

CEREBRAL AMYLOID ANGIOPATHY AND ALZHEIMER'S DISEASE

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Abstract Cerebral amyloid angiopathy (CAA) is increasingly recognized as a major contributor of Alzheimer's disease (AD) pathogenesis. To date, vascular deposits and not parenchymal plaques appear more sensitive predictors of dementia. Amyloid deposition in and around cerebral blood vessels plays a central role in a series of response mechanisms that lead to changes in the integrity of the blood-brain barrier, extravasations of plasma proteins, edema formation, release of inflammatory mediators and matrix metalloproteases which, in turn, produce partial degradation of the basal lamina with the potential to develop hemorrhagic complications. The progressive build-up of amyloid deposits in and around blood vessels chronically limits blood supply and causes focal deprivation of oxygen, triggering a secondary cascade of metabolic events several of which involve the generation of nitrogen and oxygen free radicals with consequent oxidative stress and cell toxicity. Many aspects of CAA in early- and late-onset AD –the special preference of A β 40 to deposit in the vessel walls, the favored vascular compromise associated with many A β genetic variants, the puzzling observation that some of these vasculotropic variants solely manifest with recurrent hemorrhagic episodes while others are mainly associated with dementia– await clarification. Non-A β cerebral amyloidosis reinforce the viewpoint that plaque burden is not indicative of dementia while highlighting the relevance of non-fibrillar lesions and vascular involvement in the disease pathogenesis. The lessons learned from the comparative study of A β and non-A β cerebral amyloidosis provide new avenues and alternative models to study the role of amyloid in the molecular basis of neurodegeneration.

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Alzheimer's disease (AD) is the most common form of dementia in humans over the age of 65, affecting more than 50% of individuals 85 or older. It is a debilitating neurodegenerative disorder that affects millions of people constituting one of the major Public Health concerns in all developed countries. Neuropathological hallmarks of AD are the presence of intraneuronal neurofibrillary tangles (NFT) -deposits of hyperphosphorylated protein tau in the form of paired helical filaments-together with the existence of parenchymal extracellular deposits composed of both diffuse pre-amyloid lesions and mature amyloid plaques.

Although its significance was ignored for decades, together with these lesions, fibrillar amyloid deposition is also commonly observed in medium-sized and small cerebral vessels, a feature known as Cerebral Amyloid Angiopathy (CAA). Cerebrovascular and parenchymal lesions are composed of self-aggregates of A β protein generated by proteolytic cleavage of a larger precursor APP by the so-called β and γ secretases¹⁻³⁾. In difference to β -secretase, the multiprotein γ secretase complex has broader specificity and is able to cleave APP at multiple sites within its transmembrane domain generating A β peptides ranging in length from

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38 to 42 residues⁴). Nearly 90% of secreted A β ends at residue 40, whereas A β 42 accounts for only <10%, and peptides ending at residues 38 are minor components⁵). Notably, the pattern and distribution of these species varies among the different topographical lesions. Parenchymal deposits consist of A β 42 as the major component, whereas vascular A β - particularly in the large leptomeningeal vessels - is primarily composed of A β 40 species organized in large concentric sheets and replacing the smooth muscle cell layer⁶). Amyloid associated with arterioles and small cortical arteries contains a mixture of A β 40 and A β 42, while deposits affecting the capillary network are mainly composed of A β 42⁷). The reasons for this selectivity as well as its importance for the pathogenesis of the disease remain unclear.

A β and non-A β CAA genetic variants

Although the most common protein associated with amyloid deposition in the vasculature is by far A β present in sporadic late-onset AD as well as in normal aging, numerous hereditary conditions are known today which primarily associate with CAA. These familial diseases are characterized by the presence of genetic variants of both A β and non-A β proteins as the main components of the CAA deposits. Among the many A β variants described today, those located in the hot spot comprised between positions 21-23 typically show strong vascular compromise and primarily associate with CAA, hemorrhagic strokes and dementia. The first described and likely the most studied of these variants is A β E22Q, found in a condition known as hereditary cerebral hemorrhage with amyloidosis, Dutch type (HCHWA-Dutch)^{8,9}). Carriers of the mutation develop recurrent episodes of cerebral hemorrhages correlating with massive amyloid deposition in the walls of leptomeningeal and cortical arteries and arterioles as well as in vessels in the brainstem and cerebellum¹⁰), a

phenotype recapitulated, albeit at old age, in transgenic mice carrying the mutation¹¹). In addition to the vascular involvement, parenchymal amyloid deposits resembling the diffuse preamyloid lesions seen in AD are also observed in Dutch familial cases, while dense-core plaques and neurofibrillary tangles are rare or even completely absent⁸).

