WHERE DO ALZHEIMER’S PLAQUES AND TANGLES COME FROM? AGING-INDUCED PROTEIN DEGRADATION INEFFICIENCY

Ming Chen and Hugo L. Fernandez

1,2 Neuroscience Research Laboratory, Medical Research Service (151), Bay Pines VA Medical Center, Bay Pines, Florida 33744.
1 Department of Pharmacology and Therapeutics; 2 Department of Neurology and Department of Physiology and Biophysics, University of South Florida College of Medicine, Tampa, Florida, USA

TABLE OF CONTENTS
1. Abstract
2. Introduction
3. Metabolic inefficiency is a natural event in aging
4. Mechanism of amyloid plaque formation
   4.1. Three possible scenarios for plaque formation
   4.2. Slowdown of APP α-processing underlies Aβ overproduction
   4.3. Regulation mechanism of APP α-processing
   4.4. Why do APP and presenilin mutations cause the same plaques?
5. Mechanism of tangle formation
   5.1. Normal degradation mechanism of tau
   5.2. Inefficient degradation of tau underlies tangle formation
6. Risk factors can explain why plaques and tangles are more abundant in AD
7. Promoting basic metabolisms will slowdown neurodegeneration
8. Conclusions
9. Acknowledgements
10. References

1. ABSTRACT

Amyloid plaques and neurofibrillary tangles are prominent lesions in the aging brain and they may be responsible for cell death in Alzheimer’s disease. But a basic question has not been answered: why and how do plaques and tangles form during aging? In this study, we approach this question by first examining what happens in the aging body. Plaques and tangles do not come alone, but together with many other aging markers in the body (cholesterol deposition, gallstones, hair graying, and bone loss, etc.). Because these aging markers occur to a certain extent in all elderly and at about the same time in life, it is reasonable to conceive that they originate from a common cause, that is, aging-induced metabolic inefficiency. If cholesterol and gallstone depositions are the results of inefficient degradation/clearance of lipids and minerals, then similarly plaque and tangle formation in most people would be the results of inefficient normal degradation of β-amyloid precursor protein (APP) and tau, respectively. By this view, our studies should focus on the enzymes responsible for APP and tau normal degradation and their natural changes in aging, rather than on presumed pathological factors. Whatever precise mechanisms underlying their depositions, plaques and tangles are the natural products of aging, thus fundamentally different from pathological events such as cancer growth in concept.

2. INTRODUCTION

Amyloid plaques and neurofibrillary tangles are prominent lesions in aging brains and they are widely thought to be responsible for neurodegeneration and cell death in Alzheimer disease (AD). Along this line, considerable research efforts have been devoted to block their negative effects, but little is known about the mechanism of their origins (1–4). In this study, we approach this question from a new perspective.

Conceptually, there are two groups of cellular lesions in our body that are disease-associated. The first group includes cancer growth or virus proliferation, etc., which are caused by conventional pathogens (gene defects, metabolic errors, infectious agents, etc.). The second group includes age-related cholesterol deposition, gallstones, bone loss, etc. Both groups of lesions can lead to diseases, but their origins may be different.

It is a common assumption today that plaques and tangles are the results of “pathological” processing of β-amyloid precursor protein (APP) and tau protein, respectively. This model may be comparable to the mechanism of cancer growth. Following this concept, many studies today are focused on searching potential pathogens or metabolic errors (2–4). But, our experimental
Where do plaques and tangles come from?

Figure 1. Where do these aging markers come from? They have the same origin (aging), but differ only in the individual biochemical pathways that underlie their formation. These pathways will become inefficient during aging thus aging markers will appear in essentially everyone and at about the same time in life.

findings (5) and comprehensive analyses of current data (6-11) have suggested that plaques and tangles are the results of metabolic inefficiency (especially in Ca\(^{2+}\) signaling), a natural event in aging. By this view, their mechanisms of origin would be similar to that of cholesterol deposition, a process that may be triggered by aging itself, but if progressed into advanced stages and enhanced by risk factors, it can lead to clinical disease.

This finding has led us to a new model for AD origin, that is, advanced aging intensified by risk factors, but not conventional pathogens, may underlie late-onset sporadic AD (12, 13). Since this model contrasts to the current belief that AD is a discrete disease “independent of aging”, it has provoked a scholarly debate about the medical nature of AD (14-17). This debate has prompted us to further examine the origins of plaques and tangles from a broader background. In this study, we start by first considering what happens in the body during aging process.

