Published in final edited form as:

Acta Neuropathol. 2010 May ; 119(5): 523–541. doi:10.1007/s00401-010-0679-9.

Intraneuronal β -amyloid accumulation and synapse pathology in Alzheimer's disease

Gunnar K. Gouras¹, Davide Tampellini¹, Reisuke H. Takahashi², and Estibaliz Capetillo-Zarate¹

¹Department for Neurology and Neuroscience, Weill Cornell Medical College, New York, NY, USA

²Department of Pathology, Tokyo Medical University Hospital, Tokyo, Japan

Abstract

The aberrant accumulation of aggregated β -amyloid peptides (A β) as plaques is a hallmark of Alzheimer's disease (AD) neuropathology and reduction of A β has become a leading direction of emerging experimental therapies for the disease. The mechanism(s) whereby A β is involved in the pathophysiology of the disease remain(s) poorly understood. Initially fibrils, and subsequently oligomers of extracellular A β have been viewed as the most important pathogenic form of A β in AD. More recently, the intraneuronal accumulation of A β has been described in the brain, although technical considerations and its relevance in AD have made this a controversial topic. Here we review the emerging evidence linking intraneuronal A β accumulation to the development of synaptic pathology and plaques in AD, and discuss the implications of intraneuronal β -amyloid for AD pathology, biology, diagnosis and therapy.

Introduction

The anatomically selective and differential accumulation of A β plaques and neurofibrillary tangles (NFTs) are neuropathological hallmarks of AD [193, 207]. Brains of those afflicted by AD are additionally characterized by aging-related cerebral atrophy, loss of neurons, inflammation and typically also amyloid angiopathy. Although not routinely analyzed in clinical neuropathological examination of AD brains, research studies have reported that loss of synaptic markers, specifically of the pre-synaptic protein synaptophysin, is the best brain correlate of cognitive decline in AD [40, 42, 168, 184, 192]. Over the past two to three decades significant progress has been made in understanding the genetics, biology and pathology of AD [11, 44, 65, 148]. The short, hydrophobic, and up to 42/43 amino acid long A β peptide has taken center stage in AD research, with the majority of emerging experimental therapies directed at reduction of cerebral A β . Although traditionally viewed as causing only extracellular pathology, the past decade has provided increasing evidence for a critical role for the accumulation of A β peptides within neurons.

Central role of A β in Alzheimer's disease

The A β peptide moved to the center of AD research when converging genetic, biological and pathological clues pointed to its importance. Specifically, all autosomal dominant

Correspondence: Dr. Gunnar K. Gouras, Department of Neurology and Neuroscience, Weill Cornell Medical College, New York, NY 10065, USA, Phone: 1 212 746-6598, Fax: 1 212 746-6598, gkgouras@med.cornell.edu.

Conflict of interest statement: The authors declare that they have no commercial or financial relationship that could be construed as a potential conflict of interest.

mutations associated with rare familial forms of early onset AD in the amyloid precursor protein (APP) and presenilin (PS) 1 and 2 were shown to increase the proportion of A β 42 to A β 40 peptides. In addition, the added copy of APP in trisomy 21 was known to be associated with the early and invariable development of AD pathology in Down syndrome [52]. Moreover, familial AD (FAD) associated with triplication of wild type APP was reported [153]. Current evidence argues against another APP cleavage product being more important than specifically the A β 42 isoform in AD. First, the location of FAD mutations in APP point to the role of A β , since they localize either to the β - or γ -cleavage sites in APP involved in A β generation or within the A β domain itself (Fig. 1). Second, changes in other APP metabolites, such as the APP intracellular domain (AICD) and APP C-terminal fragments (CTFs)[161], are not as consistent as the increased A β 42 to A β 40 ratio in FAD [71]. At the same time, the most important genetic risk factor for the development of AD is apolipoprotein E (apoE) [30, 166]. Present in humans as a combination of two apolipoprotein ϵ 2, ϵ 3 and/or ϵ 4 alleles, it is specifically the ϵ 4 allele that markedly increases the relative risk for the development of AD. Although the biological mechanism(s) of apoE's involvement in the disease process is at a relatively early stage of understanding [17, 111], apoE ϵ 4 has been consistently associated with increased cerebral A β load [88].

Notably, the slightly longer A β 42 species that is specifically linked with FAD is also the first A β species deposited in cerebral plaques [82]. Interestingly, the earliest A β 42 in plaques appears to be N-truncated [98]. It is known that progressive N-truncation increases the propensity of aggregation [146, 165] and toxicity [23], although the most important N-terminus of highly aggregable A β x-42 peptides in plaque formation remains unclear. In fact, a diverse set of N- and C-terminally truncated A β peptides exist in the brain [131]. In addition, β -cleavage of APP normally generates both N-terminal A β Asp1 and Glu11 peptides [21, 59, 197]. Overall, data suggest that pyroglutamate A β 3-42 [68, 157, 206] and A β 11-42 [131, 155], among others, might be particularly important in AD.

Additional support for the importance of A β has come from therapeutic studies on AD transgenic mouse models. Experimental therapies, such as A β immunotherapy [8, 53, 138, 164, 178], were not only shown to reduce cerebral A β , but even more importantly, to improve behavior in transgenic mouse models of AD-like cerebral β -amyloidosis. Whether such A β -targeted therapies can also be effective in human AD, not consistently linked with elevated A β production as AD transgenic mice, remains less clear [75, 176].

At the same time, one needs to be vigilant about assigning too much of a role to one small peptide. Aging is considered the most important risk factor driving the development of AD. There are many hypotheses for aging that are also active areas of investigation in the field of AD research. Oxidative stress and mitochondrial dysfunction are considered to play major roles in aging and to promote the development of age-related neurodegenerative diseases [102, 104, 109, 144, 183]. Inflammation [128, 208] and the immune system [178] are also thought to modulate the disease process and are under investigation for their role in AD.

The main constituent of NFTs, the microtubule-associated protein tau, has received somewhat less attention compared to A β , mainly because tau pathology is less specific to AD than A β pathology, and because research has shown that in human AD and transgenic mouse models of AD, tau follows A β pathology rather than vice versa [60, 138]. Moreover, mutations in tau, rather than associated with AD, have been linked with familial forms of frontotemporal dementia (FTD; [132]), a less common form of dementia with a different clinical presentation and neuropathology that is not characterized by amyloid plaques. Evidence in experimental AD mouse models supports that A β acts via tau, particularly in promoting neurodegeneration [56, 101, 140, 151, 160].

Current data suggest a hierarchy in the development of AD with aging, that emerging evidence increasingly supports begins with declining levels of A β 42 in cerebrospinal fluid (CSF) and accumulation of intraneuronal A β 42, although the precise steps linking A β to synaptic dysfunction, cognitive decline and neurodegeneration remain to be determined.

Intraneuronal A β in Alzheimer's disease

A few intriguing early studies using newly developed antibodies directed at A β peptides had noted immuno-labeling of some neurons and NFTs in the brain [4, 62, 80, 115]. It is important to understand that these pan-A β antibodies directed at the A β domain detect not only A β peptides but also the normally much more abundant full-length APP, and additionally, other A β -containing APP fragments such as β -secretase cleaved APP β CTFs (Fig. 1). Hereafter, pan-A β antibodies will be referred to as A β /APP antibodies. This reactivity of A β /APP antibodies to APP has contributed to considerable misunderstanding with regards to the presence of intraneuronal A β in the field. Widely used A β /APP antibodies for detection of plaques should label all cells, since APP, in contrast to A β , is normally present at relatively high levels in cells. Since cell biological studies in the late 1980s and early 90s did not support the presence of intraneuronal A β , labeling of neurons and tangles with A β /APP antibodies was eventually ascribed to cross-reactivity with lipofuscin [7] or to an artifactual shared epitope with tau [4]. Furthermore, the wide use of well-established A β /APP antibodies (such as 4G8 and 6E10) was optimized for plaque detection in diagnostic neuropathology, where short 3,3'-Diaminobenzidine (DAB)/peroxidase reaction times typically reveal minimal labeling next to plaques in AD brain. The subsequent development of antibodies directed at the free C-terminal ends of A β 40 or A β 42 [82] led to the ability to differentiate A β from APP labeling, since such antibodies now did not label the A β domain within APP as A β /APP antibodies had done (Fig. 1). In contrast, while antibodies to the free Asp1 N-terminus of A β 1-x do not cross-react with full-length APP, these antibodies (for example antibody 3D6) do still detect the 1st product of amyloidogenic APP processing, β -cleaved β CTFs, which has the same N-terminus as A β 1-x peptides [76]. Confocal microscopy of mouse [129] and human (Fig. 2) primary neurons in culture underscores the complexity and differences in subcellular labeling of APP and its various metabolites, including A β .

In 1993 the first biological paper appeared on the presence of A β within cells [201]. Subsequent work showed that particularly the disease-linked A β 42 peptide, rather than the more abundantly secreted A β 40 peptide, increased with differentiation and time in culture within clonal NT2 cells that can develop a neuron-like phenotype [174, 195]. In addition, marked up-regulation of newly generated intracellular A β 42 was reported following treatment of cells with extracellular A β 1-42 [211]. AD transgenic mice showed physiological [32, 74, 77, 124] and neuritic alterations [22, 125] prior to plaques, shifting the emphasis from fibrillar A β to soluble, and potentially intracellular, A β 42 oligomers. Similarly, biochemical studies on human AD brain indicated that the soluble pool of A β 42 correlated best with degree of cognitive loss [108, 118, 130]. Intriguing *in vitro* and *in vivo* studies showed that intraneuronal A β 1-42 was particularly neurotoxic [213], although lack of plaque formation in intraneuronal A β 42 accumulating transgenic mice with concomitant neurodegeneration [36, 93] limited conclusions that could be drawn to the human disease, since pathological diagnosis of AD requires A β plaques. An intracellular environment should be more favorable for allowing A β aggregation, since intracellular organelles can have a lower pH, higher metal ion concentrations and provide a limited space. These and other considerations led to the hypothesis that the intracellular pool of A β may be quite important in the disease process [69, 203].

Intraneuronal A β in brain by light microscopy

Two publications appeared in January 2000 on the intraneuronal accumulation of particularly A β 42 by light microscopy within neurons of human brain. One of these [123] showed A β 42 positive non-pyramidal neurons near plaques in advanced AD brain, although on closer inspection nuclei are not evident in these neuron-like structures that more resemble NFTs. The other publication [60] studied intraneuronal A β 40 and A β 42 in Down syndrome, non-neurological controls, mild cognitive impairment (MCI) and mild, moderate and severe AD. This publication described prominent early A β 42 labeling of AD vulnerable neuron cell bodies, but not their nuclei, in pre-plaque Down syndrome brains and also non-Down syndrome brains, particularly those with early AD neuropathological changes. This publication noted a tendency for increased intraneuronal A β 42 labeling with increasing age in Down syndrome prior to plaques and also in normal aging; the most marked intraneuronal A β 42 accumulation was noted with early AD plaque and tangle pathology in patients with MCI or mild dementia. Intriguingly, this and subsequent studies noted an apparent decline in intraneuronal A β 42 labeling with advancing plaque pathology [95, 139]. Intraneuronal A β 42 accumulation has since been reported by many groups in brain tissue of human AD [24, 38, 45, 64, 126, 141, 185, 204] and Down syndrome [19, 24, 64, 126], and virtually all transgenic mouse [47, 89, 106, 114, 137, 140, 172, 182, 188, 196, 205] and rat [99] models of AD.

Major support for the importance of intraneuronal A β came with the development of new transgenic mouse models. In particular, the triple transgenic mouse harboring mutations in APP, PS1 and tau was shown to develop prominent intraneuronal A β accumulation that coincided with the emergence of behavioral, physiological and synaptic abnormalities several months prior to A β plaques [12, 140]. Additionally, PS1/APP mutant transgenic mice were shown to have prominent intraneuronal A β accumulation associated with neuron cell death [23, 35, 167]. Another transgenic mouse over-expressing human APP harboring 5 different FAD mutations was shown to have intraneuronal A β 42 fibrilization, indicated by thioflavin S staining, associated with neuronal death [137]. Confirmation for the presence of intraneuronal A β was provided by a study that showed the disappearance of intraneuronal A β in AD transgenic mice when A β levels were reduced either by treatment with γ -secretase inhibitor or by crossing the mice with BACE knockout mice [145].