To the moment, of the dozen of A β genetic variants reported half of them are located within the 21-23 amino acid cluster. Interestingly, only one of the intra-A β variants located outside this region of the molecule -the L34V Piedmont mutant- associates with cerebral hemorrhage and exhibits a comparable, albeit less aggressive, clinical phenotype to A β E22Q. The clinical features of the disease include recurrent hemorrhagic strokes, weakness and parasthesias together with confusional states. Cognitive impairment is infrequent as a presenting symptom but is observed after various episodes of intracerebral hemorrhages. Neuropathological examination of the few available cases showed severe CAA with compromise of small and medium-size arteries as well as capillaries in all lobes of the brain, particularly the occipital and cerebellar regions. The vascular involvement includes vessel-within-the-vessel configurations, microhemorrhages, microaneurisms, microthrombi, and lymphocytic infiltration of the vessel walls. Diffuse and dense-core plaques as well as neurofibrillary pathology were notably absent¹²).

A number of familial forms of CAA are caused by deposition of non-A β amyloid proteins. The deposited molecules comprise an array of different molecules including cystatin C, gelsolin, transthyretin, prion, and BRI-2 related proteins. For space constraints we will limit our review to two of these categories which exemplify the striking diverse clinical phenotypes associated with CAA. In one extreme cystatin C-CAA,

which correlates with massive cerebral hemorrhages in the other end, BRI2-related dementias, which in spite of the severe vascular involvement, correlates only with cognitive impairment in total absence of hemorrhagic episodes.

Cystatin C-related cerebral amyloidosis

Hereditary cerebral hemorrhage with amyloidosis, Icelandic type (HCHWA-Icelandic), is a disorder described in individuals from small rural communities of Iceland¹³. The disease is associated with a point mutation that translates into a Leu for Gln change at position 68 of cystatin C¹⁴, a ubiquitously expressed inhibitor of cysteine proteases. The 110-residue-long amyloid subunit constituting the amyloid deposits not only bears the mutated amino acid residue but it is also degraded at the N terminus, starting at position 11 of the normal cystatin C^{15,16}. The main clinical hallmark of the disease is cerebral hemorrhage with fatal outcome in the third to fourth decade of life in approximately 50% of the cases. Cognitive decline followed by dementia may occur in those cases that survive the hemorrhagic episodes. Neuropathologically, the mutation is associated with massive amyloid deposition within small arteries and arterioles of leptomeninges, cerebral cortex, basal ganglia, brainstem, and cerebellum. Although brain involvement is the main clinicopathological feature, silent amyloid deposits have also been described in peripheral tissues, such as skin, lymph nodes, spleen, salivary glands, and seminal vesicles.

The biochemical and structural properties of the variant form of cystatin C have been extensively studied. The mutated residue is located within the hydrophobic core of the protein and the amino acid substitution affects the stability of the molecule destabilizing alpha-helical structures and yielding a more unfolded molecule with higher tendency to form dimeric assemblies compared with the

wild type counterpart¹⁷. The crystal structure of the molecule revealed that dimerization occurs through 3-dimensional domain swapping¹⁸ and, in turn, through dimer association, results in the formation of larger amyloid-like structures with involvement of intermolecular β -sheet interactions¹⁹. The N-terminal truncation of the molecule found *in vivo* in the amyloid deposits of the Icelandic patients seems not to be crucial for the overall domain-swapped dimer formation; however the absence of the N-terminal decapeptide appears to facilitate the subsequent association of the protein via β -sheet interactions through intermolecular contacts²⁰.