3. METABOLIC INEFFICIENCY IS A NATURAL EVENT IN AGING

Current view about the ultimate reason for aging is a “genetic clock” coming to term, and this clock governs human lifespan as a biological species (18, 19). Since this clock has not been identified, it has been conceived that the first prominent expression of aging is perhaps the decline of energy metabolisms (10, 20-22). This view is consistent with the concept that life itself is a continuous process of free energy accumulation and consumption, and energy is the ultimate driving force for all life processes (23). Energy levels (mainly the rate of ATP generation and utilization) in humans culminate in young adult (ages 18-25), but start to decline after about age 30. This, in turn, will trigger myriad metabolic pathways to slowdown.

Why will metabolisms slowdown during aging? Because the end point of life is a full-stop of metabolisms (death), therefore, our body must “prepare” for it during the period preceding it (aging) by reducing energy consumption and metabolic rate. This will slowly and progressively diminish the basic life-supporting metabolisms (Kreb cycle, electron transportation, signal transduction, etc.). In biochemical terms, this process will be necessarily associated with diminished consumption of glucose/oxygen, reduced production of hormones, growth factors, neurotransmitters and many other vital metabolites (18, 19).

This metabolic slowdown, in turn, will result in many signs of aging in the body. For example, after age 30, one will be unable to run as fast or remember new information (such as foreign languages) as efficiently as a teenager. This decline of the bodily functions will intensify throughout the years that follow. When these changes accumulate over time, they will give rise to numerous aging markers, such as hair graying, bone loss, cholesterol deposit, plaques and tangles. These aging markers occur to a certain extent in everyone as a necessity after ages 40-50 (with slight individual variations in time course; but in terms of human lifespan, they essentially occur at the same time)(Figure 1).

It may be argued that metabolic overactivation can also happen in some age-related events such as cancer growth (where some enzymes will be activated), so how can metabolic inefficiency be said to be a necessity in aging? It should be point out that cancer occurs only in a minority group of people (18), thus although its incidence increases with aging, it is not a marker for normal aging.

Since normal aging markers occur universally and at about the same time in life, it is reasonable to assume that they originate from a common course (aging), but differ only in the individual biochemical pathways that underlie their formation. For example, although multiple factors are involved, cholesterol deposition can be attributed ultimately to the inefficient normal degradation of lipids; likewise, gallstones to the inefficient degradation/clearance of metals, and so on (18)(Figure 1). Thus, it appears that these aging markers are all the physiological products of aging (though unwanted). Notably, aging markers also include such cellular events as random errors.
Where do plaques and tangles come from?

Figure 2. Two pathways in APP processing. The two pathways compete for the same APP pool. Note that: (a) $\beta$-secretase comes from intact APP, so no $\beta$-secretase can be overly generated unless more APP has escaped $\alpha$-secretase attack; (b) $\alpha$-processing overwhelms $\beta/?$-pathway; and (c) $\alpha$-pathway is a regulated process, so it should be more vulnerable to aging and other insults than $\beta/?$-pathway.

4. MECHANISM OF AMYLOID PLAQUE FORMATION

4. 1. Three possible scenarios for plaque formation

Plaques and tangles are the most prominent markers in aging and AD brains, so any theories about AD origins need to offer a reasonable explanation for their origin. According to our model, their appearance would be similar to other aging markers, that is, simply due to inefficient normal processing of APP and tau, respectively (6, 7)(Figure 1). Is this concept reasonable? We first examine the potential mechanism of the genesis of $\beta$-secretase, the core of amyloid plaques.

Physiologically, APP is processed by two pathways: normal $\alpha$-processing mediated by a putative protease known as a-secretase, or abnormal amyloidogenic processing by two other proteases, $\beta$- and $?\beta$-secretases (4)(Figure 2). From this overall picture, it can be inferred that there are three most reasonable mechanisms for $\beta$-secretase overproduction during aging. They are: (i) APP gene overexpression; (ii) overactivation of both $\beta$- and $?\beta$-secretases (so more APP would enter amyloidogenic pathway); and (iii) inactivation of a-secretase (so APP will accumulate thereby providing more substrate for $\beta/?$-secretases to overproduce $\beta$-secretase).

Of the three scenarios, “APP gene overexpression” has been explored first, but largely ruled out as a common cause for most people (though it can occur in certain cases such as Down’s or head injury)(24, 25). So currently, the scenario of “$\beta/?$-secretases overactivation” is most attractive. Indeed, most studies today on APP processing are focused on these two secretases and the development of their inhibitors (2-4, 26-30). These studies are based on a central assumption that these proteases are somehow overactivated during aging thus inhibiting them will reduce the production of $\beta$-secretase. However, few, if any, of these studies have provided a necessary rationale: why and how can $\beta/?$-secretases become “overactivated” during normal aging in the first place?