Intraneuronal A β in brain by immuno-electron microscopy

Immuno-electron microscopy (immuno-EM) was utilized to determine the subcellular localization of A β in neurons of the brain. Remarkably, neurons in normal wild-type mouse, rat and human brains showed localization of A β 42 primarily associated with endosomal organelles, often identifiable on electron micrographs as late endosomal multivesicular bodies (MVBs; [188]). Importantly, A β 42 labeling was shown to be absent in neurons of APP knockout mouse brains and the monoclonal A β 42 antibody used (MBC42) was determined by Western blot to bind A β 42, but not A β 40, full-length APP or APP C-terminal fragments (CTFs). In addition, dual-immuno-EM further indicated that APP has a different subcellular localization (primarily Golgi apparatus) compared to A β 42 (primarily endosomes). Endosomal A β 42 labeling by immuno-EM has since been confirmed by other groups [49, 95, 143]. In addition, A β 42 labeling in endosomes was reported in neurons in culture [2, 154] and brain [24, 196].

Consecutive immuno-EM for A β 42 in aging, A β plaque-forming AD transgenic mice (Tg2576 harboring the FAD Swedish mutation [78]) during a time period known to show marked increases in brain A β [86] but prior to plaques, revealed that A β 42 accumulated particularly in endosomes within distal axons and dendrites (neurites), and their pre- and post-synaptic compartments, where with marked accumulation it was, at times, directly

associated with intraneuronal pathology [188]. Confirmatory data were obtained in human AD brain tissue. Moreover, a subsequent immuno-EM study demonstrated that intraneuronal accumulation of higher molecular weight A β 42 oligomers (antibody M16; [210]) was invariably associated with neuritic degeneration, even prior to plaques, in vulnerable distal neurites and synaptic compartments in brains of AD transgenic mice and human AD [187]. These immuno-EM studies were the first to directly associate A β with synaptic and neuritic damage in the brain.

Concerns about intraneuronal A β accumulation in AD

A β antibody specificity and immunostaining

Although the presence of intraneuronal A β accumulation has now been widely detected in brains of human Down syndrome and AD, and transgenic models of AD, skepticism persists. A major initial concern was that intraneuronal A β 42 labeling in the brain was an artifact. There are different aspects to this concern. Many investigators, including clinical neuropathologists, did not appreciate intraneuronal A β immuno-labeling in postmortem AD brains. Not seeing intraneuronal A β in brain can be due to many factors other than its absence. In diagnostic neuropathology, typically brief development times for DAB/ peroxidase reactions are used with A β /APP antibodies. This leads to "clean" plaque staining without "background", although as noted above, one should be aware that APP, in contrast to A β , normally is an abundant protein in cells. Therefore, neurons should, and eventually do, stain with A β /APP antibodies that also detect APP, if one uses appropriate immuno-histochemical procedures, including allowing for longer reaction times.

As noted above, the use of A β /APP antibodies has been an issue in the acceptance of intraneuronal A β . Some investigators inadvertently led to confusion by using such antibodies to describe intraneuronal A β labeling in the brain. In the case of the triple transgenic mouse, the first transgenic mouse shown to develop both neuropathological hallmarks of the human disease, staining with an A β /APP antibody was compared to that of an APP C-terminal antibody with an epitope outside of the A β domain [140]. Since the APP antibody did not show similar intracellular accumulation, it was logical to conclude that it was A β rather than APP that was accumulating. It is preferable to directly use A β end-specific antibodies for determination of intraneuronal A β , although it is important to be aware that not all C-terminal end-specific A β 40 and A β 42 antibodies are optimal. The quality of antibodies is of major importance, particularly for A β detection. For example, it is not common knowledge that commercial antibodies can change. Particularly polyclonal antibodies can have batch to batch variations that can significantly affect interpretation. A significant development that further complicated interpretation of results with A β antibodies was that of A β antibody conformational specificity [87]. As A β aggregates from monomers to oligomers and then fibrils, the conformation and antibody accessibility of peptide epitopes within A β can change. Diverse new antibodies have been developed against different conformations of A β . For example, C-terminal specific A β 42 antibodies typically are best at detecting A β 42 monomers, and have minimal reactivity against oligomers larger than trimers [187]. One also needs to be cautious when interpreting immunohistochemistry with antibodies against the N-terminus of A β 1-x peptides. Although these antibodies do not cross-react with full-length APP, they do also react against the same N-terminus on APP β CTFs. N-terminal A β 1-x/ β CTF-specific antibodies also fail to label N-truncated A β 42 peptides that appear to be particularly important in AD [60, 98, 157, 158, 206].

Thus, the above caveats further underscore that the absence of labeling does not have to reflect absence of A β within neurons. Detection of intraneuronal A β is even more challenging in human brain, where in contrast to AD transgenic mice, APP is not over-expressed and FAD mutations that elevate A β 42 are rare. Moreover, intraneuronal A β is best

appreciated at early stages of pathology in AD transgenic mouse and human AD brains, while postmortem human AD brains are more often examined at end stages of disease. It should be noted that there also is variability in the degree of intraneuronal A β labeling [60], which is more pronounced in human compared to AD transgenic mouse brains. AD transgenic mice allow for considerably less variability, since not only can genetically identical mice be studied, but time to, and length of, fixation can be carefully controlled. Overall, detection of intraneuronal A β can be challenging, and the use of complementary methods and APP knockout mice [214] are helpful controls. While there appeared to be an increase in intraneuronal A β 42 labeling with aging in AD vulnerable neurons in normal human brains, individual differences in neuroanatomical involvement and relative intensities of A β 40 compared to A β 42 labeling were noted [60]. For example, particularly prominent intraneuronal A β 42 labeling was detected in the CA1 hippocampus in one young case of Down syndrome (3 year old; [60]) with concurrent tetralogy of Fallot (personal observations). Given the influence of, among others, head injury on APP metabolism and A β deposition, coincident illness and agonal state likely contribute to variability of intraneuronal A β labeling in human brain.

Additional concerns for the acceptance of intraneuronal A β are applicable to all immunohistochemical data. Postmortem interval, pre-treatments, counter-stains, fixation and post-fixation, detergent, and blocking solutions, all can influence interpretation of immunohistochemistry, and also that of intraneuronal A β [34, 39]. For a detailed protocol for A β immunohistochemistry and immuno-electron microscopy see Gouras and Takahashi, 2005 [57].

Biochemical evidence on intraneuronal A β accumulation

The relative dearth of recent biochemical evidence on intraneuronal A β has contributed in delaying the acceptance of intraneuronal A β . The aggregation-prone nature of A β makes the accurate biochemical analysis of intraneuronal A β particularly challenging. The limitation of a standard and widely used A β assay was highlighted in a study using an enzyme-linked immunosorbent assay (ELISA) [181]. When the same amounts of A β 1-42 were incubated over time, levels of A β 42 assayed by a standard non-denaturing ELISA appeared to decline as a function of time, while denaturing Western blotting indicated that levels were actually unchanged; this occurred because ongoing A β aggregation progressively blocked access of the A β 42 antibody in the ELISA. Furthermore, other biochemical A β detection methods, such as immunoprecipitation, can vastly underestimate total A β levels, since spiking of samples with a defined amount of A β 1-42 reveals that only a very small fraction of the added A β can subsequently be detected (personal observations).

Biochemical determination of A β levels in brain can also show considerable variation between studies. For example, the predominance of A β 40 compared to A β 42 in brain can vary, with some studies showing more and others less A β 42 than A β 40 in normal brain [131, 191]. It is particularly challenging to determine intra- compared to extra-cellular A β in the brain using biochemical methods. Studies have increasingly differentiated between soluble and insoluble pools of A β within the brain, where it is the increase in the soluble pool of A β 42 that tracks best with cognitive decline in AD [108, 118, 130]. Attempts have been made at further separating the soluble pool of A β in the brain as soluble intracellular-enriched, membrane-associated or extracellular-enriched pools [100], although the feasibility of separating these pools of A β after brain homogenization, which breaks apart cell membranes, is questionable. It is intriguing to note that a recent study indicated that cognitive decline in AD tracked best with the membranous pool of A β 42 in brain tissue from human AD [180]; it was concluded that this membrane-associated A β 42 was cell associated and reflected intraneuronal A β 42. Very interesting recent biochemical studies used laser capture microdissection combined with sensitive A β ELISA to demonstrate elevated levels

of A β 42 and an increased A β 42 to A β 40 ratio in both AD vulnerable CA1 hippocampal pyramidal and relatively AD resistant cerebellar Purkinje neurons in both familial and sporadic AD compared to controls, although the amount of A β 42 was markedly higher in CA1 pyramidal compared to cerebellar Purkinje neurons in AD [5, 70].

A β in mitochondria

Another challenge for the acceptance of intraneuronal A β accumulation were subsequent reports for different subcellular locations of accumulating A β than endosomes, which included the plasma membrane [136, 209] and mitochondria [109]. In particular A β localization to mitochondria has received considerable recent attention. There is a large group of investigators who have for years emphasized the importance of energy metabolism, oxidative stress and mitochondria in aging and AD. Thus, the localization of A β to mitochondria was an exciting new link between their compelling prior work on the role of mitochondria in aging and AD with the peptide most linked with AD, and questions of how A β might traffic to mitochondria were of less concern. APP trafficking and processing are not typically thought to involve mitochondria [159, 175, 194]. Overall, the data for the early accumulation of A β in mitochondria appear weaker compared to that for endosomes. In fact, the initial study on A β localization to mitochondrial [109] looked at much older AD transgenic mice with abundant plaques compared to studies on A β in endosomes [188]. Nevertheless, A β localization to mitochondria may still be quite important in AD pathogenesis. Thus, while aberrant A β 42 accumulation begins in endosomes, mitochondrial A β may be a critical contributor in the pathological cascade leading to neuronal dysfunction and pathology in AD [33, 103, 149]. In fact, we have noted the presence, at times, of A β -accumulating endosomes adjacent to mitochondria by immuno-EM (personal observations), which could suggest a link between endosomal and mitochondrial A β [67]. At the same time, the role of mitochondria and oxidative stress in AD might be even more important upstream, rather than downstream, of A β , since aging is the most important risk factor for A β accumulation and AD. Indeed, elevated oxidative stress was reported to promote A β plaques in the brains of AD transgenic mice [102].

Familial AD mutations

It is widely thought that FAD mutations in APP or the presenilins cause increased secretion of A β , although the common denominator among FAD mutations is not an increase in A β secretion, but an increase in the A β 42 to A β 40 ratio [71]. It is less widely known that FAD mutations actually cause a relatively greater increase in intracellular compared to secreted A β ([202], and unpublished observations). FAD mutations can even decrease secretion of the most abundantly released A β isoform, A β 40, while still increasing the A β 42 to A β 40 ratio [90, 134, 173]. Emerging data from AD transgenic mice support that A β 40 might even be protective against cerebral amyloid [117]. The Arctic FAD mutation within the mid-region of A β [133] primarily increase the propensity for A β aggregation rather than secretion. In fact, FAD transgenic mouse models with the highest level of amyloidogenic A β display particularly prominent early intraneuronal A β accumulation [89, 106, 196]. Remarkably, a recent E693Delta mutation in APP was reported to be associated with an AD clinical phenotype in the absence of plaques; cells harboring this mutation were shown to have reduced A β secretion and increased intracellular A β accumulation and oligomerization [134].

Relevance of intraneuronal A β to AD pathogenesis

A concern that has been raised is that even if intraneuronal A β 42 accumulation does occur, it may be irrelevant to the disease. Emerging data, such as FAD mutations noted above, and the direct association of intraneuronal A β 42 with subcellular pathology [187, 188], cell death [23, 167], and behavioral and physiological abnormalities, even prior to plaques, in

AD transgenic mouse models [140], are providing increasing evidence for the importance of intraneuronal A β . On the other hand, the apparent decrease in intraneuronal A β 42 with advancing plaque pathology noted in several of the initial reports on intraneuronal A β accumulation [60, 64, 140, 205], might suggest that while intraneuronal A β accumulation is an early event, it unlikely is the driving force in AD pathology, since in that case it should be progressive and correlate with cognitive decline. One possible explanation for this concern is that A β aggregation leads to conformational changes and thereby the reduced ability of A β antibodies in detecting their antigens. In fact, as noted above, standard A β 42 C-terminal end-specific antibodies typically are only optimal at detecting A β 42 monomers, and then lose their reactivity after dimers and trimers [187]. Another challenge is that the most marked accumulation of intraneuronal A β 42 occurs in distal neurites and synaptic compartments as determined by immuno-EM [188] that cannot be visualized effectively by light or standard immuno-fluorescence microscopy. It remains unclear whether and, if indeed the case, why cell body A β 42 by light microscopy seems to decline with AD progression in AD vulnerable neurons such as CA1 pyramidal neurons, while by immuno-EM it continues to increase particularly within neurites and synapses [186]. It could, among other possibilities, be a combination of loss of A β epitopes in cell bodies as A β 42 aggregates, increased synaptic A β as cell body A β declines, and even increased ability to degrade cell body compared to neurite-associated A β 42.

