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Neurons bearing presenilins: weapons for defense or suicide?

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Abstract

Apoptotic machinery designed for cell's organized self-destruction involve different systems of proteases which cleave vital proteins and disassemble nuclear and cytoplasmic structures, committing the cell to death. The most studied apoptotic proteolytic system is the caspase family, but calpains and the proteasome could play important roles as well. Alzheimer's disease associated presenilins showed to be a substrate for such proteolytic systems, being processed early in several apoptotic models, and recent data suggest that alternative presenilin fragments could regulate cell survival. Mutations in genes encoding presenilins proved to sensitize neurons to apoptosis by different mechanisms e.g. increased caspase-3 activation, oxyradicals production and calcium signaling dysregulation. Here we review the data involving presenilins in apoptosis and discuss a possible role of presenilins in the regulation of apoptotic biochemical machinery.

Keywords: apoptosis - presenilin - caspase - neuron - Alzheimer's disease

Introduction

Apoptosis regulation has gained an increasing research interest in the last decade, based on two sides of a death coin: uncontrolled proliferation of cancer cells and extensive loss of cells in various pathologic situations. A possible 'beneficial' feature of apoptosis as compared with necrosis is an opposite temporal profile, in which hours are needed to complete the complex cellular breakdown, this providing the chance for a therapeutic rescue after the initiation of pathological stimuli. Many different molecules were identified to play a role as proapoptotic or antiapoptotic factors, but no key cellular signaling could be used for a suitable therapeutic intervention yet.

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Several lines of evidence place the Alzheimer's disease (AD) associated presenilins (PSs) among the proteins involved in apoptosis as caspase substrates and as cell survival regulators. PSs were brought into research focus by the finding that autosomal dominant familial AD (FAD) pedigrees showed to bear mutations of PSs genes. Considering that the molecular basis of AD were extensively reviewed elsewhere [1-3], this review will focus on the importance of PSs in the apoptosis machinery.

Presenilins structure and expression

In 1995, genes encoding presenilin1 (PS1) and presenilin 2 (PS2) were identified on chromosome 14 and chromosome 1, [4,5] and mutations of these genes were correlated with an important number of cases of familial Alzheimer disease (FAD). To date, more than 50 pathological mutations are known for PS1 (for review, see [2]) contrasting with only two mutations of PS2 detected in FAD cases [6]. PSs mutations showed also to sensitise cells to apoptosis triggered by several stimuli (Table 1), suggesting the hypothesis that neuronal loss in AD could be due to apoptosis.

PS1 and PS2 are integral proteins sharing 60% amino acid sequence homology and are predicted to contain 6 to 8 transmembrane (TM) domains [7]. Between TM domains 6 and 7 they contain a large hydrophilic loop, on the cytoplasmic side of the membrane. The cytoplasmic loop includes the sites where different proteolytic cleavages of PSs occur (Fig.1). A physiological endoproteolytic cleavage by an elusive protease known as 'presenilinase' occurs within the exon 9 region of PSs, between

Table 1 Experimental models where PSs mutations showed to sensitize cells to apoptosis triggered by different inducers.

	Mutation	Apoptosis inducer	Cells/animals	Reference
PS1	L286V	Trophic factor deprivation β-amyloid	PC12 cells	77
	L286V	β-amyloid	PC12 cells	78,79
	M146V	β-amyloid	PC12 cells	79
	H115Y	Anti-Fas receptor antibody	Jurkat cells	82
	L286V	3-nitropropionic acid malonate	PC12 cells	36
	M146V	β-amyloid	Rat hippocampal neurons	80
	M146V	β-amyloid	PC6 cells	81
	L286V	β-amyloid	PC6 cells	81
	A246E	None (spontaneous)	Rat hippocampal neurons	83
	A246E	None (spontaneous)	PC12 cells	83
	C410Y	None (spontaneous)	Rat hippocampal neurons	83
	C410Y	None (spontaneous)	PC12 cells	83
	A246E	Staurosporine	H4 cells	85
	L286V	Staurosporine	H4 cells	85
	Exon 9 deletion	Staurosporine	H4 cells	85
	L250S	Glucose	SH-SY5Y cells	86
	Exon 9 deletion	Glucose	SH-SY5Y cells	86
	Exon 9 deletion	Ca^{2+} ionophore A23187	SH-SY5Y cells	60
	M146V	Focal cerebral ischemia	PS1 mutant knock-in mice	87
PS2	N141I	Trophic factor deprivation β-amyloid	PC12	89
	N141I	None (spontaneous apoptosis)	HeLa	61