BRI2 gene-related dementias

This novel group of hereditary disorders, also known as chromosome 13 dementias, and composed of familial British and Danish dementias (FBD and FDD, respectively), presents with cognitive impairment as one of the main defining clinical phenotypes^{21,22}. Both diseases, share many features with AD including the presence of neurofibrillary tangles, parenchymal preamyloid and amyloid deposits, extensive cerebral amyloid angiopathy and a variety of co-localized amyloid-associated proteins and inflammatory components. These early-onset conditions, as described below, are linked to specific mutations at or near the stop codon of the *BRI2* gene that cause generation of longer-than-normal protein products.

Interesting new findings have demonstrated a cross-talk between BRI2 and APP that could bring new lights into the molecular mechanisms underlying neurodegeneration. Although BRI2 has still an unknown function, it was recently shown to specifically interact with APP. As a result of this binding interaction BRI2 masks the cleavage sites of β - and α -secretase on APP and the γ -secretase docking site on the APP C-terminal fragment C99. As a consequence, BRI2 modulates APP processing inhibiting A β formation and deposition properties, a feature

observed in both cell culture and mouse models of AD²³⁻²⁶.

Familial British dementia

This hereditary disorder is the first described cerebral amyloidosis in the Western world²⁷ and affects an extensive pedigree of British origin which spans over nine generations²¹. The disease presents typically around the fifth decade of life, being its earliest manifestations personality changes followed by cerebellar ataxia and spastic paralysis. In the end, all patients progress to a chronic vegetative state becoming mute, unresponsive, quadriplegic, and incontinent. Neuropathologically, FBD cases exhibit severe and widespread amyloid angiopathy of the brain and spinal cord and characteristic perivascular changes including abundant vessel-associated-amyloid plaques. Notably, despite the extensive amyloid deposition in the vasculature, large intracerebral hemorrhage is a rare feature. Neuritic and non-neuritic amyloid plaques affect cerebellum, hippocampus, amygdala, and occasionally, cerebral cortex. Neurofibrillary degeneration is indistinguishable from that observed in AD cases. As it occurs in other forms of non-A β cerebral amyloidosis, FBD presents systemic thioflavin-S-positive deposits in many organs²⁸; however, this systemic deposition appears to be asymptomatic since clinical phenotypes of all described cases are only related to the cerebral compromise.

The disease is associated with a T to A substitution at codon 267 of *BRI2* which results in the presence of an Arg residue in place of the stop codon normally occurring in the wild-type precursor molecule and a longer open-reading frame of 277 amino acids instead of 266²⁹. Furin-like processing of this longer precursor releases a 34 amino acids-long C-terminal fragment, named ABri, which is found constituting the characteristic cerebral and systemic deposits in FBD^{28,29}.

Familial Danish dementia

Familial Danish dementia (FDD) is an early onset disorder originating in the Djursland peninsula in Denmark and also associated with a genetic mutation in the *BRI2* gene³⁰. A 10-nucleotide duplication insertion mutation between codons 265 and 266 abolishes the normal stop codon and results, as in the case of FBD, in an extended precursor protein composed of 277 amino acids instead of the normal 266^{30,31}. The 34-amino-acid-long C-terminal peptide ADan, as the ABri counterpart, is cleaved from the mutated precursor protein by a furin-like process, and readily forms the deposited amyloid fibrils. The disease, identified in a single family and spanning three generations, is clinically characterized by the development of cataracts, hearing loss and progressive cerebellar ataxia before the age of 40 with subsequent paranoid psychosis and dementia. Death occurs in most patients during their fifth or sixth decade. Neuropathological characteristics, similarly to those seen in FBD, include widespread amyloid angiopathy and severe neurofibrillary compromise which is more pronounced than that seen in FBD. Abnormal neurites, as seen in some other forms of CAA, mainly cluster around the vascular deposits and are absent around nonfibrillar diffuse parenchymal lesions³².