Many questions remain for the role of intraneuronal A β in Alzheimer's disease

Substantial questions remain for the role of intraneuronal A β , and also for A β in general, in AD pathogenesis. Notably, a subset of patients from the aborted Wyeth/ELAN active A β vaccine trial that were studied at autopsy showed A β plaque removal in the absence of cognitive improvement [75]. This study has been highlighted as evidence against the importance of A β in AD. Additionally, it remains unclear whether the decline in CSF A β 42 seen early in the conversion from normal to MCI and AD [48, 66], continues with progressive cognitive decline in AD. At the same time, increases in soluble A β 42 levels in the brain have been shown to track with decline in AD [108, 118, 130]. The combination of increasing soluble A β 42 in brain, declining CSF A β 42, and immuno-EM evidence of concomitant intraneuronal A β 42 accumulation, seem to strongly suggest that the soluble pool of A β 42 in the brain that correlates with disease progression mainly reflects intraneuronal A β 42. We note that one of the biochemical studies on soluble A β 42 levels correlating with AD progression [130] studied the same postmortem human brains analyzed in the initial immuno-histochemical study on intraneuronal A β 42 accumulation [60]. It will be important for future clinical-pathological studies to investigate for a correlation of biochemical and immuno-histochemical data on intraneuronal A β with cognitive decline. Additional correlation with levels of CSF A β 42 will also be important, particularly in individual AD patients over time, since population based comparisons might fail to appreciate progressive changes, because of, for example, person-to-person differences in baseline levels of CSF A β 42.

A central question is why intraneuronal A β 42 increases in the first place. A more precise understanding of how aging and other injurious processes, such as cardiovascular disease or traumatic brain injury, can modulate A β levels will be important. The mechanism whereby intraneuronal A β 42 first adversely affects the biology of neurons is a central unanswered question. At the most basic level it seems that A β 42 could alter the normal function of endosomes near synapses. In one study, A β -induced abnormalities in MVB endocytic sorting were demonstrated in APP mutant neurons, which appeared to be due to impairment of the ubiquitin proteasome system (UPS; [2]). The role of extracellular A β on intracellular A β accumulation and plaque formation also remains unclear, as well as what proportion of intraneuronal A β is derived from the inside compared to the outside of neurons.

Implications of intraneuronal A β for Alzheimer's disease

Since accumulation of A β 42 within AD vulnerable neurons has been associated with physiological, pathological and behavioral abnormalities even prior to plaques [12, 140, 187, 188], the evidence is mounting that intraneuronal A β 42 is the driving force in A β -related AD pathogenesis [9, 58, 92]. Cumulative pathological data on intraneuronal A β accumulation support a scenario of increased A β 42 in vulnerable distal neurites and synapses leading to their destruction from within (Fig. 3). The subsequent formation of more amorphous plaques reflect remnants of this destruction further shaped by activated inflammatory cells. Reactive inflammatory cells and plaques may have both protective and detrimental roles that contribute to AD pathogenesis. It is possible that the localized and suddenly high levels of extracellular A β 42 following neuritic degeneration from within can then propagate A β pathology to neighboring neurites via a prion-like ability of extracellular A β 42 [121] to up-regulate intracellular A β 42 [51, 58]. The lack of correlation between A β plaques and cognitive decline in AD may relate to person-to-person differences in the ability of inflammatory cells to effectively remove A β plaques from the brain. This scenario of A β -related pathogenesis makes the cell biological study of the molecular and biological mechanism(s) whereby intraneuronal A β accumulation leads to synaptic dysfunction of central importance. Intraneuronal A β accumulation can also illuminate many ongoing questions and paradoxes in the field.

Pathology

Pathological relation between A β , tau and synapses in Alzheimer's disease

The implications for intraneuronal A β in AD include providing new insights into a link between A β and tau pathologies that were formerly considered to be separate and how these two pathologies relate to synapse pathology [186]. By beginning within neurons, A β is more directly accessible to altering tau, the principle component of the other neuropathological hallmark of AD. Specifically, immuno-histochemical data support that neuron cell bodies with early intraneuronal A β 42 accumulation are those that go on to develop NFTs [60, 140], while their terminal fields are sites of early plaque pathology. An example of this is the early accumulation of A β 42 within layer II neuron cell bodies of the entorhinal cortex and early plaques at their axonal terminal fields in the outer molecular layer of the dentate in the hippocampus [60]. A more recent study investigated the subcellular link between intracellular A β and tau pathologies [186]. It had been known that even in APP mutant transgenic mice hyperphosphorylated tau can be seen within some dystrophic neurites around plaques [14]. Since A β 42 accumulates within dystrophic neuritis [188], A β 42 and phospho-tau were examined by dual-immunofluorescence and -EM in FAD mutant transgenic mouse and human AD brains. Remarkably, dystrophic neurites around plaques showed co-localization of phospho-tau with A β 42 in AD transgenic mice and human AD [186]. Since the triple transgenic mouse expressing mutant APP, PS1 and tau develops early A β accumulation in CA1 hippocampal neurons and later tau paired helical filaments [140], the stratum lacunosum-moleculare (SLM) of hippocampus containing the CA1 distal apical dendrites was selectively examined by immuno-EM. Remarkably, the SLM showed not only prominent early A β 42 increases but also the earliest labeling with antibodies against phospho-tau, such as the well-established antibody AT8. By dual-immuno-EM phospho-tau was specifically evident in markedly A β 42 accumulating, distal CA1 apical dendrites and their post-synaptic compartments in the SLM [186]. Thus, tau, normally localized to axons, showed early mislocalization specifically in aberrant A β 42 accumulating distal dendrites. These data build on previous studies to provide an early temporal and spatial relationship of the main constituents of the two neuropathological hallmarks of AD and thereby also link them with synapse pathology in AD.

Additional evidence for synapses as sites of Alzheimer's disease pathology

Synapses have long been viewed as critical sites of AD pathology [6, 28, 112, 119, 168] and diverse evidence had supported that synapses are major sites of A β pathology and also generation. Lesioning of the perforant pathway originating in the early AD prone entorhinal cortex leads to reduced plaque pathology at terminal synaptic fields in the hippocampus [96, 171]. In contrast, lesioning of another projection pathway, that from the locus coeruleus, was reported to increase plaque pathology in terminal fields of the cerebral cortex [72]. APP is transported down both axons and dendrites and A β appears to be generated preferentially at synapses [25, 54, 85]. In fact synaptic activity was recently shown to promote APP transport down neurites to synapses [190]. Furthermore, extracellular A β 1-42 oligomers preferentially target synapses when added to neurons [43, 91]. Immuno-EM for PS1 localized this critical component of the γ -secretase complex particularly to endosomes in distal neurites, including in both pre- and post-synaptic compartments [94]. Indirect support for synapse-specific A β generation also comes from PS1 conditional knockout mice, which showed aberrant accumulation of APP β CTFs in distal axons/pre-synaptic compartments [162] and also some anatomically selective accumulation in distal dendrites/post-synaptic compartments (personal observations).

Overall, emerging work on intraneuronal A β is most consistent with the scenario of aging, and other genetic and environmental risk factors, such as apoE ϵ 4 and head injury, respectively, leading to up-regulation and then oligomerization of intraneuronal A β 42 in distal neurites and synapses, which then leads directly to destruction of neurites and synapses from within (Fig. 3). Immuno-EM images of completely abnormal A β 42 accumulating dystrophic neurites [187, 188], lacking the normal microtubule tracks required for organelle trafficking, secretion and exocytosis, argue against the active secretion of aggregating A β 42 from neuritis prior to the dissolution of the plasma membrane [95]. Thus, the nidus of A β plaques develops from within neurites at a time course that could also fit that of plaque formation determined by multiphoton microscopy (MPM) occurring within a day [120]. Remarkably, this MPM study of AD transgenic mouse brain *in vivo* also described the emergence of dystrophic neurites in areas distant from plaques that appeared and, at times, even disappeared, although intraneuronal A β was not examined in these neurites. Plaque development in terminal processes can help explain the lack of neuron cell death in most AD transgenic mice in the presence of considerable plaque pathology, since neuron cell bodies can survive following loss of some neurites and synapses. At the same time, a subset of plaques appear to emerge directly from intracellular A β accumulating neuron cell bodies [38, 60]. While A β immuno-EM provides evidence for all forms of plaques, including diffuse plaques, representing destruction of neurites, there are several possible reasons for why degree of plaque pathology is not as good a measure of cognitive dysfunction compared to soluble, and we hypothesize intraneuronal, A β 42. Degree of intraneuronal A β 42 might tightly correlate with synaptic dysfunction, whereas synapse-independent factors might dissociate plaque burden from cognitive decline, for example, genetic heterogeneity may exist in the ability of inflammatory cells to remove plaques.

Head trauma and intraneuronal A β

Intraneuronal A β can provide new insights into head trauma as a risk factor for AD [116, 127]. Individuals, including children, who died following coma not long after severe head trauma were shown to develop amorphous cerebral A β plaques [152]. At first this seems difficult to reconcile with recent data showing that patients in coma have marked reductions in extracellular A β 42, which rises and falls with cognitive improvement and decline, respectively [16]. We have observed marked up-regulation of intraneuronal A β 42 in surrounding neurites and neuron cell bodies near needle track injuries in the rodent brains 48 to 72 hours following brain injury (Fig. 4a). By immuno-EM these increases in A β 42

occurred predominantly in endosomes (Fig. 4b). Thus, head trauma provides an example of extracellular-appearing amyloid developing in the setting of what has been shown to be a state of reduced interstitial, extracellular A β and, as experimental evidence now indicates, concomitant intraneuronal A β 42 accumulation. In addition, dementia pugilistica that develops in professional boxers [31], while characterized by significant tauopathy and comparably less amyloid pathology, limited mainly to diffuse plaques [27], also shows considerable intraneuronal A β 42 (Fig. 4c).

Questions for intraneuronal A β in Alzheimer's disease neuropathology

Many questions remain for the role of intraneuronal A β in AD pathogenesis. It remains unclear whether and how A β accumulation and pathology might differ in axons and pre-synaptic compartments compared to dendrites and post-synaptic compartments. Overall, more prominent increases in A β 42 accumulation were observed in distal dendrites compared to axons [188], although there are anatomical differences, and it remains possible that either axons or dendrites are the initial site of intraneuronal A β pathology. Immuno-histochemical and -EM observations support that both distal and proximal dendritic arborizations are important sites of A β 42 accumulation and plaque formation (Fig. 3).

What determinants are involved in the selective vulnerability of certain neurons and their processes to A β 42 accumulation is another unanswered question. Differential anatomical levels of secretases [84] and chronic sites of synaptic activity [18] may be important in the selective vulnerability of AD. The cause of differences in plaque morphology and the involvement of inflammatory cells in plaque formation are also not fully clear [37]. In particular dense core plaques appear to be derived from neuron cell bodies, although even here it is possible that dendritic A β 42 accumulations are the inciting event. A recent report on *in vitro* plaque formation in cultured microglia [49] suggests the possibility that similar intracellular A β accumulation and pathology as described in neurons might also occur in inflammatory cells in AD and thereby further contribute to plaque formation.

The involvement of the more abundantly secreted A β 40 in intraneuronal A β accumulation and plaque formation is less clear. Although not apparently present in early A β 42 plaques as determined in Down syndrome [98], A β 40 typically is the predominant peptide in amyloid angiopathy. An intriguing study showed that shifting the ratio of A β 42 to A β 40 in APP mutant transgenic mice under a neuron-specific promoter shifted the preponderance of parenchymal A β 42 plaques to vascular A β 40 amyloid [73]. This supports that amyloid angiopathy derives from perivascular drainage of A β 40 generated by neurons [61, 200]. Likewise, more A β 42 over-producing mice, by retaining A β 42 within neurites, can therefore be hypothesized to have less amyloid angiopathy.

Future comprehensive clinical-pathological studies that carefully compare and examine various intraneuronal A β 40 and A β 42 isoforms in different brain regions from cases with normal aging, mild cognitive impairment and different stages of AD, according to apoE genotype and intercurrent illness, and also in relation to neuropsychological testing, positron emission tomography (PET) amyloid and functional brain imaging, and CSF A β levels, will be important to help clarify the role of intraneuronal A β accumulation in AD [1].