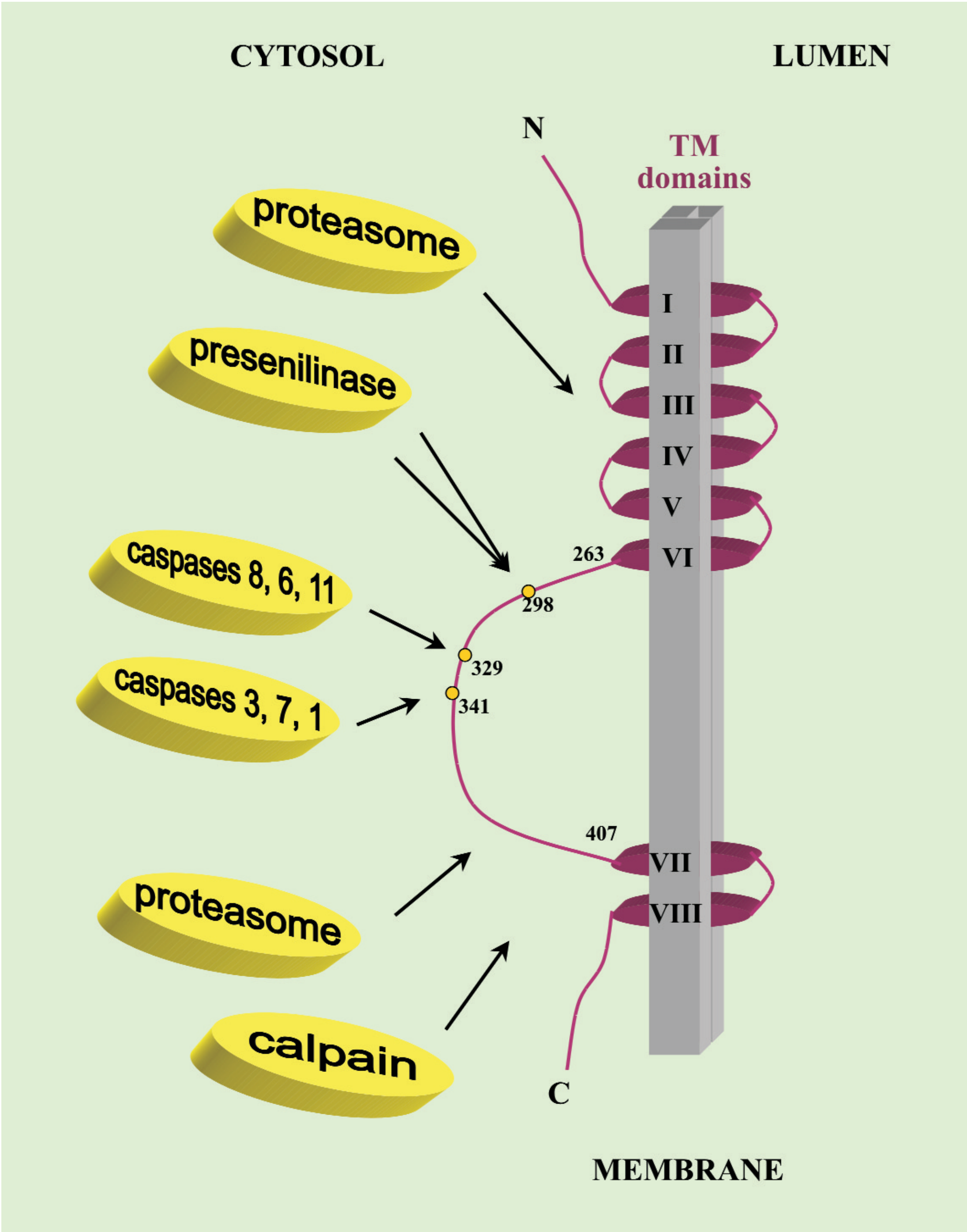

Fig. 1 Topology and proteolytic processing of PS1. PS1 is an integral protein, which contains 6-8 transmembrane (TM) domains. A large hydrophilic loop (between amino acids 263-407), the N-terminal and C-terminal regions of PS1 are located on the cytoplasmic side of the membrane. PS1 is physiologically processed by 'presenilinase' to N- and C-terminal fragments that are stable in heterodymeric complexes. Caspases cleave PS1 at two identified sites within the loop. When are not part of heterodymeric complexes, N- and C-terminal fragments of PS1 are degraded by the proteasome. Calpain, another protease involved in cell death, also attacks C-terminal fragment of PS1. The residues where PS1 is processed by proteasome and calpain are not yet known.

residues Thr_{291} and Ala_{298} [8]. From this cleavage result a ~30 kDa N-terminal fragment and a ~20 kDa C-terminal fragment, which accumulate in cells with a stoichiometry of 1:1 and represent the main PSs species in human tissues [8]. The levels of N-terminal and C-terminal PSs derivatives seem to be tightly regulated by limiting cellular factors [9] and overexpression of PSs holoproteins do not increase correspondingly the levels of N- and C-terminal fragments.

PSs are highly conserved among species, from flies like *Drosophila melanogaster* [10, 11] or worms like *Caenorhabtidis elegans* [12, 13] to mammals. There are no known proteins with a sequence similar to PSs in unicellular organisms.

PSs are ubiquitous proteins, being identified in most human organs, e.g. brain, lung, heart, liver and muscle [14]. Within the brain, PSs are present in variable amounts in all regions, mainly in the neocortex, hippocampal pyramidal neurons and magnocellular basal forebrain neurons [15, 16], areas affected by Alzheimer's disease. PS1 and PS2 mRNAs were identified to a greater extent in neurons but are also detectable in glial cells and no difference in the two related proteins distribution was found [15].

Within the cells, PSs are mainly located in endoplasmic reticulum (ER), Golgi apparatus [17, 18] and nuclear envelope [19] but they were also identified in various amounts in plasma membrane [20] and mitochondrial inner membrane (M.Ankarcrona, unpublished data). At the neuronal level, immunostaining detect PS2 exclusively within neuronal bodies while PS1 is present in cell bodies and dendrites [21].

Presenilins in development of nervous system