An interesting feature observed in FDD cases is the deposition of variable amounts of A β in blood vessels and, to a lesser extent, in brain parenchyma in the form of pre-amyloid deposits, either in combination with ADan or in isolated lesions³³. Biochemical analysis of brain extracted amyloid revealed that CAA deposited A β is an N-terminal truncated form of A β 42, a surprising finding in view of the prevalence of A β ending at position 40 in vascular deposits observed in sporadic and familial AD, Down syndrome, and normal aging³⁴. Detailed mass spectrometry analysis of extracted brain amyloid revealed that the deposited ADan species, similarly to

the ABri counterparts, are post-translationally modified at the N-terminus^{28,34}, as described in detail below.

Biochemical features of CAA deposits

The biochemical composition of the amyloid lesions has been assessed at the molecular level in only a few instances, being the A β -Dutch deposits perhaps the most thoroughly studied^{35,36}. The currently available data indicate that all CAA deposits share a number of biochemical similarities irrespective of the nature of the primary component of the deposits being the high heterogeneity one of the most striking characteristics.

Co-deposition of mutated and wild type subunits

Familial cases associated with specific mutations exhibit a remarkable co-deposition of mutated and non-mutated species, a feature observed in A β hereditary forms³⁶ and prion diseases³⁷, as well as in systemic forms of amyloidosis³⁸⁻⁴². It is likely that the deposition of the respective mutated species exerts a seeding effect –or a conformational mimicry– enhancing the fibrillization and subsequent co-deposition of the wild type counterparts, as previously proposed^{34,43,44}. Other common finding among the different familial CAA forms is the presence of numerous post-translational modifications, and an abundance of associated components, known as amyloid associated proteins.

Post-translational modifications

Among the many post translational modifications identified in cerebral amyloidoses, phosphorylation, isomerization, racemization, oxidation and cyclation occurring in conjunction with proteolytic fragmentation are the most relevant^{6,28,34,45-48}. Hyperphosphorylation is the most common post-translational modification of intracellular tau composing the paired helical filaments accumulating as NFTs in

several neurodegenerative disorders⁴⁹. Heavy isomerization and racemization of the aspartyl residues at positions 1 and 7 have been reported for A β with more than half of the residues being composed of L-isoAsp in some AD cases⁴⁵. A similar modification of Asp residues was found in the Iowa FAD variant cases⁵⁰. Additional isomerization at Asp 23 was reported not only in sporadic AD⁵¹ but also in Iowa patients⁵², although at a seemingly lower ratio than in the other positions. Our own biochemical studies showed that overall ~10 to 30% of the total deposited molecules in Iowa- brain tissues contain isoAsp23. In general terms, Asn deamidation with the consequent generation of isoAsp is associated with conformational changes and aging^{53,54}. In this sense, not only do A β molecules bearing isoAsp23 show enhanced *in vitro* fibrillization kinetics compared to wt molecules⁵¹, but the presence of isoAsp typically translates into a decreased sensitivity for proteolytic degradation⁵⁵, features that may well contribute to disease pathogenesis.

Perhaps one of the most frequent post-translational modifications is the formation of N-terminal pyroglutamate which is also believed to confer resistance against N-terminal peptidases delaying and/or preventing their removal from the lesions. Interestingly, the formation of pyroglutamate seems to take place via two different molecular mechanisms according to the N-terminal amino acid involved. If the amino acid is glutamine, as observed with many immunoglobulin molecules, pyroglutamate arise through a process of deamination; however, if the N-terminus is aspartic acid, as seen in A β species truncated at position 3 and 11 as well as in the ABri and ADan molecules, pyroglutamate formation occurs through a process of dehydration^{28,34}. According to some reports, pyroglutamate- containing peptides account for more than 50% of the truncated A β species

accumulated in plaques, being particularly enriched with A β 3(pE)40/42^{46,56}, a component not only present in senile plaques but also in pre-amyloid lesions⁵⁷. A β 3(pE) appears more resistant than full-length peptides to proteolytic cleavage *in vitro*, confers enhanced tendency to oligomerization⁵⁸, and exerts higher toxicity for both neuronal and glial cell cultures⁵⁹. Pyroglutamate derivatives of A β are not major species in plasma or CSF, suggesting that their formation takes place at the site of deposition. In cases of familial British and Danish dementias, circulating ABri and ADan peptides feature only glutamate at their N-terminus whereas their pyroglutamate counterparts are the heavily dominant species in the amyloid deposits. Since the conversion to pyroglutamate is chemically stable and poorly reversible, the presence of glutamate-only species in plasma is a clear indication that the circulating material does not represent a clearance mechanism for the cerebral deposits, but rather their immediate precursors²¹. Another post-translational modification, the oxidation of methionine (in the form of methionine sulfoxide) at position 35 of the A β peptide has been widely documented in AD⁶⁰⁻⁶³. Interestingly, A β peptides containing Met35 sulfoxide are able to induce oxidative stress and are more cytotoxic⁶⁴.