Biology

Aberrant accumulation of A β within neurons in AD underscores the importance of uncovering the cellular and molecular mechanisms by which this pool of A β leads to synaptic dysfunction. That this pathological accumulation is most prominent in distal neurites and synapses of AD transgenic mice provided the first direct association between A β and synapse pathologies in the brain [188]. Since accumulating A β 42 localizes

particularly to the outer limiting membrane of endosomes, and endosomes are known to reside near synapses [29], altered function of these organelles [135] might be important for A β -induced synaptic dysfunction. Fortuitously, it was possible to model intraneuronal A β accumulation in cultured primary neurons derived from AD transgenic mice [187]. Comparing AD transgenic to wild-type neurons allowed for biological studies to begin elucidating early intraneuronal A β -related abnormalities in neurons. Remarkably, APP mutant neurons showed differential alterations in synaptic proteins [3] that resembled those that have been described in AD brain, such as early loss of the pre-synaptic protein synaptophysin [113, 163, 192] and the post-synaptic protein PSD-95 [63]. It is of interest that among the earliest and most significantly A β -altered synaptic proteins are AMPA glutamate receptor subunits [3, 79], that are particularly important for synaptic plasticity.

In an effort to elucidate dysfunction of A β -accumulating endosomes, a dynamic cell biological approach was used to compare APP mutant to wild-type neurons [2]. Using the well established endocytosis of the epidermal growth factor receptor (EGFR), this study demonstrated that specifically the MVB sorting pathway, and not internalization or the recycling pathway, was altered in A β accumulating neurons. The study went on to provide evidence that abnormalities in MVB sorting were due to A β -dependent alterations in the ubiquitin-proteasome system (UPS). The UPS is increasingly being linked to neurodegenerative diseases of aging [10]. Best known for its role in degrading cytosolic proteins, the UPS also has important roles in synaptic plasticity [46] and lysosome-dependent degradation of trans-membrane proteins [105].

An area of considerable interest has been how extracellular A β alters synapses. Many *in vitro* and *in vivo* studies have shown that exogenous application of A β alters synaptic plasticity [170, 198] and important synaptic proteins [3, 79, 177], although how precisely this occurs remains unclear. Remarkably, it was shown that extracellular A β 1-42 was not toxic to APP knockout neurons [107] and that even point mutations in the NPXY motif in the C-terminus of APP block toxicity [169]. In addition, alterations of extracellular A β on selective synaptic proteins mirrored those from intraneuronal A β accumulation [3]. Recently, additional evidence pointed to the importance of amyloidogenic processing of APP, and therefore potentially intraneuronal A β , in extracellular A β -induced synapse dysfunction [190]. Work in the 1990s had shown that extracellular A β 1-42 led to marked up-regulation of endogenously generated A β 42 [51, 211], which also occurs in neurons [190]. Although it is thought that only a small amount of extracellular can be internalized into neurons [156, 211], A β 42 appears to have a prion-like ability of transferring an altered aggregation state from the outside to the inside of cells [51]. It is possible that small amounts of extracellular A β are internalized into neurons via APP at synapses, where extracellular A β preferentially binds [91] and APP is enriched, particularly with synaptic activation [190].

We are at an early stage of understanding the molecular mechanism(s) whereby accumulating A β 42 can alter normal synaptic function. Many questions remain next to figuring out the critical first step in intraneuronal A β -induced synaptic dysfunction. Elucidating the normal function of APP [199, 212] and probably even of A β [147] are additionally of major interest.

Diagnosis

Recently it is becoming apparent that the earliest diagnostic change indicating that an individual without known cognitive deficits will go on to decline cognitively and develop AD is a drop in levels of A β 42 in the CSF [15, 48]. That the best predictor for conversion to MCI and then AD is a decrease in the levels of extracellular A β 42 might have seemed surprising to proponents of the prevalent extracellular A β cascade hypothesis. The

explanation put forward to keep these data in line with the extracellular A β hypothesis was that high levels of extracellular A β lead to progressive oligomerization and eventually plaque formation, with plaques then sequestering secreted A β . There is a problem with this argument. It seems the extracellular A β hypothesis wants it both ways. On one hand, toxic extracellular A β oligomers are thought to be released from plaques to damage nearby, although still physically removed, neurites, while on the other hand, extracellular A β levels are supposed to be reduced because of increased sequestration to plaques. A more parsimonious and novel explanation, that also is in line with the A β 42 immuno-EM neuropathological data in brain and new insights relating to head injury described above, is that extracellular A β 42 declines because secretion of A β 42 is reduced as it accumulates instead within neurons.

At the same time, postmortem neuropathological studies and PET amyloid imaging indicate that individuals without known cognitive dysfunction can nevertheless have considerable plaque pathology. This has been used to argue that A β and plaques are not themselves important for neurodegeneration [83]. One needs to be cautious with this interpretation, since considerable brain pathology can occur without detectable cognitive deficits. For example, relatively large strokes or tumors in strategic locations can fail to show deficits on bedside neurological examination. In fact, it is well known that most elderly patients will have signs of microvascular ischemic changes/damage on brain magnetic resonance imaging (MRI) even in the absence of known neurological deficits. Thus, microscopic A β plaque pathology should also be able to go unnoticed, at least for a while. Furthermore, a recent imaging study supports that the presence of plaques by Pittsburgh compound B (PiB) PET amyloid imaging even in those diagnosed as cognitively normal does reflect deficits on functional imaging [179]. It needs to be stressed again that by immuno-EM high molecular weight A β 42 oligomers are consistently associated with pathology within neurites even prior to plaques [187]. Intraneuronal A β 42 accumulation therefore clearly can be detrimental already prior to plaques and obvious cognitive decline. One can even speculate that intracellular A β contributes to the well-known normal age-related decline in cognitive function that is accounted for by declining normative scores with age in neuropsychological testing. Similarly, intraneuronal A β accumulation with advancing age may be important for what is termed benign forgetfulness of aging. The triple transgenic mouse supports the notion that elevated intraneuronal A β can cause cognitive decline prior to plaques and tangles [12].

Therapy

Most clinical trials now in progress, and even more that are planned, are targeted at reduction of brain A β . Pharmacological inhibition of A β generation by inhibiting β - or γ -secretase has turned out to be more challenging than was originally hoped [13, 41]. Although reduction of the neuropathological hallmark and peptide most linked with the disease, A β plaques and A β peptides, is reasonable, a misunderstanding into the pathological role of A β can have negative repercussions. For example, screening for small molecule chemical inhibitors of specifically A β secretion could select compounds that are ineffective, and potentially even detrimental, for AD, since A β secretion may not be harmful. The neuropathological and biological evidence on intraneuronal A β accumulation increasingly suggest that this is the critical pool of A β that needs to be contained. A recent neurobiological study showed that it was reduction of intraneuronal rather than extracellular A β that correlated with synaptic improvement [190]. Remarkably, it was demonstrated that A β antibodies can reduce the intraneuronal pool of A β *in vivo* [138] and *in vitro* [189], which for the latter was shown to require A β antibody internalization. It is possible that the Wyeth/ELAN A β vaccine trial failed precisely because it was not able to effectively reduce this critical pool of intraneuronal A β . One can hypothesize that the abundant A β in cerebral

plaques might have sequestered the A β antibodies and thereby prevented removal of the important intraneuronal pool of A β . The concept that extracellular antibodies can be internalized and specifically alter intracellular proteins is not fully appreciated in clinical medicine and has therapeutic implications beyond AD.

Role of synaptic activity in Alzheimer's disease

Intraneuronal A β can also illuminate a major current paradox in the field regarding the role of synaptic activity in AD. Synaptic activity has been shown to increase the secretion of A β in hippocampal slices and living rodent brain [26, 85]. Furthermore, A β secretion is also increased with cognitive activity, since patients in coma showed elevations in A β with cognitive improvement; a subsequent decline in cognitive function correlated with decline in interstitial/extracellular A β levels [16]. Hyperexcitability is present in AD brain [142] and epilepsy was shown to promote plaque pathology [110]. Accordingly, dampening of synaptic activity is under consideration for the treatment of AD. The A β /synaptic activity paradox arises when one considers that cognitive activity is encouraged as potentially protective for patients with MCI and AD, and environmental enrichment was shown to reduce plaques in AD transgenic mice [55, 97]. Moreover, synaptic activity was recently shown to reduce the intraneuronal pool of A β *in vitro* and *in vivo* [190]. Furthermore, functional brain imaging shows reduced resting brain activity in subjects with AD, and even in genetically at-risk subjects decades prior to predicted cognitive symptoms [150]. Thus, reduced cerebral activity with AD should imply reduced A β secretion in AD brain. The more widespread cortical activity seen by functional brain imaging in AD patients performing a specific task [122] could be used to argue for anatomical spread of AD along these now more hyperactive cortical areas then secreting more A β with disease progression. Nevertheless, such task-specific spread in cortical activation is seen as a compensatory response with other brain injuries, and patients with AD would still be expected to have reduced cerebral activity most of the time. Another explanation for the potential A β /synaptic activity paradox is that sites of high cerebral activity, in the default system, are indeed sites of early AD neuropathology [18, 179], but not necessarily because of increased A β secretion. Rather these sites of high metabolic activity also generate high levels of reactive oxygen species that might contribute to a progressively decreased ability to effectively degrade intraneuronal A β 42 with aging. The protease shown to most efficiently degrade A β , neprilysin [81], was reported to be critical in synaptic activity-induced intraneuronal A β 42 reduction [190]. Given that neprilysin localizes to synapses and declines with aging [20, 50], declining neprilysin may be important in the age-related intraneuronal accumulation of A β in synapses.

Conclusion

Reviews on AD have for years typically started with the statement: "Alzheimer's disease is characterized by extracellular β -amyloid plaques and intracellular neurofibrillary tangles". Emerging evidence on intraneuronal A β strongly argues that this needs to change. Similar to tau aggregation developing within neurons but then as ghost tangles no longer within living neurons, A β accumulation also begins intracellularly and ends up extracellular following neuritic degeneration. The intraneuronal origin of A β plaques makes the pursuit of the neurobiology of A β within neurons all the more important. AD is a tremendously complex disease that incorporates some of the most intricate processes that we know of: aging, memory and synapses. Finding more effective therapy for AD sooner may hinge on overcoming, but not just replacing, our own obstacles and dogmas, and pressing on with work to elucidate the molecular neuropathology and neurobiology of this most common neurodegenerative disease of aging.

Acknowledgments

The support of National Institutes of Health grants AG028174 and AG027140, and Alzheimer's Association New Investigator award (D.T.) and Zenith award (G.K.G.). We thank Xun (Julie) Lian for helpful technical assistance.

References

1. Alafuzoff I, Pikkarainen M, Arzberger T, et al. Inter-laboratory comparison of neuropathological assessments of beta-amyloid protein: a study of the BrainNet Europe consortium. *Acta Neuropathol.* 2008; 115:533–546. [PubMed: 18343933]
2. Almeida CG, Takahashi RH, Gouras GK. Beta-amyloid accumulation impairs multivesicular body sorting by inhibiting the ubiquitin-proteasome system. *J Neurosci.* 2006; 26:4277–4288. [PubMed: 16624948]
3. Almeida CG, Tampellini D, Takahashi RH, et al. Beta-amyloid accumulation in APP mutant neurons reduces PSD-95 and GluR1 in synapses. *Neurobiol Dis.* 2005; 20:187–198. [PubMed: 16242627]
4. Allsop D, Haga S, Bruton C, Ishii T, Roberts GW. Neurofibrillary tangles in some cases of dementia pugilistica share antigens with amyloid beta-protein of Alzheimer's disease. *Am J Pathol.* 1990; 136:255–260. [PubMed: 2407121]
5. Aoki M, Volkmann I, Tjernberg LO, Winblad B, Bogdanovic N. Amyloid betapeptide levels in laser capture microdissected cornu ammonis 1 pyramidal neurons of Alzheimer's brain. *Neuroreport.* 2008; 19:1085–1089. [PubMed: 18596605]
6. Arendt T. Synaptic degeneration in Alzheimer's disease. *Acta Neuropathol.* 2009; 118:167–179. [PubMed: 19390859]
7. Bancher C, Grundke-Iqbal I, Iqbal K, Kim KS, Wisniewski HM. Immunoreactivity of neuronal lipofuscin with monoclonal antibodies to the amyloid beta-protein. *Neurobiol Aging.* 1989; 10:125–132. [PubMed: 2657463]
8. Bard F, Cannon C, Barbour R, et al. Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nat Med.* 2000; 6:916–919. [PubMed: 10932230]
9. Bayer A, Wirths O. Intracellular accumulation of amyloid-beta - a predictor for synaptic dysfunction and neuron loss in Alzheimer's disease. *Front Ag Neurosci.* 2010; 2:8.
10. Bence NF, Sampat RM, Kopito RR. Impairment of the ubiquitin-proteasome system by protein aggregation. *Science.* 2001; 292:1552–1555. [PubMed: 11375494]
11. Bertram L, Tanzi RE. Thirty years of Alzheimer's disease genetics: the implications of systematic meta-analyses. *Nat Rev Neurosci.* 2008; 9:768–778. [PubMed: 18802446]
12. Billings LM, Oddo S, Green KN, McGaugh JL, LaFerla FM. Intraneuronal A β causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice. *Neuron.* 2005; 45:675–688. [PubMed: 15748844]
13. Bittner T, Fuhrmann M, Burgold S, et al. Gamma-secretase inhibition reduces spine density in vivo via an amyloid precursor protein-dependent pathway. *J Neurosci.* 2009; 29:10405–10409. [PubMed: 19692615]
14. Blanchard V, Moussaoui S, Czech C, et al. Time sequence of maturation of dystrophic neurites associated with A β deposits in APP/PS1 transgenic mice. *Exp Neurol.* 2003; 184:247–263. [PubMed: 14637096]
15. Blennow K, Hampel H, Weiner M, Zetterberg H. Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. *Nat Rev Neurol.* 2010; 6:131–144. [PubMed: 20157306]
16. Brody DL, Magnoni S, Schwetye KE, et al. Amyloid-beta dynamics correlate with neurological status in the injured human brain. *Science.* 2008; 321:1221–1224. [PubMed: 18755980]
17. Bu G. Apolipoprotein E and its receptors in Alzheimer's disease: pathways, pathogenesis and therapy. *Nat Rev Neurosci.* 2009; 10:333–344. [PubMed: 19339974]
18. Buckner RL, Snyder AZ, Shannon BJ, et al. Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. *J Neurosci.* 2005; 25:7709–7717. [PubMed: 16120771]