The nervous system development during embryogenesis is known to be a fascinating arena of neuronal network modeling by mitosis and apoptosis. Oppenheim showed that in several mammalian species approximately 50% of postmitotic neurons die naturally during embryonic or fetal development and their death is due to apoptosis [22]. Caspases, mainly caspase 9 and caspase 3, seem to actively participate in neural apoptosis of developing brain (for review, see [23]).

Several groups reported the identification of PSs within the developing nervous system of different species. Lee and co-authors showed by reverse-transcribed polymerase chain reaction (RT-PCR) a wide distribution of PS1 and PS2 mRNAs in the mouse and human developing brain, with a higher level of expression in neurons of hippocampus and entorhinal cortex [15]. Another interesting finding of their work was that PSs expression level is changing as a function of age. A different report [24] studied the expression of PS1 in developing rat nervous system and reached similar results and additional data that open new working hypotheses. First, they found that during late embryogenesis, PS1 was highly expressed at the level of the ventricular zone, which is probably shaped by apoptosis, as soon as caspase-9 deficient mice present obstructed lateral and third ventricles [25]. This specific location of PS1 expression rises the question whether PSs could be active in some pathway of the physiological apoptosis during nervous system development, which remains to be determined. Second, they noticed a peak of PS1 expression at postnatal day 10, mainly in the cerebellum and hippocampus, where during this period neuronal migration and synapse formation take place, finding that suggests a role of PS1 in neural network formation.

Presenilins and Ca^{2+} signaling

Calcium ion is broadly used as a signal transduction element by an enormous variety of cells, ranging from bacteria to neurons [26]. During the last decades it became a generally accepted fact that alteration of intracellular calcium concentration ($[Ca^{2+}]_i$) plays an important role in cell death. In neurons, rising of $[Ca^{2+}]_i$ can trigger necrosis, apoptosis or both [27], depending on the mitochondrial energy charge. There are multiple mechanisms mediated by Ca^{2+} in cell killing, e.g. mitochondrial dysfunction, activation of proteases, alteration of cytoskeletal network (for review see [28]). Calcium signalling deficits in aging brain were reported by many groups (for reviews see [29-32]).