Amyloid associated proteins

Complex mixtures of unrelated molecules, collectively known as amyloid associated proteins, colocalize with all amyloid deposits (including those of A β) not being a structural part of the final fibril [reviewed in ref 2]. Serum amyloid P component (SAP), α 1-antichymotrypsin (ACT), apoE, apoJ, complement components, vitronectin, glycosaminoglycans, interleukins and extracellular matrix proteins are among the many amyloid associated proteins so far described in all forms of cerebral and systemic amyloidosis^{21,65-73}. To the moment, it is still

unclear whether these molecules are innocent bystanders or whether their presence is related to the mechanism of amyloidogenesis. Several lines of investigation favor the latter notion, at least for some of them. For example, apoE and SAP have been found in all immunoglobulin-light-chain fibrillar deposits whereas their presence cannot be demonstrated in the non-fibrillar, Congo red negative, immunoglobulin deposits in cases of Light Chain Deposition Disease^{74,75}. Similar findings have been reported in A β and non-A β cerebral amyloidosis in which SAP and activation-derived products of the complement system are present in amyloid deposits but consistently absent in non-fibrillar pre-amyloid lesions^{74,76}. Mice knockout for either SAP or apoE exhibit fewer amyloid lesions and delay in their onset although neither SAP nor apoE gene ablation prevents the formation of amyloid deposits^{77,78}. Studies mostly limited to A β indicate that many amyloid associated proteins have also the ability to modulate the formation of amyloid fibrils *in vitro*. Some of them (e.g. C1q, apoE4, SAP, ACT) enhance A β fibril formation⁷⁹⁻⁸³ while others (e.g. apoJ) contribute to the peptide solubility precluding fibrillogenesis *in vitro*^{68,84}. In the latter, this protecting effect has been proposed to contribute to the enhanced production of slowly sedimenting A β -derived diffusible ligands (ADDLs) highly toxic to neurons in culture at nanomolar concentrations⁸⁵.

CAA contribution to disease pathogenesis

It is clear that more than a single factor is associated with the pathogenic mechanisms resulting from cerebral amyloid deposition. Mounting evidence indicates that, in addition to a direct effect on cell viability related to the formation of oligomers / protofibrils and their ability to assemble into functional ion channel-like structures in lipidic environments, amyloids are also able to induce apoptosis/necrosis, to trigger