Overactivation of enzymes during aging cannot occur without a reason. So it has been suggested that this may be due to some preceding cellular damages such as membrane disruption, pH or metal imbalance, or as widely believed, mistakes in APP intracellular trafficking (2-4, 31). However, these are apparently not the answer, because another question will immediately follow: what has caused these damages? This way, one would be eventually led to an initial “mistake” (gene mutation, metabolic error, etc.). If this is the case, then plaque formation would be similar to cancer growth. However, cancer usually affects only a minority group of people, thus this scenario is inconsistent with the universality of amyloid plaques in normal aging brains. Apparently, amyloid plaques are an integral part of normal aging, therefore any proposed mechanisms, if they are not part of normal aging, should be logically ruled out.

It may be argued that although a mistake/pathogen responsible for plaque formation has not been found, this does not mean that it does not exist. Indeed, how do we know that a mistake/pathogen will not be found in the future?

A theoretical consideration may be helpful for this question. Mistake/pathogen, if it strikes us, is an “accident” in medical nature, or an exception from majority/normality. This is because the probability that our body is overtaken by a mistake/pathogen, or the number of the victims in the population, must be low. Therefore, pathogen-caused diseases usually occur in low incidence, such as Down’s, AIDS or cancer (no more than a few percent). As a low incident event, mistake/pathogen cannot explain common lesions such as plaques, tangles, gray hair or wrinkled skin. Logically, the latter lesions should be related to an intrinsic defect that occurs normally during aging. Metabolic inefficiency meets this criterion.

It is also known today that in addition to aging, $\beta$-secretase is also overproduced by numerous pathological insults including: head trauma (32), ischemic injury (33, 34), energy metabolism inhibition (35), gene mutations on presenilins or APP (2, 3), experimentally induced apoptosis (36), aluminum (37) and mercury toxicity (38).

Why do these insults all cause $\beta$-secretase overproduction? Because although their mechanisms are heterogeneous, they have a common action, that is, inhibiting or disrupting normal cellular metabolisms (otherwise cells would not be dysfunctioned). It thus follows that, whatever its precise mechanism is, $\beta$-secretase overproduction, in essence, is the result of cellular dysfunction or inefficiency, which will necessarily slowdown protein normal metabolisms among many other basic metabolisms. Hence, the cellular consequence of aging would be similar to that of other insults, but also
Where do plaques and tangles come from?

4.2. Slowdown of APP a-processing underlies Aβ accumulation

This reasoning would point to the scenario of “a-processing inactivation” to be a more reasonable model for Aβ overproduction (6, 7). Why will a-processing become inactivated during aging? Because secreted APP (APPs) is only one of the many secretory proteins that are physiologically released during cell growth, differentiation or maintenance (23). As cell growth and metabolisms will slowdown during aging (as well as during the actions of the pathological insults), so will the secretion of APPs.

Slowdown of APP, secretion will result in some APP unclipped and accumulated, and this, in turn, will allow other proteases, including β/β-secretases, to attack it. As proposed in Figure 3, the N-terminal portion of APP is extracelluar, so many proteases there will act on it non-specifically to generate Aβ-containing fragments of varying lengths and such fragments have been well-observed (39). These attacks will continue throughout the aging process until they reach the core of APP (Aβ), which is aggregating and resistant to further attacks. So, in the end of this “progressive trimming”, APP will become uniform Aβ aggregates, and remain as such until after the person’s death. Thus, postmortem examination of the plaques will find mainly the uniform Aβ (Figure 3, bottom). This, at first glance, would convey an impression that a specific β-secretase has “selectively” and “actively” cleaved Aβ out of APP [the role of ?-secretase is discussed in ref. 6]. But, critically absent in this concept is the underlying and invisible changes in a-secretase activity which determines the availability of intact APP, the necessary substrate for Aβ genesis.

This novel model can also be reached if the two APP processing pathways are examined more carefully. Figure 2 reveals two critical but sometimes overlooked features. First, a-processing prevents Aβ formation, indicating that no Aβ will be generated unless some intact APP has first escaped a-secretase attack. This means that the two pathways are not independent of each other, but compete for the same APP pool (i.e., increase in one pathway will be accompanied by a corresponding decrease in the other). Such a reciprocal relationship between APPs and Aβ has been observed by several laboratories (40-44).