19. Busciglio J, Pelsman A, Wong C, et al. Altered metabolism of the amyloid beta precursor protein is associated with mitochondrial dysfunction in Down's syndrome. *Neuron*. 2002; 33:677–688. [PubMed: 11879646]
20. Caccamo A, Oddo S, Sugarman MC, Akbari Y, LaFerla FM. Age- and region-dependent alterations in Abeta-degrading enzymes: implications for Abeta-induced disorders. *Neurobiol Aging*. 2005; 26:645–654. [PubMed: 15708439]
21. Cai H, Wang Y, McCarthy D, et al. BACE1 is the major beta-secretase for generation of Abeta peptides by neurons. *Nat Neurosci*. 2001; 4:233–234. [PubMed: 11224536]
22. Capetillo-Zarate E, Staufenbiel M, Abramowski D, et al. Selective vulnerability of different types of commissural neurons for amyloid {beta}-protein-induced neurodegeneration in APP23 mice correlates with dendritic tree morphology. *Brain*. 2006; 129:2992–3005. [PubMed: 16844716]
23. Casas C, Sergeant N, Itier v, et al. Massive CA1/2 neuronal loss with intraneuronal and N-terminal truncated Abeta42 accumulation in a novel Alzheimer transgenic model. *Am J Pathol*. 2004; 165:1289–1300. [PubMed: 15466394]
24. Cataldo AM, Petanceska S, Terio NB, et al. Abeta localization in abnormal endosomes: association with earliest Abeta elevations in AD and Down syndrome. *Neurobiol Aging*. 2004; 25:1263–1272. [PubMed: 15465622]
25. Cirrito JR, Kang JE, Lee J, et al. Endocytosis is required for synaptic activitydependent release of amyloid-beta in vivo. *Neuron*. 2008; 58:42–51. [PubMed: 18400162]
26. Cirrito JR, Yamada KA, Finn MB, et al. Synaptic activity regulates interstitial fluid amyloid-beta levels in vivo. *Neuron*. 2005; 48:913–922. [PubMed: 16364896]
27. Clinton J, Ambler MW, Roberts GW. Post-traumatic Alzheimer's disease: preponderance of a single plaque type. *Neuropathol Appl Neurobiol*. 1991; 17:69–74. [PubMed: 2057052]
28. Coleman PD, Yao PJ. Synaptic slaughter in Alzheimer's disease. *Neurobiol Aging*. 2003; 24:1023–1027. [PubMed: 14643374]
29. Cooney JR, Hurlburt JL, Selig DK, Harris KM, Fiala JC. Endosomal compartments serve multiple hippocampal dendritic spines from a widespread rather than a local store of recycling membrane. *J Neurosci*. 2002; 22:2215–2224. [PubMed: 11896161]
30. Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science*. 1993; 26:921–923. [PubMed: 8346443]
31. Corsellis JA, Bruton CJ, Freeman-Browne D. The aftermath of boxing. *Psychol Med*. 1973; 3:270–303. [PubMed: 4729191]
32. Chapman PF, White GL, Jones MW, et al. Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. *Nat Neurosci*. 1999; 2:271–276. [PubMed: 10195221]
33. Chen X, Yan SD. Mitochondrial Abeta: a potential cause of metabolic dysfunction in Alzheimer's disease. *IUBMB Life*. 2006; 58:686–694. [PubMed: 17424907]
34. Christensen DZ, Bayer TA, Wirths O. Formic acid is essential for immunohistochemical detection of aggregated intraneuronal Abeta peptides in mouse models of Alzheimer's disease. *Brain Res*. 2009; 1301:116–125. [PubMed: 19751708]
35. Christensen DZ, Kraus SL, Flohr A, Cotel MC, Wirths O, Bayer TA. Transient intraneuronal A beta rather than extracellular plaque pathology correlates with neuron loss in the frontal cortex of APP/PS1KI mice. *Acta Neuropathol*. 2008; 116:647–655. [PubMed: 18974993]
36. Chui DH, Tanahashi H, Ozawa K, et al. Transgenic mice with Alzheimer presenilin 1 mutations show accelerated neurodegeneration without amyloid plaque formation. *Nat Med*. 1999; 5:560–564. [PubMed: 10229234]
37. D'Andrea M, Nagele R. Morphologically distinct types of amyloid plaques point the way to a better understanding of Alzheimer's disease pathogenesis. *Biotech Histochem*. 2010
38. D'Andrea MR, Nagele RG, Wang HY, Peterson PA, Lee DH. Evidence that neurones accumulating amyloid can undergo lysis to form amyloid plaques in Alzheimer's disease. *Histopathology*. 2001; 38:120–134. [PubMed: 11207825]
39. D'Andrea MR, Reiser PA, Polkovitch DA, et al. The use of formic acid to embellish amyloid plaque detection in Alzheimer's disease tissues misguides key observations. *Neurosci Lett*. 2003; 342:114–118. [PubMed: 12727331]

40. Davies CA, Mann DM, Sumpter PQ, Yates PO. A quantitative morphometric analysis of the neuronal and synaptic content of the frontal and temporal cortex in patients with Alzheimer's disease. *J Neurol Sci.* 1987; 78:151–164. [PubMed: 3572454]
41. De Strooper B, Vassar R, Golde T. The secretases: enzymes with therapeutic potential in Alzheimer disease. *Nat Rev Neurol.* 2010; 6:99–107. [PubMed: 20139999]
42. DeKosky ST, Scheff SW. Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. *Ann Neurol.* 1990; 27:457–464. [PubMed: 2360787]
43. Deshpande A, Kawai H, Metherate R, Glabe CG, Busciglio J. A role for synaptic zinc in activity-dependent Abeta oligomer formation and accumulation at excitatory synapses. *J Neurosci.* 2009; 29:4004–4015. [PubMed: 19339596]
44. Duyckaerts C, Delatour B, Potier MC. Classification and basic pathology of Alzheimer disease. *Acta Neuropathol.* 2009; 118:5–36. [PubMed: 19381658]
45. Echeverria V, Cuellar AC. Intracellular A-beta amyloid, a sign for worse things to come? *Mol Neurobiol.* 2002; 26:299–316. [PubMed: 12428762]
46. Ehlers MD. Activity level controls postsynaptic composition and signaling via the ubiquitin-proteasome system. *Nat Neurosci.* 2003; 6:231–242. [PubMed: 12577062]
47. Espana J, Gimenez-Llort L, Valero J, et al. Intraneuronal beta-amyloid accumulation in the amygdala enhances fear and anxiety in Alzheimer's disease transgenic mice. *Biol Psychiatry.* 2010; 67:513–521. [PubMed: 19664757]
48. Fagan AM, Head D, Shah AR, et al. Decreased cerebrospinal fluid Abeta(42) correlates with brain atrophy in cognitively normal elderly. *Ann Neurol.* 2009; 65:176–183. [PubMed: 19260027]
49. Friedrich RP, Tepper K, Ronicke R, et al. Mechanism of amyloid plaque formation suggests an intracellular basis of A{beta} pathogenicity. *Proc Natl Acad Sci U S A.* 2010; 107:1942–1947. [PubMed: 20133839]
50. Fukami S, Watanabe K, Iwata N, et al. Abeta-degrading endopeptidase, neprilysin, in mouse brain: synaptic and axonal localization inversely correlating with Abeta pathology. *Neurosci Res.* 2002; 43:39–56. [PubMed: 12074840]
51. Glabe C. Intracellular mechanisms of amyloid accumulation and pathogenesis in Alzheimer's disease. *J Mol Neurosci.* 2001; 17:137–145. [PubMed: 11816787]
52. Goate AM, Haynes AR, Owen MJ, et al. Predisposing locus for Alzheimer's disease on chromosome 21. *Lancet.* 1989; 1:352–355. [PubMed: 2563508]
53. Golde TE, Das P, Levites Y. Quantitative and mechanistic studies of Abeta immunotherapy. *CNS Neurol Disord Drug Targets.* 2009; 8:31–49. [PubMed: 19275635]
54. Goldsbury C, Mocanu MM, Thies E, et al. Inhibition of APP trafficking by tau protein does not increase the generation of amyloid-beta peptides. *Traffic.* 2006; 7:873–888. [PubMed: 16734669]
55. Gortz N, Lewejohann L, Tomm M, et al. Effects of environmental enrichment on exploration, anxiety, and memory in female TgCRND8 Alzheimer mice. *Behav Brain Res.* 2008; 191:43–48. [PubMed: 18433890]
56. Gotz J, Chen F, van Dorpe J, Nitsch RM. Formation of neurofibrillary tangles in P301 tau transgenic mice induced by Abeta 42 fibrils. *Science.* 2001; 293:1491–1495. [PubMed: 11520988]
57. Gouras GK, Takahashi RH. Immunohistochemical analysis of amyloid precursor protein and its derivatives. In: Xia, W.; Xu, H., editors. *Amyloid Precursor Protein A Practical Approach.* Florida: CRC Press; 2005. p. 155-160.
58. Gouras GK, Almeida CG, Takahashi RH. Intraneuronal Abeta accumulation and origin of plaques in Alzheimer's disease. *Neurobiol Aging.* 2005; 26:1235–1244. [PubMed: 16023263]
59. Gouras GK, Xu H, Jovanovic JN, et al. Generation and regulation of beta-amyloid peptide variants by neurons. *J Neurochem.* 1998; 71:1920–1925. [PubMed: 9798916]
60. Gouras GK, Tsai J, Naslund J, et al. Intraneuronal Abeta42 accumulation in human brain. *Am J Pathol.* 2000; 156:15–20. [PubMed: 10623648]
61. Grinberg LT, Thal DR. Vascular pathology in the aged human brain. *Acta Neuropathol.* 2010
62. Grundke-Iqbal I, Iqbal K, George L, Tung YC, Kim KS, Wisniewski HM. Amyloid protein and neurofibrillary tangles coexist in the same neuron in Alzheimer disease. *Proc Natl Acad Sci U S A.* 1989; 86:2853–2857. [PubMed: 2649895]

