γ -secretase-dependent ϵ -cleavage that generates E-cad/CTF2 results in the dissociation of E-cadherin from the cytoskeleton and its release to the cytosol in complex with β -catenin, addressing its role in the disassembly of cadherin junc-

Fig. 2. PS1/ γ -secretase cleavage of N-cadherin is involved in CREB-mediated gene expression. PS1 interacts with N-cadherin and is localized at synaptic sites [37] (left of the vertical dashed line). Membrane depolarization or NMDA receptor agonists induce PS1/ γ -secretase cleavage of N-cadherin, leading to the production of N-cad/CTF2. N-cad/CTF2 represses CREB-mediated transcription, possibly by binding to CBP, and promoting its degradation [59] (right of the vertical dashed line). PS1 FAD mutations do not produce N-cad/CTF2, so there is no repression of CREB-dependent gene activation.

tions. It is interesting that PS1 possesses two distinct roles in relation with the functional integrity and stability of the adhesion: at sites of cell-cell contact it stabilizes the cadherin/catenin adhesion complex and under conditions that facilitate E-cadherin catabolism (apoptosis, Ca^{2+} imbalance), it regulates disassembly of the junctions (fig. 1). In addition, the PS1/ γ -secretase-dependent release of the complex E-cad/CTF2 with β -catenin possibly links the action of PS1 with transcription regulation that can take place after the release of β -catenin. Under certain conditions β -catenin can be released from its complex with E-cad/CTF2, translocate to the nucleus and induce transcription in complex with the lymphocyte enhancer-binding factor 1 (LEF-1)-responsive transcription factor [53]. These observations are in accordance with a study which indicates that β -catenin binding to the cadherin cytoplasmic tail either in the membrane or in the nucleus can inhibit β -catenin degradation and efficiently block its transactivation capacity [54].

Regulated Intramembrane Proteolysis, PS and Transcriptional Activation

Cell-surface receptors transmit their signals via ion influxes or complicated protein kinase signaling cascades. A number of receptors, however, are processed by a rather different signaling mechanism called regulated intramem-

brane proteolysis 'RIP' [55]. A number of studies have described RIP as two-step proteolysis receptors that release their intracellular fragments to the cytosol. These fragments alone or in association with other proteins can potentially translocate to the nucleus and regulate gene activation. An increasing piece of evidence suggests that the release of the intracellular fragments from cell surface receptors is PS1/ γ -secretase dependent and takes place at the interface of their transmembrane and cytoplasmic domains (ϵ -cleavage) [55]. Indeed, Notch, APP [56], receptor tyrosine kinase ErbB4 [57], cell adhesion molecules CD44 [58], E [35]- and N-cadherin [59] and low-density-lipoprotein-receptor-related protein (LRP) [60] have been reported to release their intracellular soluble fragment in a PS1/ γ -secretase-dependent manner.

One of the substrates undergoing PS1/ γ -secretase-dependent intramembrane proteolysis is N-cadherin. PS1 forms complexes with N-cadherin in neurons catalyzing the production of an N-cadherin C-terminal fragment (N-cad/CTF2) [59] after induction by membrane depolarization or stimulation of the NMDA receptor (fig. 2).

A number of different experimental procedures suggest that the N-cad/CTF2 interacts with CBP (CREB binding protein) and promotes its proteasomal degradation. CBP binds to CREB and regulates the initiation of the CREB-mediated transcription of target genes [61]. CREB is involved in a number of normal cellular events and diseases including human neurodegenerative disorders [61]

and among the genes that are expressed under its control are those implicated in the plasticity of the nervous system [62]. Since CBP is mainly localized in the nucleus, it has not been determined whether N-cad/CTF2 is able to 'recruit' the nuclear CBP pool to the cytosol or whether it interacts with newly synthesized CBP. The interaction of N-cad/CTF2 with CBP results in suppressed CREB-dependent gene activation, as reported by the specific reduction in the expression of the gene *c-fos*, which is controlled by the CREB/CBP activity. The above findings imply a model of PS-mediated transcriptional regulation (fig. 2). A remarkable observation is that a number of different PS1 mutants associated with AD do not promote N-cad/CTF2 production and fail to suppress CRE-mediated transactivation and *c-fos* expression.

Therefore, a key role for PS1 as a repressor of transcriptional activity is proposed, and could be related to a number of recent studies that point out the idea that PS1/ γ -secretase-dependent intramembrane proteolysis of receptors is a more generic mechanism of cell surface-to-nucleus signal transduction [55, 63].

AD and Transcriptional Dysregulation

The 'amyloid hypothesis' has been accepted for more than a decade as the main theory according to which the involvement of A β peptide, a proteolytic fragment of APP, is the main causative agent of the AD progression [64, 65]. However, a number of studies are inconsistent with this theory and argue that the amyloid hypothesis

does not adequately explain the correlation between amyloid deposition and the pathophysiology of the disease [66, 67]. Some of the most important arguments are the presence of brain amyloid deposition in nondemented elderly people [68] and the study of transgenic mice models, where mice expressing mutated forms of human APP and/or PS show only some of the neuropathological and behavioral abnormalities characteristic of AD [69–72]. However, the mice did not present all the symptoms of the disease and they cannot be regarded as having the AD phenotype. In addition, there is the fact that almost 95% of people having AD without APP and PS1 mutations cannot be accounted for by the amyloid hypothesis. It is, therefore, easy to appreciate that AD is a multifactorial neurodegenerative disorder and further studies are required in order to elucidate the causes of the neuronal function impairment of AD patients.

Thus, the involvement of PS1 in transcriptional regulation as described earlier enhances our view on the contribution of PS1 mutations to AD, suggesting that these mutations are responsible for neuronal dysfunction by impairing the activity of specific genes, and not only by interfering with β -amyloid production [73].

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