Second, the two pathways in APP processing are not equally executed, but a-processing dominates over the other. Thus, any minor changes in a-pathway would be expected to affect Aβ levels substantially. The fact that most intact APP ends up as APPs, but not Aβ, indicates that the activities of β/β-secretases are so low that normally they can only cleave a tiny fraction of APP. So, unless a-secretase is inactivated and more intact APP becomes available, Aβ will not be overly produced during aging. Therefore, the state of a-secretase is a primary determinant for the outcomes of APP processing. Although this model is entirely new, it is a simple analogue to the mechanism of cholesterol deposition: any deposited cholesterol during aging must come from the failed normal degradation of the same molecules (Figure 4; compare to Figure 2).

Because APPs is much more abundant than Aβ, it would not be unexpected that minor changes in APPs, sometimes can be more difficult to measure than the prominent changes in Aβ (45). This situation can also be compared to cholesterol in the body (large pool) and the same molecule deposited on the vessel walls (a tiny fraction of the pool). Any increase in the latter (readily
Where do plaques and tangles come from?

Table 1. Correlative effects of various agents on APPs secretion and Ca\(^{2+}\) activity*

<table>
<thead>
<tr>
<th>Reagent</th>
<th>on APP(_{\beta})</th>
<th>on Ca(^{2+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 NGF</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>EGF</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>FGF</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>2 Glutamate</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Serotonin</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>AMPA</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>3 Carbachol</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Thapsigargin</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Elect.depolarizn.</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Thrombin</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Phorbol ester</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Estrogen</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Vasopressin</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Ca(^{2+}) ionophore</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>

* Group 1, growth factors; 2, excitatory neurotransmitters, 3, Ca\(^{2+}\) agonists; 4, Ca\(^{2+}\) suppressors. ↑, increase; ↓, decrease. References are listed in ref. 6 and 9.

measured) should be associated with a corresponding but minor decrease in the large pool. But evidently, this minor decrease sometimes can be difficult to measure.

In this context, A\(\beta\) genesis would be a by-product of the inefficient APP processing, therefore not an essential part of life. But, both APP, and A\(\beta\) are generated physiologically, so how can one be said to be essential for life whereas the other due to inefficiency? Notably, a key criterion can distinguish the two: essential products of the body will always decrease during aging (APP, growth factors, hormones, ATP, etc.), whereas those due to inefficiency will increase (A\(\beta\), deposited cholesterol, gallstones, cataracts, glucose in the urine, etc.).

If A\(\beta\) is not essential for life, then why do we naturally produce it? This is because our body is not 100% efficient and thus will allow some molecules to escape normal processing. For example, glucose is an essential nutrient and should be completely digested in concept, but in reality, trace amounts of it are always wasted in the urine (proteins and amino acids are as well)(23). So, for the same reason, the imperfect a-processing will spare some APP even in young people, and this inefficiency will intensify during aging (as will lipid and mineral catabolism, etc.).

This reasoning will lead to following logical outcomes:

[a] Increased A\(\beta\) levels in aging can be explained by naturally inactivated a-secretase, but not necessarily by its “overactivated” \(\beta/\?\)-counterparts.

[b] Similar to the knowledge that promoting cholesterol normal degradation is the best way to reduce its deposition (18), re-activating APP a-processing in the elderly will not only increase APP\(_{\beta}\), but also reduce A\(\beta\).

[c] Studying A\(\beta\) origin by focusing exclusively on \(\beta/\?\)-secretes would be similar to studying cholesterol deposition by assuming some “abnormal” enzymes that are “overactivated” during aging thus creating an alternative pathway that is independent of cholesterol normal metabolism. Such an independent pathway perhaps has compared A\(\beta\) deposition, a physiological process, to pathological events such as cancer growth or HIV proliferation.

[d] Inhibiting \(\beta/\?\)-secretes, two natural proteases, in the elderly may need a second thought. It is known that age-related diseases in general can be delayed or alleviated by replenishing hormones, growth factors, vitamins, and other life-supporting factors (18). Evidently, these factors exert their effects by re-activating the diminished metabolisms, but not inhibiting them. These factors are naturally reduced during aging, so the reactions they are responsible for must become inefficient.

4.3. Regulation mechanism of APP a-processing

If A\(\beta\) overproduction is the result of inefficient APP normal processing, then identification of a-secretase would be important for an in-depth understanding of APP metabolism. In this regard, we have shown that calpain(s), a well-known Ca\(^{2+}\)-dependent protease, is a reasonable candidate for a-secretase (5, 9). Many other proteases have also been suggested to be the candidates for this enzyme (46-49), why is calpain so unique?