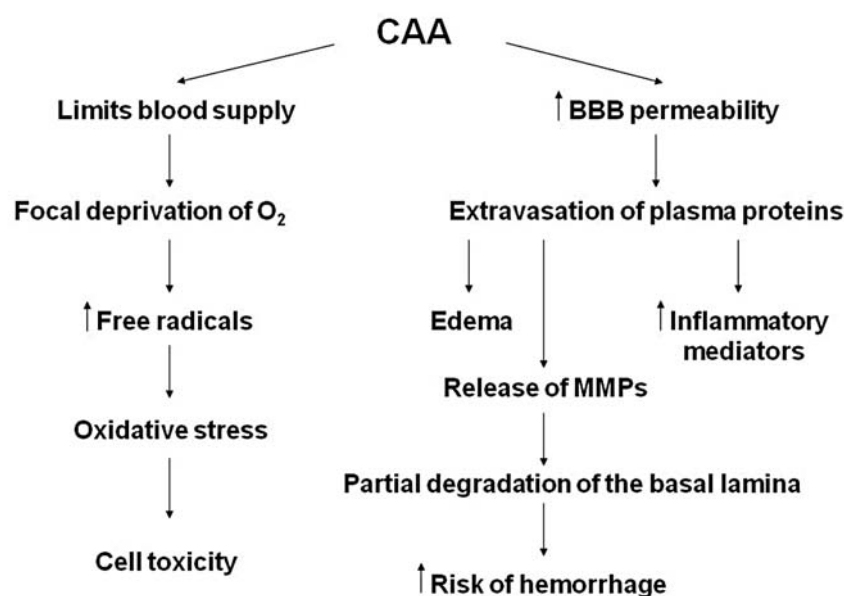


Figure 1 Molecular mechanisms associated with amyloid deposition in the cerebrovascular vessels. Schematic representation of the diverse molecular pathways affected by CAA, leading to stroke, ischemia, and the development of hemorrhagic complications.

oxidative stress, to generate an inflammatory response and/or to activate the complement cascade. In turn, these pathways, separately or synergistically, are capable to produce different levels of cell damage and modulate cytotoxicity.

Focal ischemia, oxidative stress, inflammation, and hemorrhagic complications

The vascular dysfunction resulting from amyloid deposition at the cerebral vessel walls is considered today an active player in the mechanism of neurodegeneration and a major contributor to the disease pathogenesis⁸⁶⁻⁹⁰. CAA can actively contribute to cognitive decline as a result of the deposit-related cerebral ischemia and microhaemorrhagic complications⁹¹⁻⁹⁷. As illustrated in **Figure 1**, amyloid deposition in the cerebral vessel wall limits blood supply causing a focal deprivation of oxygen. The resulting focal ischemia, in turn, elicits the release of free radicals inducing oxidative stress mechanisms and leading to enhanced cellular toxicity. Degenerative changes secondary to amyloid deposition also predispose blood vessels to rupture, which

is a major cause of spontaneous, frequently recurrent, lobar cerebral haemorrhages in the elderly⁹⁸. The alteration in blood brain barrier permeability caused by the deposits is aggravated by the induction of inflammatory mechanisms and release of proteases, in particular matrix metalloproteases, which contribute to the partial degradation of the basal lamina and subsequent hemorrhagic complications.

CAA and apoptotic bio-signaling

Recent studies have also brought light into the intricate molecular mechanisms elicited by specific CAA proteins on cells of the cerebral vessel wall. **Figure 2** illustrates the two main pathways -extrinsic and intrinsic- leading to apoptosis in mammalian cells. The latter, modulated by the Bcl-2 family of proteins and typically initiated by oxidative stress and calcium dysregulation, involves mitochondrial outer membrane permeabilization allowing the release of proteins, including cytochrome c, to the cytoplasm. These events, in turn, facilitate downstream cell death cascades leading to sequential activation of

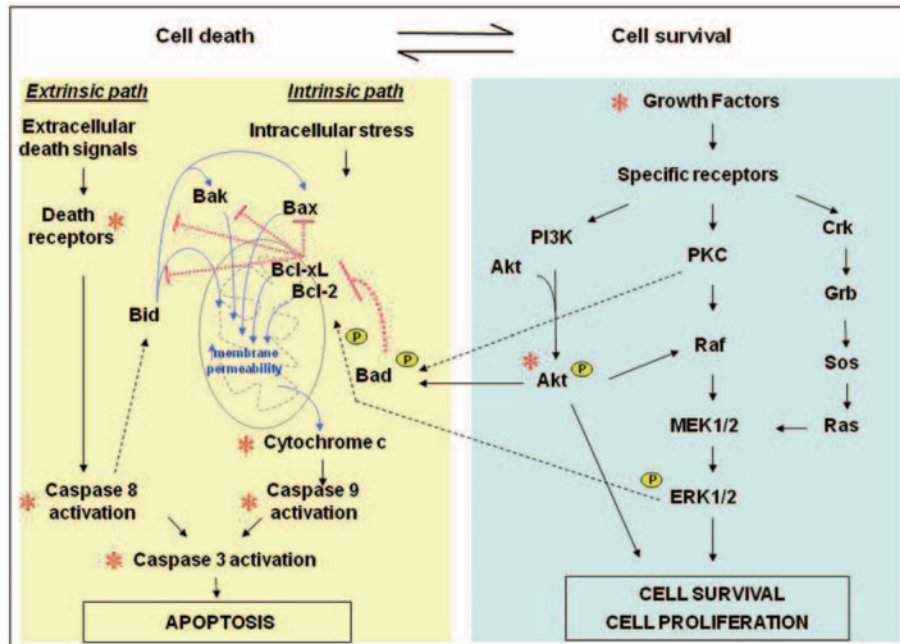


Figure 2 Cellular apoptotic pathways.