63. Glyls KH, Fein JA, Yang F, Wiley DJ, Miller CA, Cole GM. Synaptic changes in Alzheimer's disease: increased amyloid-beta and gliosis in surviving terminals is accompanied by decreased PSD-95 fluorescence. *Am J Pathol.* 2004; 165:1809–1817. [PubMed: 15509549]
64. Gyure KA, Durham R, Stewart WF, Smialek JE, Troncoso JC. Intraneuronal abeta-amyloid precedes development of amyloid plaques in Down syndrome. *Arch Pathol Lab Med.* 2001; 125:489–492. [PubMed: 11260621]
65. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Biol.* 2007; 8:101–112. [PubMed: 17245412]
66. Hampel H, Teipel SJ, Fuchsberger T, et al. Value of CSF beta-amyloid1-42 and tau as predictors of Alzheimer's disease in patients with mild cognitive impairment. *Mol Psychiatry.* 2004; 9:705–710. [PubMed: 14699432]
67. Hansson Petersen CA, Alikhani N, Behbahani H, et al. The amyloid beta-peptide is imported into mitochondria via the TOM import machinery and localized to mitochondrial cristae. *Proc Natl Acad Sci U S A.* 2008; 105:13145–13150. [PubMed: 18757748]
68. Harigaya Y, Saido TC, Eckman CB, Prada CM, Shoji M, Younkin SG. Amyloid beta protein starting pyroglutamate at position 3 is a major component of the amyloid deposits in the Alzheimer's disease brain. *Biochem Biophys Res Commun.* 2000; 276:422–427. [PubMed: 11027491]
69. Hartmann T. Intracellular biology of Alzheimer's disease amyloid beta peptide. *Eur Arch Psychiatry Clin Neurosci.* 1999; 249:291–298. [PubMed: 10653285]
70. Hashimoto M, Bogdanovic N, Volkmann I, Aoki M, Winblad B, Tjernberg LO. Analysis of microdissected human neurons by a sensitive ELISA reveals a correlation between elevated intracellular concentrations of Abeta42 and Alzheimer's disease neuropathology. *Acta Neuropathol.* 2010
71. Hecimovic S, Wang J, Dolios G, Martinez M, Wang R, Goate AM. Mutations in APP have independent effects on Abeta and CTFgamma generation. *Neurobiol Dis.* 2004; 17:205–218. [PubMed: 15474359]
72. Heneka MT, Ramanathan M, Jacobs AH, et al. Locus ceruleus degeneration promotes Alzheimer pathogenesis in amyloid precursor protein 23 transgenic mice. *J Neurosci.* 2006; 26:1343–1354. [PubMed: 16452658]
73. Herzig MC, Winkler DT, Burgermeister P, et al. Abeta is targeted to the vasculature in a mouse model of hereditary cerebral hemorrhage with amyloidosis. *Nat Neurosci.* 2004; 7:954–960. [PubMed: 15311281]
74. Holcomb L, Gordon MN, McGowan E, et al. Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nat Med.* 1998; 4:97–100. [PubMed: 9427614]
75. Holmes C, Boche D, Wilkinson D, et al. Long-term effects of Abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. *Lancet.* 2008; 372:216–223. [PubMed: 18640458]
76. Horikoshi Y, Sakaguchi G, Becker AG, et al. Development of Abeta terminal end-specific antibodies and sensitive ELISA for Abeta variant. *Biochem Biophys Res Commun.* 2004; 319:733–737. [PubMed: 15184044]
77. Hsia AY, Masliah E, McConlogue L, et al. Plaque-independent disruption of neural circuits in Alzheimer's disease mouse models. *Proc Natl Acad Sci U S A.* 1999; 96:3228–3233. [PubMed: 10077666]
78. Hsiao K, Chapman P, Nilsen S, et al. Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science.* 1996; 274:99–102. [PubMed: 8810256]
79. Hsieh H, Boehm J, Sato C, et al. AMPAR removal underlies Abeta-induced synaptic depression and dendritic spine loss. *Neuron.* 2006; 52:831–843. [PubMed: 17145504]
80. Hyman BT, Van Hoesen GW, Beyreuther K, Masters CL. A4 amyloid protein immunoreactivity is present in Alzheimer's disease neurofibrillary tangles. *Neurosci Lett.* 1989; 101:352–355. [PubMed: 2671814]

81. Iwata N, Tsubuki S, Takaki Y, et al. Identification of the major Abeta1-42-degrading catabolic pathway in brain parenchyma: suppression leads to biochemical and pathological deposition. *Nat Med.* 2000; 6:143–150. [PubMed: 10655101]
82. Iwatsubo T, Odaka A, Suzuki N, Mizusawa H, Nukina N, Ihara Y. Visualization of A beta 42(43) and A beta 40 in senile plaques with end-specific A beta monoclonals: evidence that an initially deposited species is A beta 42(43). *Neuron.* 1994; 13:45–53. [PubMed: 8043280]
83. Jack CR Jr, Knopman DS, Jagust WJ, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol.* 2010; 9:119–128. [PubMed: 20083042]
84. Jin LW, Shie FS, Maezawa I, Vincent I, Bird T. Intracellular accumulation of amyloidogenic fragments of amyloid-beta precursor protein in neurons with Niemann-Pick type C defects is associated with endosomal abnormalities. *Am J Pathol.* 2004; 164:975–985. [PubMed: 14982851]
85. Kamenetz F, Tomita T, Hsieh H, et al. APP processing and synaptic function. *Neuron.* 2003; 37:925–937. [PubMed: 12670422]
86. Kawarabayashi T, Younkin LH, Saido TC, Shoji M, Ashe KH, Younkin SG. Age-dependent changes in brain, CSF, and plasma amyloid (beta) protein in the Tg2576 transgenic mouse model of Alzheimer's disease. *J Neurosci.* 2001; 21:372–381. [PubMed: 11160418]
87. Kaye R, Head E, Sarsoza F, et al. Fibril specific, conformation dependent antibodies recognize a generic epitope common to amyloid fibrils and fibrillar oligomers that is absent in prefibrillar oligomers. *Mol Neurodegener.* 2007; 2:18. [PubMed: 17897471]
88. Kim J, Basak JM, Holtzman DM. The role of apolipoprotein E in Alzheimer's disease. *Neuron.* 2009; 63:287–303. [PubMed: 19679070]
89. Knobloch M, Konietzko U, Krebs DC, Nitsch RM. Intracellular Abeta and cognitive deficits precede beta-amyloid deposition in transgenic arcAbeta mice. *Neurobiol Aging.* 2007; 28:1297–1306. [PubMed: 16876915]
90. Kumar-Singh S, De Jonghe C, Cruts M, et al. Nonfibrillar diffuse amyloid deposition due to a gamma(42)-secretase site mutation points to an essential role for N-truncated A beta(42) in Alzheimer's disease. *Hum Mol Genet.* 2000; 9:2589–2598. [PubMed: 11063718]
91. Lacor PN, Buniel MC, Chang L, et al. Synaptic targeting by Alzheimer's-related amyloid beta oligomers. *J Neurosci.* 2004; 24:10191–10200. [PubMed: 15537891]
92. LaFerla FM, Green KN, Oddo S. Intracellular amyloid-beta in Alzheimer's disease. *Nat Rev Neurosci.* 2007; 8:499–509. [PubMed: 17551515]
93. LaFerla FM, Tinkle BT, Bieberich CJ, Haudenschild CC, Jay G. The Alzheimer's A beta peptide induces neurodegeneration and apoptotic cell death in transgenic mice. *Nat Genet.* 1995; 9:21–30. [PubMed: 7704018]
94. Lah JJ, Heilman CJ, Nash NR, et al. Light and electron microscopic localization of presenilin-1 in primate brain. *J Neurosci.* 1997; 17:1971–1980. [PubMed: 9045726]
95. Langui D, Girardot N, El Hachimi KH, et al. Subcellular topography of neuronal Abeta peptide in APPxPS1 transgenic mice. *Am J Pathol.* 2004; 165:1465–1477. [PubMed: 15509518]
96. Lazarov O, Lee M, Peterson DA, Sisodia SS. Evidence that synaptically released beta-amyloid accumulates as extracellular deposits in the hippocampus of transgenic mice. *J Neurosci.* 2002; 22:9785–9793. [PubMed: 12427834]
97. Lazarov O, Robinson J, Tang YP, et al. Environmental enrichment reduces Abeta levels and amyloid deposition in transgenic mice. *Cell.* 2005; 120:701–713. [PubMed: 15766532]
98. Lemere CA, Blusztajn JK, Yamaguchi H, Wisniewski T, Saido TC, Selkoe DJ. Sequence of deposition of heterogeneous amyloid beta-peptides and APO E in Down syndrome: implications for initial events in amyloid plaque formation. *Neurobiol Dis.* 1996; 3:16–32. [PubMed: 9173910]
99. Leon WC, Canneva F, Partridge V, et al. A Novel Transgenic Rat Model with a Full Alzheimer's-Like Amyloid Pathology Displays Pre-Plaque Intracellular Amyloid-beta-Associated Cognitive Impairment. *J Alzheimers Dis.* 2010
100. Lesne S, Koh MT, Kotilinek L, et al. A specific amyloid-beta protein assembly in the brain impairs memory. *Nature.* 2006; 440:352–357. [PubMed: 16541076]
101. Lewis J, Dickson DW, Lin WL, et al. Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. *Science.* 2001; 293:1487–1491. [PubMed: 11520987]

102. Li F, Calingasan NY, Yu F, et al. Increased plaque burden in brains of APP mutant MnSOD heterozygous knockout mice. *J Neurochem*. 2004; 89:1308–1312. [PubMed: 15147524]
103. Lin MT, Beal MF. Alzheimer's APP mangles mitochondria. *Nat Med*. 2006; 12:1241–1243. [PubMed: 17088888]
104. Lin MT, Beal MF. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature*. 2006; 443:787–795. [PubMed: 17051205]
105. Longva KE, Blystad FD, Stang E, Larsen AM, Johannessen LE, Madshus IH. Ubiquitination and proteasomal activity is required for transport of the EGF receptor to inner membranes of multivesicular bodies. *J Cell Biol*. 2002; 156:843–854. [PubMed: 11864992]
106. Lord A, Kalimo H, Eckman C, Zhang XQ, Lannfelt L, Nilsson LN. The Arctic Alzheimer mutation facilitates early intraneuronal Abeta aggregation and senile plaque formation in transgenic mice. *Neurobiol Aging*. 2006; 27:67–77. [PubMed: 16298242]
107. Lorenzo A, Yuan M, Zhang Z, et al. Amyloid beta interacts with the amyloid precursor protein: a potential toxic mechanism in Alzheimer's disease. *Nat Neurosci*. 2000; 3:460–464. [PubMed: 10769385]
108. Lue LF, Kuo YM, Roher AE, et al. Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. *Am J Pathol*. 1999; 155:853–862. [PubMed: 10487842]
109. Lustbader JW, Cirilli M, Lin C, et al. ABAD directly links Abeta to mitochondrial toxicity in Alzheimer's disease. *Science*. 2004; 304:448–452. [PubMed: 15087549]
110. Mackenzie IR, Miller LA. Senile plaques in temporal lobe epilepsy. *Acta Neuropathol*. 1994; 87:504–510. [PubMed: 8059603]
111. Mahley RW, Weisgraber KH, Huang Y. Apolipoprotein E: structure determines function, from atherosclerosis to Alzheimer's disease to AIDS. *J Lipid Res*. 2009; 50 Suppl:S183–S188. [PubMed: 19106071]
112. Masliah E, Crews L, Hansen L. Synaptic remodeling during aging and in Alzheimer's disease. *J Alzheimers Dis*. 2006; 9:91–99. [PubMed: 16914848]
113. Masliah E, Mallory M, Hansen L, DeTeresa R, Alford M, Terry R. Synaptic and neuritic alterations during the progression of Alzheimer's disease. *Neurosci Lett*. 1994; 174:67–72. [PubMed: 7970158]
114. Masliah E, Sisk A, Mallory M, Mucke L, Schenk D, Games D. Comparison of neurodegenerative pathology in transgenic mice overexpressing V717F beta-amyloid precursor protein and Alzheimer's disease. *J Neurosci*. 1996; 16:5795–5811. [PubMed: 8795633]
115. Masters CL, Multhaup G, Simms G, Pottgiesser J, Martins RN, Beyreuther K. Neuronal origin of a cerebral amyloid: neurofibrillary tangles of Alzheimer's disease contain the same protein as the amyloid of plaque cores and blood vessels. *Embo J*. 1985; 4:2757–2763. [PubMed: 4065091]
116. Mayeux R, Ottman R, Tang MX, et al. Genetic susceptibility and head injury as risk factors for Alzheimer's disease among community-dwelling elderly persons and their first-degree relatives. *Ann Neurol*. 1993; 33:494–501. [PubMed: 8498827]
117. McGowan E, Pickford F, Kim J, et al. Abeta42 is essential for parenchymal and vascular amyloid deposition in mice. *Neuron*. 2005; 47:191–199. [PubMed: 16039562]
118. McLean CA, Cherny RA, Fraser FW, et al. Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Ann Neurol*. 1999; 46:860–866. [PubMed: 10589538]
119. Mesulam MM. Neuroplasticity failure in Alzheimer's disease: bridging the gap between plaques and tangles. *Neuron*. 1999; 24:521–529. [PubMed: 10595506]
120. Meyer-Luehmann M, Spires-Jones TL, Prada C, et al. Rapid appearance and local toxicity of amyloid-beta plaques in a mouse model of Alzheimer's disease. *Nature*. 2008; 451:720–724. [PubMed: 18256671]
121. Meyer-Luehmann M, Coomaraswamy J, Bolmont T, et al. Exogenous induction of cerebral beta-amyloidogenesis is governed by agent and host. *Science*. 2006; 313:1781–1784. [PubMed: 16990547]
122. Miller SL, Celone K, DePeau K, et al. Age-related memory impairment associated with loss of parietal deactivation but preserved hippocampal activation. *Proc Natl Acad Sci U S A*. 2008; 105:2181–2186. [PubMed: 18238903]