Because it fits in with several key features of APP a-processing. First, protein normal secretion in general is under the control of Ca\(^{2+}\) signaling (23). Consistent with this concept, APP a-processing is long known to be regulated by signal transduction (50) and, indeed, it is up-regulated by many Ca\(^{2+}\) activators such as growth factors, hormones, and excitatory neurotransmitters, etc., but inhibited by several Ca\(^{2+}\) suppressors (6, 9)(Table 1). These highly consistent reports support the possibility that a-secretase is a Ca\(^{2+}\)-dependent protease (6). Second, Ca\(^{2+}\) signaling occurs in the cytosol but APP is a-cleaved at cell surface. This suggests that a-secretase should respond to Ca\(^{2+}\) in the cytosol meanwhile be able to cleave proteins at cell surface (Figure 3, top), a key feature that calpain has been known of (51-53), but other Ca\(^{2+}\)-dependent proteases have not. Third, abundant production of APP, by various types of cells suggest that a-secretase should be a major and ubiquitous protease. Calpain fulfills these three major criteria altogether perhaps better than other proposed candidates, though further studies are required to establish this model (9).

Interestingly, the essence of this model also fits in with a current concept that a-processing is regulated by protein kinase C (PKC) (4, 51). This concept is based on the fact that a-processing is activated by phorbol esters, potent PKC activators (51). However, it must be noted that the first cellular action of phorbol esters is to stimulate Ca\(^{2+}\) signaling (54) which, in turn, activates PKC (note “C” in PKC stands for Ca\(^{2+}\)(54). As Ca\(^{2+}\) activators, phorbol esters are also potent calpain activators (53, 55). Thus, a more reasonable interpretation for the observed action of phorbol esters is that Ca\(^{2+}\) stimulated by phorbol esters activates both PKC and calpain, but it is calpain that is directly responsible for a-
Where do plaques and tangles come from?

cleavage of APP (Figure 3, top). Notably, APP a-processing is not only activated by phorbol esters, but also by many other agents (Table 1). The correlative actions of these agents can be uniformly explained by Ca\(^{2+}\)-calpain as a common denominator, but not by PKC. Also, Ca\(^{2+}\) is perhaps the only known factor that can sensitively and reversibly regulate proteases (though many other factors can affect protease activities)(23).

If a-processing is a Ca\(^{2+}\)-dependent process, then the mechanism of Ca\(^{2+}\) changes during aging would be a key for understanding the age-related alteration in APP processing. In this regard, a prevailing view today is that intracellular Ca\(^{2+}\) levels constantly increase during aging leading to cell death (56). But, upon careful scrutiny, it came to our attention that this concept is inconsistent with the fact that Ca\(^{2+}\)-mediated processes in the body are all reduced during aging (cell growth, muscle contraction, neuro-transmission, etc.)(23). This indicates that Ca\(^{2+}\) signaling potency (i.e., its final output) must be reduced.

But why are higher Ca\(^{2+}\) levels usually measured in aged cells? This may be because the usually measured Ca\(^{2+}\) “levels” do not always correspond to its potency. It is well-known today that Ca\(^{2+}\) acts by changing its spike frequency and amplitude (like radio waves)(57, 58), but not steady-state “levels” (like water in swimming pool as conventionally conceived). Based on this new knowledge, we proposed that the Ca\(^{2+}\) spike frequency and amplitude will diminish during aging as a result of energy level decline (action potentials and Ca\(^{2+}\) extrusion pumps are both highly energy-dependent) and this will lead to a reduced Ca\(^{2+}\) spike frequency (11). Nevertheless, such a reduced frequency (occur in sub-millisecond) may exhibit as a prolonged Ca\(^{2+}\) stay in the cytosol or a slightly “increased level” if Ca\(^{2+}\) is measured in second or minute time intervals (as in most studies). But, such an increased Ca\(^{2+}\) “level” actually means a reduced signaling potency (11).

Although this novel model awaits experimental confirmation, it is important to note that Ca\(^{2+}\) signaling is a basic mechanism for life, therefore its potency should be naturally reducing during aging, as are any other basic mechanisms of life. The naturally reduced Ca\(^{2+}\) signaling will progressively diminish the a-secretase activity, thereby explaining the natural origin and universality of the amyloid plaques among many other aging markers (Figure 1).

4. 4. Why do APP and presenilin gene mutations cause the same plaques?

If a-secretase is the primary determinant for the outcomes of APP processing, then this model should also explain why various gene mutations in familial AD lead to the same (but more severe) amyloid deposition as in aging. The simple substrate/enzyme relationship (APP/a-secretase; Figure 3, top) suggests that either a changed substrate or changed enzyme activity will end up with the same result: reduced APP a-processing. By this model, mutations in APP gene would act by the former mechanism (changed substrate; detailed discussion in ref. 6). On the other hand, presenilin gene mutations, which account for most early-onset familial AD (2, 3), would cause amyloid deposition by the latter mechanism (reduced a-secretase activity).