In 1996, Guo and coworkers reported that L286V PS1 mutation perturbs Ca^{2+} homeostasis in

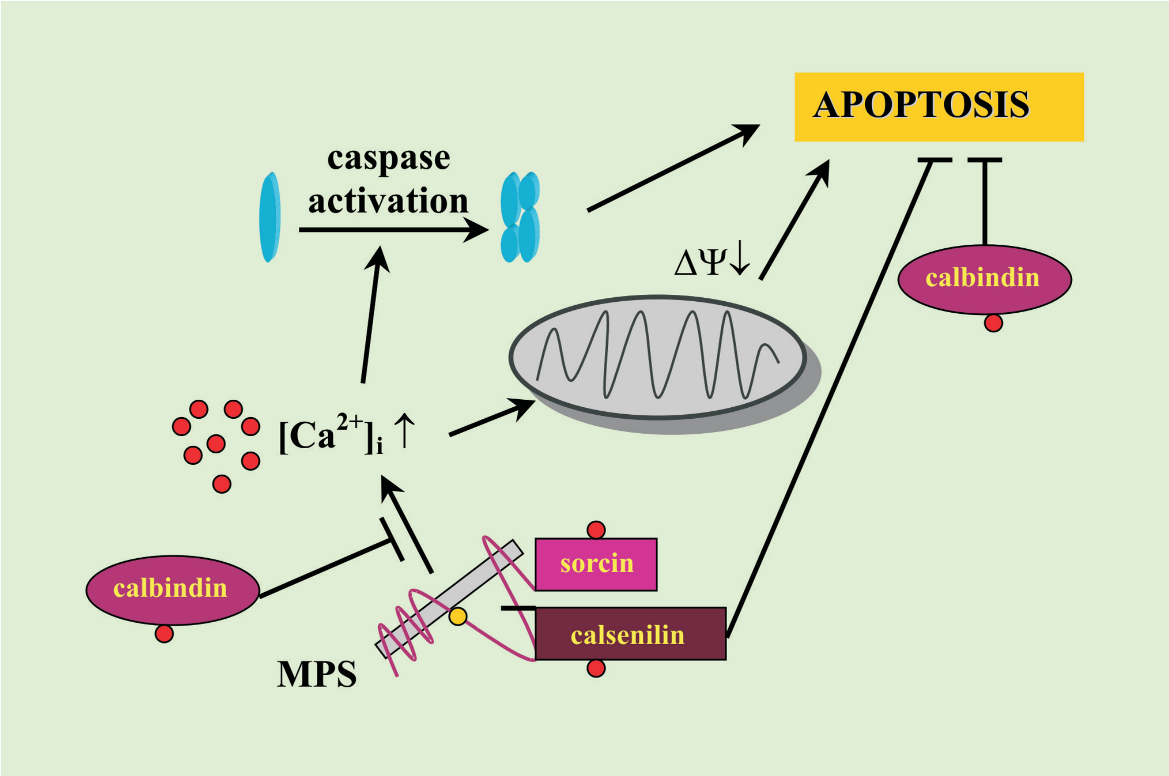

Fig. 2 Ca^{2+} signaling is altered by mutated PSs (MPS). MPS trigger a rise in $[Ca^{2+}]_i$, which activates caspases and decreases mitochondrial potential ($\Delta\Psi$), driving the cell to apoptosis. Calsenilin and sorcin are PSs and Ca^{2+} binding proteins with antiapoptotic properties. Calbindin is another Ca^{2+} binding protein, which counteract the proapoptotic effect of MPS and induce the cleavage of PS to a C-terminal fragment similar to ALG-3. Yellow dot represents the PS mutation. Red dots symbolize calcium ions.