Left panel: diagram of the molecular events triggered by the induction of intrinsic and extrinsic apoptotic pathways. *Right panel:* schematic representation of the main cell survival and proliferation mechanisms. * indicates the molecular events affected by CAA-amyloid in cerebral vessel wall cells.

caspase-9 followed by the effector caspase-3, DNA fragmentation and formation of apoptotic bodies. The extrinsic path, normally activated through specific cell receptors, involves multiple partners and complex mechanisms and is typically centered in the initiator caspase-8 before the downstream activation of effector caspases common to both intrinsic and extrinsic pathways⁹⁹. Both mechanisms are not completely independent and, once activated, caspase-8 can also result in involvement of the mitochondrial path through its proteolytic effect on Bid leading to Bax translocation, oligomerization and insertion onto the mitochondria with subsequent leakage of cytochrome c. The PI3K pathway and its downstream effector Akt play a primordial role in EC homeostasis constituting active participants in the cross-talk between the cell survival and apoptotic mechanisms. Phosphorylated Akt allows Bad phosphorylation which, in turn, inhibits the activation of Bcl-2 and Bcl-xL, precluding the translocation of Bid, Bax and Bak to the

mitochondria and, therefore, preventing the activation of the intrinsic apoptotic pathway. Under physiologic conditions cell homeostasis is tightly maintained through a rigorous control of the balance regulating survival and apoptotic signaling.

Recent data demonstrate that A β vasculotropic variants are strong inducers of the apoptotic mitochondrial pathway in cells composing the vessel wall, and highlight their role in various downstream cascades including Bax translocation and cytochrome c release to the cytosol. These events subsequently trigger caspase-9 activation, followed by activation of caspase-3, and downstream DNA fragmentation¹⁰⁰⁻¹⁰². Contributing to the induction of apoptosis our studies also indicate that A β peptides exert an additional powerful effect on Akt phosphorylation downregulating in this way cell survival signaling with the concomitant decrease in EC angiogenesis, and enhancement

of apoptosis induction¹⁰¹.

Lessons from A β and non-A β cerebral amyloidoses

The mechanisms regulating cerebral amyloidosis in general and CAA in particular, are undoubtedly very complex and likely interlink a diversity of mechanistic pathways. Although AD is by far the most common and best studied form of amyloid-related disorders in humans, recent findings on the molecular mechanisms of brain degeneration have demonstrated common features among this heterogeneous group of disorders. Histopathologic, genetic, biochemical, and physicochemical studies, together with the generation of transgenic animal models, strongly support the notion that this diverse group of pathological entities are caused by the abnormal folding, aggregation/fibrilization and subsequent tissue accumulation of particular proteins, specific for each disorder. Shared features among the different CAA-associated disorders bridge together this wide range of pathological entities and suggest unifying mechanisms of disease pathogenesis. The analysis of these common elements, detailed above, highlight a number of features: i) Amyloid peptides are of primary importance in the process of neurodegeneration, ii) The process is not restricted to A β ; other amyloid subunits are associated with similar –if not identical– neuropathological changes leading to the same scenario: neuronal loss and dementia, iii) NFTs and amyloid/pre-amyloid deposits not always co-exist and/or co-localize, pointing to a differential vulnerability for the various neuronal populations, iv) These striking neuropathological similarities in spite of differences in the amyloid subunits, are suggestive of common pathogenic pathways, v) The mechanism of neurodegeneration is complex and likely involves additional unknown factors or interrelationships among different cellular pathways. Overall, it can be envisioned that the lessons learned from

the study of non-A β cerebral amyloid disorders may bring light to the field of neurodegeneration by providing alternative models to study the molecular basis of neuronal cell death, cerebral hemorrhage, and neurodegeneration.

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