This model for mutant presenilins is conceived not by a pure imagination, but rather, based on a systematic comparison with other mutant genes in several human diseases. For example, mutations in LDL receptor gene cause familial atherosclerosis with cholesterol deposition similar to that in sporadic/senile atherosclerosis. Mutations in glucokinase gene lead to familial diabetes similar to sporadic diabetes. Sickle-cell mutations in hemoglobin cause familial anemia similar to sporadic anemia (60, 61).

Why do these mutations all give rise to the similar pathologies as in sporadic diseases? This is because LDL receptor mutations lead to the same (but more severe) end result as aging: reduced cholesterol normal degradation (mutations reduced the LDL-binding ability of the receptor). Glucokinase mutations lead to the same result as sporadic factors/aging: reduced glucose catabolism (mutations reduced the glucose phosphorylation ability of the kinase). Likewise, sickle-cell mutations and sporadic anemia both result in a reduced hemoglobin function (mutations changed hemoglobin which carries O2 through iron; and iron is deficient in sporadic anemia)(60, 61).

If these best known gene mutations all act by reducing the normal function of the proteins, then the role of presenilin mutations in AD should be understood on the same basis. But how? Because molecular structures of presenilins are highly channel-like and have amino acid sequence similarity to an actual Ca\(^{2+}\)-channel (59), it is conceivable that presenilins act as Ca\(^{2+}\) or cation channels in vivo. If this is the case, then numerous point mutations, which all locate within or near transmembrane domains, would all disrupt their normal Ca\(^{2+}\) channeling ability. This will reduce Ca\(^{2+}\)/a-secretase activity leading to the same (but more severe) amyloid deposition as in aging (no extra intact APP, no A\(_\beta\) can be overproduced; A\(_\beta\)42 can be explained by the steric effects of the mutant proteins)(7, 8).

It is noteworthy that these other gene mutations all act by a “loss of function” mechanism, but most current models for presenilins are based on a “gain-of-function” assumption (such as mutations increase Ca\(^{2+}\) entry or activate γ-secretase, or presenilin is γ-secretase)(30, 62). Notably, our model for presenilins can explain not only amyloid deposition, but also tangle formation and neurotransmission deficit altogether in the presenilin mutant hosts (tau degradation and neurotransmitter release are also mediated by Ca\(^{2+}\”; see below).

It must also be noted that the roles of these other mutant genes have been understood only after the mechanisms of the sporadic diseases were delineated. Indeed, had the reduced cholesterol degradation, glucose consumption and O\(_2\)-transport in the sporadic diseases not been recognized, then the roles of the mutant genes would not have been so obvious. Therefore, it is clear that the role of presenilin mutations should be understood together with the potential mechanism of sporadic AD.

5. MECHANISM OF TANGLE FORMATION

5. 1. Normal degradation mechanism of tau

We have also deduced that tangle formation is the result of inefficient normal degradation of tau (7, 10). Many other models, mostly based on pathological origins,
Where do plaques and tangles come from?

Figure 5. Tau normal metabolism and changes during aging. Physiologically, tau is degraded by calpain in cooperation with PKC and calcineurin (CALN) in response to Ca\(^{2+}\) signaling. But during aging, these enzymes will become inefficient, so some tau will stay unclipped and accumulate over time. PKA, cAMP-dependent kinase.

have been proposed for tangle formation (63-65), so why do we prefer this one? Again, when a protein becomes accumulated during aging, we first suspect possible changes in its normal degradation process. What is the mechanism of tau normal degradation?

Cell division, growth and differentiation require cell shape changes, which are made possible by cytoskeleton break-ups followed by reorganization. This process involves proteolytic degradation as an essential step (23). It is also known that such proteolysis does not occur in the major cytoskeletal proteins such as actin or tubulin polymers (which is apparently too disruptive). Instead, physiological cleavages usually occur in the interlocking or cross-linking proteins such as filamin, spectrin, fodrin or tau, leading to a limited but controlled modification of cell architecture (23).

These proteins are well-known to be cleaved in vivo preferentially by calpain(s) (66-70) and this is consistent with the knowledge that cytoskeleton reorganization is a regulated process by signal transduction in response to stimuli (hormones, growth factors, etc.) (23). As such, the controlled cytoskeleton cleavage should be mediated by a regulated protease. Cell growth and differentiation are under the control of Ca\(^{2+}\) signaling (23), so it is logical to expect that the cross-linking proteins are cleaved mainly by calpain, a dominant protease in many cell types known to attack a large number of cytoskeletal and membrane proteins (66).