PS12 cells, suggesting that PS1 mutations are resulting in a gain of altered function which cause dysregulation of Ca^{2+} signaling [33]. These data correlated with older reports that showed a significant enhancement of $[Ca^{2+}]_i$ in stimulated fibroblasts of AD patients as compared with fibroblasts from age-matched healthy subjects [34, 35]. During last years, several other lines of evidence sustained the theory that altered Ca^{2+} homeostasis could be one of the mechanisms by which PSs mutations are sensitizing neurons to cell death. When apoptosis was triggered in PC12 cells with a mitochondrial toxin (3-nitropropionic acid), cells expressing mutated PS1 showed a rapid elevation of $[Ca^{2+}]_i$, followed by caspase activation and decreasing in mitochondrial potential, facts correlating with their increased sensitivity as compared with wild type cells [36]. When overexpressed, the calcium binding protein calbindin was shown to prevent apoptosis induced by β-amyloid and serum deprivation in cells expressing mutated PS1 [37]. This fact proves not only that $[Ca^{2+}]_i$ is elevated in cells expressing mutated PSs, but also that binding Ca^{2+} results in apoptosis prevention in this paradigm. Not only mutated, but also wild type PS2 showed to change the threshold of Ca^{2+} signaling. A study in *Xenopus* oocytes demonstrated that both wild type and 2 mutant variants of PS2 significantly potentiated inositol 1,4,5-triphosphate (IP3)-evoked Ca^{2+} signals [38].

Recently, two Ca^{2+}-binding proteins, sorcin and calsenilin, were found to directly interact with PSs. Sorcin is a cytosolic protein identified in multidrug-resistant cells that share substantial homology with the light chain of calpain [39]. Sorcin is found in mammalian brain associated with ryanodine receptors [40] and coexpressed with N-methyl-D-aspartate receptors [41], both involved in Ca^{2+} signaling. PS2 interacts with sorcin [42] and this finding suggests a molecular connection between PS2 and regulation of Ca^{2+} homeostasis. In order to search for new proteins that interact with PSs, Buxbaum and coworkers screened a human brain cDNA library and identified a new Ca^{2+}-binding protein, which they named calsenilin [43]. Calsenilin proved to bind to the C-terminal sequence of both PS1 and PS2. Another exciting finding of this study was that when PS2 and calsenilin were co-expressed in neuroglioma cells, PS2 was processed to a C-terminal fragment of ~ 20 kDa to an amount which paralleled the level of calsenilin expression. This C-terminal fragment of PS2 corresponded to the caspase cleaved C-terminal of PS2, also known as ALG-3, shown to have an anti-apoptotic effect in specific cellular systems [44]. Taken together, these data are allowing the hypothesis that the PSs-calsenilin molecular interaction could be a significant target for therapy strategies. A hypothetical model of PSs and Ca^{2+} signaling interactions is presented in Fig.2. Beside Ca^{2+} homeostasis alteration, several other mechanisms were proposed to explain the involvement of mutated PSs in apoptosis (Table 2).

Presenilins interact with proteins involved in mitochondrial and cytoplasmic phases of apoptosis

Recent studies reported numerous proteins directly interacting with PSs (for review, see [45]). The protein interaction approach is important in order to understand the functions of PSs in normal and stressed cells, which are still subject of debate. Here we will mention only those PSs binding proteins involved in apoptosis.

Bcl-2

Bcl-2 protein and the members of its family regulate apoptosis at the molecular level, by inhibiting

Table 2 Cellular mechanisms triggered by mutated PSs in different apoptotic models.