123. Mochizuki A, Tamaoka A, Shimohata A, Komatsuzaki Y, Shoji S. Abeta42-positive non-pyramidal neurons around amyloid plaques in Alzheimer's disease. *Lancet*. 2000; 355:42–43. [PubMed: 10615894]
124. Moechars D, Dewachter I, Lorent K, et al. Early phenotypic changes in transgenic mice that overexpress different mutants of amyloid precursor protein in brain. *J Biol Chem*. 1999; 274:6483–6492. [PubMed: 10037741]
125. Moolman DL, Vitolo OV, Vonsattel JP, Shelanski ML. Dendrite and dendritic spine alterations in Alzheimer models. *J Neurocytol*. 2004; 33:377–387. [PubMed: 15475691]
126. Mori C, Spooner ET, Wisniewsk KE, et al. Intraneuronal Abeta42 accumulation in Down syndrome brain. *Amyloid*. 2002; 9:88–102. [PubMed: 12440481]
127. Mortimer JA, French LR, Hutton JT, Schuman LM. Head injury as a risk factor for Alzheimer's disease. *Neurology*. 1985; 35:264–267. [PubMed: 3969219]
128. Mrak RE, Griffin WS. Interleukin-1, neuroinflammation, and Alzheimer's disease. *Neurobiol Aging*. 2001; 22:903–908. [PubMed: 11754997]
129. Muresan V, Varvel NH, Lamb BT, Muresan Z. The cleavage products of amyloid-beta precursor protein are sorted to distinct carrier vesicles that are independently transported within neurites. *J Neurosci*. 2009; 29:3565–3578. [PubMed: 19295161]
130. Naslund J, Haroutunian V, Mohs R, et al. Correlation between elevated levels of amyloid beta-peptide in the brain and cognitive decline. *JAMA*. 2000; 283:1571–1577. [PubMed: 10735393]
131. Naslund J, Schierhorn A, Hellman U, et al. Relative abundance of Alzheimer A beta amyloid peptide variants in Alzheimer disease and normal aging. *Proc Natl Acad Sci U S A*. 1994; 91:8378–8382. [PubMed: 8078890]
132. Neumann M, Tolnay M, Mackenzie IR. The molecular basis of frontotemporal dementia. *Expert Rev Mol Med*. 2009; 11:e23. [PubMed: 19638255]
133. Nilsberth C, Westlind-Danielsson A, Eckman CB, et al. The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced Abeta protofibril formation. *Nat Neurosci*. 2001; 4:887–893. [PubMed: 11528419]
134. Nishitsuji K, Tomiyama T, Ishibashi K, et al. The E693Delta mutation in amyloid precursor protein increases intracellular accumulation of amyloid beta oligomers and causes endoplasmic reticulum stress-induced apoptosis in cultured cells. *Am J Pathol*. 2009; 174:957–969. [PubMed: 19164507]
135. Nixon RA. Autophagy, amyloidogenesis and Alzheimer disease. *J Cell Sci*. 2007; 120:4081–4091. [PubMed: 18032783]
136. Nuntagij P, Oddo S, LaFerla FM, Kotchabhakdi N, Ottersen OP, Torp R. Amyloid deposits show complexity and intimate spatial relationship with dendrosomatic plasma membranes: an electron microscopic 3D reconstruction analysis in 3xTg-AD mice and aged canines. *J Alzheimers Dis*. 2009; 16:315–323. [PubMed: 19221421]
137. Oakley H, Cole SL, Logan S, et al. Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. *J Neurosci*. 2006; 26:10129–10140. [PubMed: 17021169]
138. Oddo S, Billings L, Kesslak JP, Cribbs DH, LaFerla FM. Abeta immunotherapy leads to clearance of early, but not late, hyperphosphorylated tau aggregates via the proteasome. *Neuron*. 2004; 43:321–332. [PubMed: 15294141]
139. Oddo S, Caccamo A, Smith IF, Green KN, LaFerla FM. A dynamic relationship between intracellular and extracellular pools of Abeta. *Am J Pathol*. 2006; 168:184–194. [PubMed: 16400022]
140. Oddo S, Caccamo A, Shepherd JD, et al. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. *Neuron*. 2003; 39:409–421. [PubMed: 12895417]
141. Ohyagi Y, Asahara H, Chui DH, et al. Intracellular Abeta42 activates p53 promoter: a pathway to neurodegeneration in Alzheimer's disease. *Faseb J*. 2005; 19:255–257. [PubMed: 15548589]
142. Palop JJ, Mucke L. Epilepsy and cognitive impairments in Alzheimer disease. *Arch Neurol*. 2009; 66:435–440. [PubMed: 19204149]

143. Pastorino L, Sun A, Lu PJ, et al. The prolyl isomerase Pin1 regulates amyloid precursor protein processing and amyloid-beta production. *Nature*. 2006; 440:528–534. [PubMed: 16554819]
144. Perry G, Nunomura A, Hirai K, Takeda A, Aliev G, Smith MA. Oxidative damage in Alzheimer's disease: the metabolic dimension. *Int J Dev Neurosci*. 2000; 18:417–421. [PubMed: 10817925]
145. Philipson O, Lannfelt L, Nilsson LN. Genetic and pharmacological evidence of intraneuronal Aβ accumulation in APP transgenic mice. *FEBS Lett*. 2009; 583:3021–3026. [PubMed: 19683527]
146. Pike CJ, Overman MJ, Cotman CW. Amino-terminal deletions enhance aggregation of beta-amyloid peptides in vitro. *J Biol Chem*. 1995; 270:23895–23898. [PubMed: 7592576]
147. Puzzo D, Privitera L, Leznik E, et al. Picomolar amyloid-beta positively modulates synaptic plasticity and memory in hippocampus. *J Neurosci*. 2008; 28:14537–14545. [PubMed: 19118188]
148. Querfurth HW, LaFerla FM. Alzheimer's disease. *N Engl J Med*. 2010; 362:329–344. [PubMed: 20107219]
149. Reddy PH. Mitochondrial medicine for aging and neurodegenerative diseases. *Neuromolecular Med*. 2008; 10:291–315. [PubMed: 18566920]
150. Reiman EM, Chen K, Alexander GE, et al. Functional brain abnormalities in young adults at genetic risk for late-onset Alzheimer's dementia. *Proc Natl Acad Sci U S A*. 2004; 101:284–289. [PubMed: 14688411]
151. Roberson ED, Searce-Levie K, Palop JJ, et al. Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. *Science*. 2007; 316:750–754. [PubMed: 17478722]
152. Roberts GW, Gentleman SM, Lynch A, Murray L, Landon M, Graham DI. Beta amyloid protein deposition in the brain after severe head injury: implications for the pathogenesis of Alzheimer's disease. *J Neurol Neurosurg Psychiatry*. 1994; 57:419–425. [PubMed: 8163989]
153. Rovelet-Lecrux A, Hannequin D, Raux G, et al. APP locus duplication causes autosomal dominant early-onset Alzheimer disease with cerebral amyloid angiopathy. *Nat Genet*. 2006; 38:24–26. [PubMed: 16369530]
154. Runz H, Rietdorf J, Tomic I, et al. Inhibition of intracellular cholesterol transport alters presenilin localization and amyloid precursor protein processing in neuronal cells. *J Neurosci*. 2002; 22:1679–1689. [PubMed: 11880497]
155. Russo C, Schettini G, Saido TC, et al. Presenilin-1 mutations in Alzheimer's disease. *Nature*. 2000; 405:531–532. [PubMed: 10850703]
156. Saavedra L, Mohamed A, Ma V, Kar S, de Chaves EP. Internalization of beta-amyloid peptide by primary neurons in the absence of apolipoprotein E. *J Biol Chem*. 2007; 282:35722–35732. [PubMed: 17911110]
157. Saido TC, Yamao-Harigaya W, Iwatsubo T, Kawashima S. Amino- and carboxyl-terminal heterogeneity of beta-amyloid peptides deposited in human brain. *Neurosci Lett*. 1996; 215:173–176. [PubMed: 8899741]
158. Saido TC, Iwatsubo T, Mann DM, Shimada H, Ihara Y, Kawashima S. Dominant and differential deposition of distinct beta-amyloid peptide species, Aβ₄₂ and Aβ₄₀, in senile plaques. *Neuron*. 1995; 14:457–466. [PubMed: 7857653]
159. Sannerud R, Annaert W. Trafficking, a key player in regulated intramembrane proteolysis. *Semin Cell Dev Biol*. 2009; 20:183–190. [PubMed: 19056506]
160. Santacruz K, Lewis J, Spires T, et al. Tau suppression in a neurodegenerative mouse model improves memory function. *Science*. 2005; 309:476–481. [PubMed: 16020737]
161. Sastre M. Troubleshooting methods for APP processing in vitro. *J Pharmacol Toxicol Methods*. 2010
162. Saura CA, Chen G, Malkani S, et al. Conditional inactivation of presenilin 1 prevents amyloid accumulation and temporarily rescues contextual and spatial working memory impairments in amyloid precursor protein transgenic mice. *J Neurosci*. 2005; 25:6755–6764. [PubMed: 16033885]
163. Scheff SW, Price DA. Synaptic pathology in Alzheimer's disease: a review of ultrastructural studies. *Neurobiol Aging*. 2003; 24:1029–1046. [PubMed: 14643375]

164. Schenk D, Barbour R, Dunn W, et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature*. 1999; 400:173–177. [PubMed: 10408445]
165. Schlentzig D, Manhart S, Cinar Y, et al. Pyroglutamate formation influences solubility and amyloidogenicity of amyloid peptides. *Biochemistry*. 2009; 48:7072–7078. [PubMed: 19518051]
166. Schmechel DE, Saunders AM, Strittmatter WJ, et al. Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proc Natl Acad Sci U S A*. 1993; 90:9649–9653. [PubMed: 8415756]
167. Schmitz C, Rutten BP, Pielen A, et al. Hippocampal neuron loss exceeds amyloid plaque load in a transgenic mouse model of Alzheimer's disease. *Am J Pathol*. 2004; 164:1495–1502. [PubMed: 15039236]
168. Selkoe DJ. Alzheimer's disease is a synaptic failure. *Science*. 2002; 298:789–791. [PubMed: 12399581]
169. Shaked GM, Kummer MP, Lu DC, Galvan V, Bredesen DE, Koo EH. Abeta induces cell death by direct interaction with its cognate extracellular domain on APP (APP 597 – 624). *FASEB J*. 2006; 20:1254–1256. [PubMed: 16636103]
170. Shankar GM, Li S, Mehta TH, et al. Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med*. 2008; 14:837–842. [PubMed: 18568035]
171. Sheng JG, Price DL, Koliatsos VE. Disruption of corticocortical connections ameliorates amyloid burden in terminal fields in a transgenic model of Abeta amyloidosis. *J Neurosci*. 2002; 22:9794–9799. [PubMed: 12427835]
172. Shie FS, LeBoeuf RC, Jin LW. Early intraneuronal Abeta deposition in the hippocampus of APP transgenic mice. *Neuroreport*. 2003; 14:123–129. [PubMed: 12544843]
173. Siman R, Reaume AG, Savage MJ, et al. Presenilin-1 P264L knock-in mutation: differential effects on abeta production, amyloid deposition, and neuronal vulnerability. *J Neurosci*. 2000; 20:8717–8726. [PubMed: 11102478]
174. Skovronsky DM, Doms RW, Lee VM. Detection of a novel intraneuronal pool of insoluble amyloid beta protein that accumulates with time in culture. *J Cell Biol*. 1998; 141:1031–1039. [PubMed: 9585420]
175. Small SA, Gandy S. Sorting through the cell biology of Alzheimer's disease: intracellular pathways to pathogenesis. *Neuron*. 2006; 52:15–31. [PubMed: 17015224]
176. Small SA, Duff K. Linking Abeta and tau in late-onset Alzheimer's disease: a dual pathway hypothesis. *Neuron*. 2008; 60:534–542. [PubMed: 19038212]
177. Snyder EM, Nong Y, Almeida CG, et al. Regulation of NMDA receptor trafficking by amyloid-beta. *Nat Neurosci*. 2005; 8:1051–1058. [PubMed: 16025111]
178. Solomon B. Clinical immunologic approaches for the treatment of Alzheimer's disease. *Expert Opin Investig Drugs*. 2007; 16:819–828.
179. Sperling RA, Laviolette PS, O'Keefe K, et al. Amyloid deposition is associated with impaired default network function in older persons without dementia. *Neuron*. 2009; 63:178–188. [PubMed: 19640477]
180. Steinerman JR, Irizarry M, Scarneas N, et al. Distinct pools of beta-amyloid in Alzheimer disease-affected brain: a clinicopathologic study. *Arch Neurol*. 2008; 65:906–912. [PubMed: 18625856]
181. Stenh C, Englund H, Lord A, et al. Amyloid-beta oligomers are inefficiently measured by enzyme-linked immunosorbent assay. *Ann Neurol*. 2005; 58:147–150. [PubMed: 15984012]
182. Stokin GB, Lillo C, Falzone TL, et al. Axonopathy and transport deficits early in the pathogenesis of Alzheimer's disease. *Science*. 2005; 307:1282–1288. [PubMed: 15731448]
183. Sultana R, Butterfield DA. Role of oxidative stress in the progression of Alzheimer's disease. *J Alzheimers Dis*. 2010; 19:341–353. [PubMed: 20061649]
184. Sze CI, Troncoso JC, Kawas C, Mouton P, Price DL, Martin LJ. Loss of the presynaptic vesicle protein synaptophysin in hippocampus correlates with cognitive decline in Alzheimer disease. *J Neuropathol Exp Neurol*. 1997; 56:933–944. [PubMed: 9258263]
185. Tabira T, Chui DH, Kuroda S. Significance of intracellular Abeta42 accumulation in Alzheimer's disease. *Front Biosci*. 2002; 7:a44–a49. [PubMed: 11897569]