Nerve cells in adult brain do not undergo division and proliferation. But during long-term potentiation and plasticity modification in memory formation, neuronal architecture will also undergo mild but permanent changes in which cleavage by calpain is long known as an essential step (70, 71). Viewed from this perspective, neuron-specific tau is only one of the many cytoskeletal proteins that undergo dynamic and regulated degradation throughout life. Memory formation is perhaps the most sensitive activity in the body, it is difficult to conceive that such a process can be mediated by unregulated proteases such as trypsin.

In addition to proteolytic cleavage, cytoskeletal proteins also undergo a dynamic phosphorylation-dephosphorylation process during reorganization, which involves a complex interplay of many protein kinases and phosphatases. But, these kinases and phosphatases usually have two types of actions (or acting on two different types of sites on their substrates): one makes cytoskeleton more vulnerable to calpain attack, the other more resistant (72-75). In this regard, it is well known that phosphorylation by PKC, or dephosphorylation by calcineurin (a Ca\(^{2+}\)-dependent phosphatase), will render cytoskeletal proteins more vulnerable to calpain cleavage (69, 72, 73). On the other hand, phosphorylation by cAMP-dependent protein kinase exhibits opposite effect (69, 74, 75).

5.2. Inefficient degradation of tau underlies tangle formation

These observations have led us to propose a phosphorylation/dephosphorylation model for the regulated proteolysis of filamin (also known as actin-binding protein) in platelet (76). In this model, PKC and calcineurin act in a cooperative manner, but on different sites, to promote filamin cleavage (during platelet activation by Ca\(^{2+}\) signals where cytoskeleton break-up is essential). On the other hand, phosphorylation by cAMP-dependent kinase will render filamin more stable (during platelet inhibition where cytoskeleton is stabilized) (69, 76) (Figure 5).

Now, it appears that the essence of this model is also applicable to tau metabolism, because similar actions of PKC, calcineurin, and cAMP-dependent kinase with respect to tau’s susceptibility to calpain have all been observed by a number of laboratories (68, 75, 77-80). According to this model (Figure 5), tau will undergo a regulatory process similar to filamin in response to Ca\(^{2+}\) signals in nerve cells throughout life. But, this process will profoundly change during aging process when basic metabolisms including Ca\(^{2+}\) signaling will decline. This, in turn, will inactivate various Ca\(^{2+}\)-dependent enzymes including calpain and calcineurin (81-84), resulting in apparent hyperphosphorylation and accumulation of tau, i.e., stabilizing it against normal degradation. In addition to calcineurin, it is also possible that inactivation of many other phosphatases in the cell may contribute to tau hyperphosphorylation during aging (84).
Where do plaques and tangles come from?

![Diagram of Tau and Ca^2+]

Figure 6. Two pathways in tau metabolism. Like any other proteins, tau will end up in one of the two outcomes which compete for the same pool. Thus, promoting normal degradation will reduce its deposition.

This model emphasizes three points. First, tangle formation is the result of inefficient catalolism of tau (Figure 6), a natural event during aging thus similar to Aβ and cholesterol deposition (Figs. 2 and 4). Second, the scenarios of “overactivation” of protein kinases leading to tau hyperphosphorylation, derived mostly from in vitro studies, may not be the case in the aging brain. Tangles are natural products of aging, so again, any proposed mechanisms, if they are not part of normal aging, will not fit in with this feature of the lesion.

Third, while most current models focus on tau phosphorylation only, we consider phosphorylation and proteolysis are both important. Altered phosphorylation will change tau conformation and function, but proteolytic failure will directly lead to its accumulation. Hyper-phosphorylation of tau can be directly observed, but it would be difficult to directly demonstrate the activity changes of calpain during aging. This is because the enzyme in the brain is controlled by dynamic Ca^2+ spikes induced mainly by action potentials which do not exist in isolated cells (11, 85). This may be why phosphorylation mechanism has attracted most research interests today.

Notably, tau cleavage by calpain has been firmly established by many laboratories (68, 74, 75, 77-80) and, to our knowledge, no any other proteases have been reported to cleave tau in vivo (in contrast to the numerous proteases being proposed as APP secretases). Given this knowledge, why has the field today largely ignored the key role of calpain? To this puzzle, we speculate that this is most likely due to the overwhelming status of the “calcium overload” hypothesis today. Under this hypothesis, tau cleavage by calpain, which points straightforwardly to a Ca^2+ signal deficit during tangle formation (68, 86), would be unfavorable. Consequently, investigators would search mechanisms other than Ca^2+ signaling to explain tangle formation. For this reason, we think that the “calcium overload” hypothesis should be a central subject for further scrutiny (7-12, 85).