Mechanism	PS mutation	Apoptosis blocking agents	Reference
Ca^{2+} homeostasis alteration	PS1L286V	Nifedipine, dantrolene	33
	PS1L286V	Bcl-2, cycloheximide	77
	PS1L286V, M146V	Calbindin D28k	79
	PS1L286V	Secreted β-APP	78
	PS1M146V	Xestospongin and dantrolene	87
	PS1M146V	-	90
Oxyradicals overproduction	PS1L286V	Propyl gallate, vitamin E	77
	PS1L286V	17β-estradiol	88
	PS1L286V	Propyl gallate, glutathione	36
	PS1L286V, M146V	Mn superoxide dysmutase, uric acid	81
Activation of Jun kinase	PS1H115Y	-	82
Caspase activation	PS1L286V	Z-Val-Ala-Asp(OMe)-CH_2F	36
	PS1M146V	Benzyloxycarbonyl-Val-Ala-Asp-fluoromethyl ketone	80
	PS1A246E, L286V, exon 9 deletion	Z-Val-Ala-Asp(OMe)-CH_2F	85
	PS1 exon 9 deletion	Z-Val-Ala-Asp(OMe)-CH_2F	60
Mitochondrial permeability transition disruption	PS1L286V	Cyclosporin A	36
Cytoskeleton breakdown (β-catenin destabilization)	PS1M146V, C410, H163R, I143T	-	54
	PS1A246E, C410Y	N-acetyl-leu-leu-norleucinal	85
Downregulation of Akt/PKB	PS1-A246E, PS1-C410Y	-	83
Perturbation of insulin-like growth factor-1 signaling	PS1L250S, exon 9 deletion	insulin-like growth factor-1	86