186. Takahashi RH, Capetillo-Zarate E, Lin MT, Milner TA, Gouras GK. Co-occurrence of Alzheimer's disease beta-amyloid and tau pathologies at synapses. *Neurobiol Aging*. 2008
187. Takahashi RH, Almeida CG, Kearney PF, et al. Oligomerization of Alzheimer's beta-amyloid within processes and synapses of cultured neurons and brain. *J Neurosci*. 2004; 24:3592–3599. [PubMed: 15071107]
188. Takahashi RH, Milner TA, Li F, et al. Intraneuronal Alzheimer abeta42 accumulates in multivesicular bodies and is associated with synaptic pathology. *Am J Pathol*. 2002; 161:1869–1879. [PubMed: 12414533]
189. Tampellini D, Magrane J, Takahashi RH, et al. Internalized antibodies to the Abeta domain of APP reduce neuronal Abeta and protect against synaptic alterations. *J Biol Chem*. 2007; 282:18895–18906. [PubMed: 17468102]
190. Tampellini D, Rahman N, Gallo EF, et al. Synaptic activity reduces intraneuronal Abeta, promotes APP transport to synapses, and protects against Abeta-related synaptic alterations. *J Neurosci*. 2009; 29:9704–9713. [PubMed: 19657023]
191. Teller JK, Russo C, DeBusk LM, et al. Presence of soluble amyloid beta-peptide precedes amyloid plaque formation in Down's syndrome. *Nat Med*. 1996; 2:93–95. [PubMed: 8564851]
192. Terry RD, Masliah E, Salmon DP, et al. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann Neurol*. 1991; 30:572–580. [PubMed: 1789684]
193. Thal DR, Del Tredici K, Braak H. Neurodegeneration in Normal Brain Aging and Disease. *Sci Aging Knowledge Environ*. 2004; 9:pe26. [PubMed: 15190177]
194. Thinakaran G, Koo EH. Amyloid precursor protein trafficking, processing, and function. *J Biol Chem*. 2008; 283:29615–29619. [PubMed: 18650430]
195. Turner RS, Suzuki N, Chyung AS, Yonkin SG, Lee VM. Amyloids beta40 and beta42 are generated intracellularly in cultured human neurons and their secretion increases with maturation. *J Biol Chem*. 1996; 271:8966–8970. [PubMed: 8621541]
196. Van Broeck B, Vanhoutte G, Pirici D, et al. Intraneuronal amyloid beta and reduced brain volume in a novel APP T714I mouse model for Alzheimer's disease. *Neurobiol Aging*. 2008; 29:241–252. [PubMed: 17112635]
197. Vassar R, Bennett BD, Babu-Khan S, et al. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science*. 1999; 286:735–741. [PubMed: 10531052]
198. Walsh DM, Klyubin I, Fadeeva JV, et al. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature*. 2002; 416:535–539. [PubMed: 11932745]
199. Wang Z, Wang B, Yang L, et al. Presynaptic and postsynaptic interaction of the amyloid precursor protein promotes peripheral and central synaptogenesis. *J Neurosci*. 2009; 29:10788–10801. [PubMed: 19726636]
200. Weller RO, Djuanda E, Yow HY, Carare RO. Lymphatic drainage of the brain and the pathophysiology of neurological disease. *Acta Neuropathol*. 2009; 117:1–14. [PubMed: 19002474]
201. Wertkin AM, Turner RS, Pleasure SJ, et al. Human neurons derived from a teratocarcinoma cell line express solely the 695-amino acid amyloid precursor protein and produce intracellular beta-amyloid or A4 peptides. *Proc Natl Acad Sci U S A*. 1993; 90:9513–9517. [PubMed: 8415732]
202. Wild-Bode C, Yamazaki T, Capell A, et al. Intracellular generation and accumulation of amyloid beta-peptide terminating at amino acid 42. *J Biol Chem*. 1997; 272:16085–16088. [PubMed: 9195901]
203. Wilson CA, Doms RW, Lee VM. Intracellular APP processing and A beta production in Alzheimer disease. *J Neuropathol Exp Neurol*. 1999; 58:787–794. [PubMed: 10446803]
204. Wirths O, Multhaup G, Bayer TA. A modified beta-amyloid hypothesis: intraneuronal accumulation of the beta-amyloid peptide--the first step of a fatal cascade. *J Neurochem*. 2004; 91:513–520. [PubMed: 15485483]

205. Wirths O, Multhaup G, Czech C, et al. Intraneuronal Abeta accumulation precedes plaque formation in beta-amyloid precursor protein and presenilin-1 double-transgenic mice. *Neurosci Lett*. 2001; 306:116–120. [PubMed: 11403971]
206. Wirths O, Bethge T, Marcello A, et al. Pyroglutamate Abeta pathology in APP/PS1KI mice, sporadic and familial Alzheimer's disease cases. *J Neural Transm*. 2010; 117:85–96. [PubMed: 19823761]
207. WorkingGroup. Consensus recommendations for the postmortem diagnosis of Alzheimer's disease. The National Institute on Aging, and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease. *Neurobiol Aging*. 1997; 18:S1–S2. [PubMed: 9330978]
208. Wyss-Coray T, Mucke L. Inflammation in neurodegenerative disease--a double-edged sword. *Neuron*. 2002; 35:419–432. [PubMed: 12165466]
209. Yamaguchi H, Maat-Schieman ML, van Duinen SG, et al. Amyloid beta protein (Abeta) starts to deposit as plasma membrane-bound form in diffuse plaques of brains from hereditary cerebral hemorrhage with amyloidosis-Dutch type, Alzheimer disease and nondemented aged subjects. *J Neuropathol Exp Neurol*. 2000; 59:723–732. [PubMed: 10952062]
210. Yang AJ, Knauer M, Burdick DA, Glabe C. Intracellular A beta 1-42 aggregates stimulate the accumulation of stable, insoluble amyloidogenic fragments of the amyloid precursor protein in transfected cells. *J Biol Chem*. 1995; 270:14786–14792. [PubMed: 7782344]
211. Yang AJ, Chandswangbhuvana D, Shu T, Henschen A, Glabe CG. Intracellular accumulation of insoluble, newly synthesized abeta1-42 in amyloid precursor protein-transfected cells that have been treated with Abeta1-42. *J Biol Chem*. 1999; 274:20650–20656. [PubMed: 10400697]
212. Yang L, Wang Z, Wang B, Justice NJ, Zheng H. Amyloid precursor protein regulates Cav1.2 L-type calcium channel levels and function to influence GABAergic short-term plasticity. *J Neurosci*. 2009; 29:15660–15668. [PubMed: 20016080]
213. Zhang Y, McLaughlin R, Goodyer C, LeBlanc A. Selective cytotoxicity of intracellular amyloid beta peptide1–42 through p53 and Bax in cultured primary human neurons. *J Cell Biol*. 2002; 156:519–529. [PubMed: 11815632]
214. Zheng H, Jiang M, Trumbauer ME, et al. beta-Amyloid precursor protein-deficient mice show reactive gliosis and decreased locomotor activity. *Cell*. 1995; 81:525–531. [PubMed: 7758106]

Fig. 1.

Schematic diagram of APP and APP metabolites, including APP β CTF and A β , cleavage sites in APP of α , β and γ -secretases, and domains recognized by representative antibodies. A β /APP antibodies, such as 6E10 and 4G8, recognize full-length APP, CTFs and A β . Antibodies P2-1 and 369 are directed against the N- and C-terminal regions of full-length APP, respectively. Antibody 3D6 is specific to the free N-terminus of A β 1-x and β CTF; this antibody will not recognize full-length APP or other APP CTFs. Antibodies MBC40 and MBC42 specifically recognize the free C-terminus of A β 40 and A β 42, respectively, and do not react against full-length APP or APP CTFs.

Fig. 2.

Confocal microscopy of endogenous APP, APP CTFs and A β in human primary neurons in culture (ScienCell; 12 days in vitro). a. Dual-immunofluorescence with N-terminal APP antibody P2-1 (green, Affinity BioReagents) and C-terminal APP antibody 369 (red). Lower panels: high power images show full-length APP (arrows) co-labeled by both N- and C-terminal APP antibodies in distal neurites. Next to full-length APP, antibody 369 also recognizes various APP CTFs and the APP intracellular domain (AICD), which is a reason for why antibody 369 gives a more diffuse pattern of staining (central panel) compared to the more punctate labeling with the N-terminal APP antibody P2-1 alone (left panel). Scale bars: 50 μ m (upper panels); 10 μ m (lower panels). b. Dual-immunofluorescence with antibody 369 (green) and A β 42 antibody 12F4 (red, Covance). Lower panels: high power images show distinct patterns of staining for antibodies 369 (left panel) and A β 42 (central panel), with no substantial co-localization (right panel). Antibody 369 labels full-length APP, various APP CTFs and AICD, while the A β 42-specific antibody labels only A β x-42 peptides. Scale bars: 25 μ m (upper panels); 5 μ m (lower panels).

Fig. 3.

Schematic representation of A β 42 accumulation within neurites leading to A β plaque formation in AD. Image at left is a low power representation of neurons with dendritic arborizations in the brain with early A β 42 accumulation; the red arrow indicates the area of a higher power view shown in the image at right. In the higher power view, accumulation of intraneuronal A β 42 (red) is associated with altered morphology of these distal neurites and synapses. Immuno-histochemical and –EM studies in AD transgenic mouse and human brains support that aberrant A β 42 accumulation in dendritic arborizations can be important determinants for the morphology of plaques.

Fig. 4.

Intraneuronal A β 42 accumulation with head injury. a. Stereotactic insertion of a needle into the brain of a young wild-type rat as a model of head injury. Increased levels of A β 42 can be seen in CA1 pyramidal neurons and their apical dendrites located near (red line) the needle track (arrow) compared to neurons located further away (blue line) 48 to 72 hours following brain injury. b. Immuno-gold staining demonstrated A β 42 accumulation particularly within endosomes (arrows) of CA1 pyramidal neurons located near to the needle track. c. Dual-immuno-staining for A β 42 and AT8 (hyperphosphorylated tau) by light microscopy revealed A β 42 positive neurons (pink, black arrow), AT8 positive neurons (blue, white arrow) and also co-localization of A β 42 and AT8 within neurons (purple, arrow head) in postmortem brain from a case with dementia pugilistica. CA1: CA1 pyramidal cell body layer, SR: stratum radiatum. Scale bars: 100 μ m (a, c); 2 μ m (b).