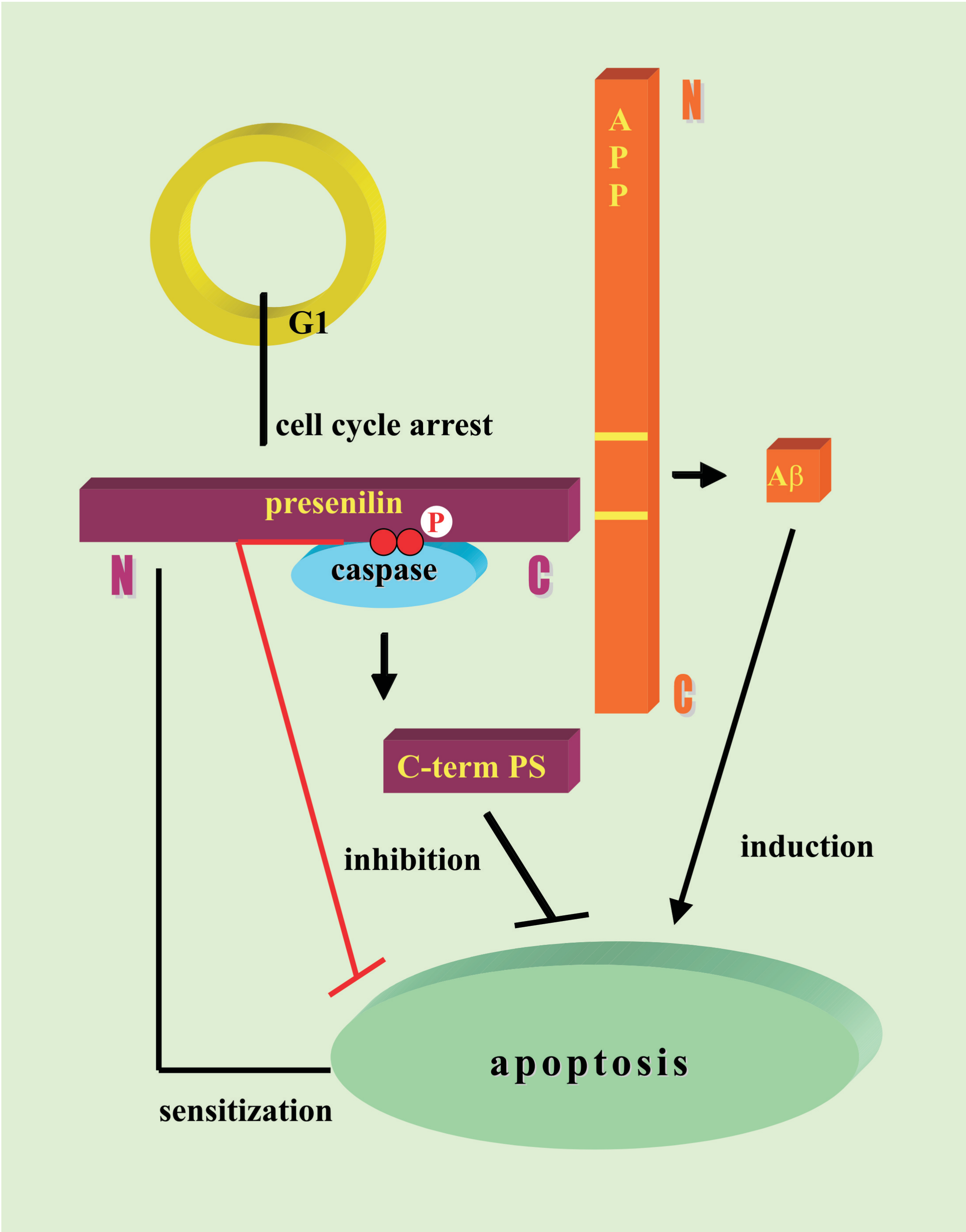

Fig. 3 Roles of PSs in apoptosis regulation. Overexpression of full-length PSs sensitizes cells to apoptosis induced by different stimuli and PS2 overexpression arrests cells in G1 phase of the cell cycle. Phosphorylation of PSs inhibits the caspase cleavage and slowers apoptosis progression. PSs caspase cleaved C-terminal fragment has an antiapoptotic effect. On the other hand, PSs exert γ-secretase activity, determining the amyloid β (Aβ) formation, which triggers apoptosis. (APP - amyloid precursor protein)

or promoting adapters that activate the caspase proteolytic cascade (for review see [46]). Alberici and colleagues reported in 1999 a direct interaction between Bcl-2 and PS1, using a yeast two-hybrid interaction system. Their hypothetical model propose a macromolecular complex containing Bcl-2 and PS1, which disassemble in response to an apoptotic stimulus, suggesting a cross-talk between Bcl-2 and PS1 during apoptosis [47]. Bcl-2 is located to inner membrane of the mitochondria, cytosol and nuclear membrane, but a functional concept of homo- and heterodimerization between the Bcl-2 family members was discussed linked to the mitochondrial localization [46]. Beside the location in ER and Golgi apparatus, PS1 seem also to localize to inner mitochondrial membrane (M. Ankarcrona, unpublished data), which is another piece of evidence supporting the idea that Bcl-2 and PS1 could functionally interact during apoptosis. Moreover, another anti-apoptotic member of Bcl-2 family, Bcl-X_L, was found to interact not only with PS1, but also with PS2 [48].

Catenins

The catenins are a family of proteins characterized by repeats of an amino acid sequence motif related to a *Drosophila Armadillo* gene product. Within the cells, catenins function in at least two ways. First, they constitute the link between actin filaments and cadherins, connecting the cytoskeleton to intercellular adhesive junctions. Second, they are switch molecules in the Wingless/Wnt signaling pathway, which is involved in development of nervous system and many other cell fate decisions (for review, see [49]). Several reports showed that β-catenin interacts with PS1 [50, 51] and the two proteins are found in high molecular weight intracellular complexes. Recently, Levesque and coworkers identified the PS1 and PS2 sequences that interact with β-catenin as being located within the cytoplasmic hydrophilic loop [52]. It was showed that not only β-catenin, but other members of catenins family, like p0071 [53] and neural plakophilin related armadillo protein (NRAP; [52]), interact with PSs. It is reported that β-catenin is more stable in complexes with PS1, and mutations of PS1 have a destabilizing effect [54]. Furthermore, β-catenin levels are decreased in AD brains [54]. Interestingly, β-catenin is also a substrate for caspases, its cleavage concurring to cytoskeletal breakdown during apoptosis [55].

Proteolytic processing of presenilins during apoptosis

Besides their physiological endoproteolytic processing, PSs are substrates for several caspases, two different caspase cleavage sites being recently identified for PS1 ($ENDD_{329}$ and $AQRD_{341}$) and one site for PS2 ($DSYD_{329}$). Caspases 8, 6 and 11 cleave PS1 after residues $ENDD_{329}$ and caspases 3, 7 and 1 after $AQRD_{341}$. PS2 is cleaved at the indicated site by caspases 8, 3, 1, 6 and 7 [56]. It was shown that calpains [57] and the 26S proteasome [58] could be responsible for other proteolytic cleavages of PS1. The role of this complex proteolytic processing of PSs (Fig.1) is not fully understood yet and two theories were formulated. The first hypothesis asserts that PSs are only ‘innocent bystanders’ in the way of activated proteolytic cascades during apoptosis. The second hypothesis state that PSs alternative fragments could play a role in signal transduction during apoptosis and is based on data shown by two different groups, which proved that PS1 [59] and PS2 [44] caspase cleaved C-terminal fragment delays apoptotic cell death in different cellular models. The observation that as compared with other caspase substrates PS1 caspase cleavage is an early apoptotic event (Popescu et al., submitted manuscript), suggests that PS1 alternative fragments could play a role in apoptosis regulation. In contrast, overexpression of full-length PS1 was reported to sensitize cells to β-amyloid induced apoptosis [33] and to calcium ionophore A23187 treatment (Popescu et al., submitted manuscript). When overexpressed, PS2 wild type holoprotein induced apoptosis in HeLa cells [61]. Recently, the same group showed that both PS1 and PS2 overexpression arrest cells in the G1 phase of the cell cycle, suggesting a possible mechanism of full-length PSs to sensitize cells to apoptosis [62, 63]. PS2 C-terminal fragment proved to be phosphorylated in vivo at residues 327 and 330, in the immediate proximity of caspase cleavage site and this phosphorylation inhibits the caspase cleavage of PS2 [64]. Phosphorylated PS2 showed also to slower apoptosis in HeLa cells treated with staurosporine [64].

These data suggest a negative feedback loop generated during apoptosis by PSs cleavage by caspases, in which the holoproteins act as a proapoptotic factor and alternative C-terminal generated fragments act as antiapoptotic factors (Fig.3). It remains to be determined if other alternative fragments of PSs, generated by different proteolytic systems, like calpains or the proteasome, play any role in apoptosis regulation.

β-amyloid generation depends on presenilins

β-amyloid peptide (Aβ) is the main component of the senile plaques, the neuropathological hallmark of AD. The source of β-amyloid is a 110-130 kDa type I integral protein, called β-amyloid precursor protein (APP). APP processing involve a complex chain of proteolytic events leading to different fragments, including Aβ (for review see [65]). Briefly, APP is degraded on two pathways: a) the α-secretase pathway, a protease that cleaves APP near residue 612, within Aβ sequence, in this way preventing the formation of Aβ; b) the β-secretase pathway, in which two proteases, β- and γ-secretases cleave APP near residues 596 and 637 respectively, giving rise to Aβ, a 4 kDa peptide with a 39 to 43 amino acid sequence.

Many studies show that Aβ induces neuronal apoptosis in different experimental models, supporting the involvement of apoptosis in neuronal loss in AD brains [66-69]. Therefore, genesis of Aβ peptide potentially represents a permanent apoptotic trigger in neurons sensitized by different factors, e.g. mutated PSs phenotype, which proved to enhance apoptosis induced by Aβ [33]. Moreover, it was reported that PSs mutations carriers show a higher Aβ plasmatic level as compared to non-carriers [70].

However, the cross-talk between PS1 and APP processing seems not to be restricted to these effects of PSs mutations on Aβ secretion or on neuronal sensitivity to Aβ. Recently, after the identification of β-secretase (BACE, Asp-2) structure [71, 72], several groups reported essential data regarding the γ-secretase activity. Embryonic stem cells generated from mice PS-null blastocysts show non-detectable Aβ production, which means a total abolition of γ-secretase activity [73, 74]. Furthermore, affinity inhibitors designed to bind to the active site of γ-secretase were found to bind covalently to PS1 [75, 76]. All these convincing data taken together make very probable the hypothesis that PSs could play in fact the role of the elusive γ-secretase.

If PSs exert γ-secretase activity, they could play complex and apparently contrasting roles in neuronal apoptosis. First, they actively participate in APP processing, and when mutated or under different cellular stresses they generate increased amounts of longer Aβ species, which could further trigger apoptosis in the neighboring cells. Second, in cells where apoptosis was triggered, PSs are proteolitically cleaved by caspases and they generate alternative fragments that have an antiapoptotic effect (Fig.3).

Conclusions

PSs were identified six years ago and data emerged due to their description led to a better understanding of AD pathogenesis. As for several other proteins, many lines of evidence support the PSs involvement in apoptosis regulation. If PSs are important only in cell death in AD or they have a more general implication in neurodegeneration or even in other pathological conditions involving apoptosis it remains to be established.

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