6. RISK FACTORS CAN EXPLAIN WHY PLAQUES AND TANGLES ARE MORE ABUNDANT IN AD

Many proteins are cleaved in vivo by calpain (66), so if calpain is inactivated, then why are only tau and Aβ accumulated and why only in the brain? We speculate that this is because the core of tau, like that of APP, is also insoluble and aggregating but other proteins may not. Brain neurons usually do not proliferate, this would allow the unclipped tau and Aβ to accumulate preferentially in the brain, but not in peripheral tissues.

It can be argued that although plaques and tangles appear in all elderly, they are apparently deposited at a faster rate thus are more abundant in AD patients. So, even if the deposition is initiated as a natural event, it is likely that the faster deposition in AD, however, is due to a pathogen/mistake additional to aging (like age-related cancers). Is this view correct? Again, we can compare this to the similar and faster deposition of cholesterol and gallstones in certain people. Although some of these cases do involve pathogens/mistakes, we know that the faster deposition, in most people, is primarily due to risk factors (advanced aging, sedentary lifestyle, diet preference, individual background, etc.)(18). Thus, it appears that the faster deposition of plaques and tangles in many cases of sporadic AD can also be explained by similar risk factors, but not always by conventional pathogens or metabolic mistakes (12).

AD is characterized by progressive neurotransmission and memory decline, thus although plaques and tangles are prominent lesions, the primary defect in AD should occur in the dynamic regulatory mechanism underlying neurotransmission and memory process. Since memory decline starts after age 30 (see above) long before plaques and tangles become visible (after ages 40-50), it is unlikely that memory decline is triggered by plaques or tangles, but rather, it is reasonable to assume that these lesions progress concurrently as aging advances. As such, they should have a common underlying defect (Figure 1).

The knowledge that Ca^2+ signaling is a central regulator in neurotransmission and memory process (23, 71), together with the roles of Ca^2+ dependent enzymes in APP and tau metabolism, allows us to deduce that this common defect most likely is Ca^2+ signaling deficit (7)(Figure 1). Such a central role of Ca^2+ in AD is akin to insulin deficiency/dysfunction in diabetes or vitamin D/calcium deficiency in bone loss (though many other factors can also be involved at various stages)(60).

7. PROMOTING BASIC METABOLISMS WILL SLOWDOWN NEURODEGENERATION

It should be emphasized that although we have pointed to Ca^2+/calpain as the primary factors in plaque and tangle formation, this view is, nevertheless, not our central theme. Indeed, regardless what specific enzymes are involved in APP and tau degradation and whatever their regulatory mechanisms are, if metabolic inefficiency (i.e., aging) underlies plaques and tangles, then this finding would point to a new mechanism for AD origin (12, 13).

Can this model be tested? It can be tested by its logic predictions. If plaques, tangles and memory decline are all related to aging-induced metabolic inefficiency, then any approaches that promote basic cellular metabolisms will slowdown their progression. This can be achieved by replenishing hormones, growth factors, and other vital metabolites (Table 1), and most importantly, by physical and brain exercises (enhancing energy and other basic metabolisms)(85). Although these strategies only have limited effects today, they can be substantially improved by modern technologies (such as gene therapy; ref. 87, 88) and enhanced by necessary social supports (89).

In this context, late-onset sporadic AD would be very similar to many other senile disorders (senile atherosclerosis, senile osteoporosis, senile muscle atrophy, etc.) where advanced aging enhanced by risk factors may play a primary and common role (18). Thus, these senile disorders are fundamentally
Where do plaques and tangles come from?

Table 2. Which rationales are more reasonable?

<table>
<thead>
<tr>
<th>Common Rationale</th>
<th>Alternative Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaques and tangles are “hallmarks of AD”</td>
<td>No aging, no plaques/tangles; during aging, everyone has them. So, they are hallmarks of aging</td>
</tr>
<tr>
<td>As hallmarks of AD, they mark AD brains only, like HIV is to AIDS</td>
<td>As aging markers, they mark AD but also non-AD brains, fundamentally different from HIV is to AIDS</td>
</tr>
<tr>
<td>As hallmarks of AD, the only question would be: what they do in the AD brain and how they kill cells there?</td>
<td>As aging markers, key questions would be: why do they come with many other aging markers in the body? And what do they also do in old but healthy brains?</td>
</tr>
<tr>
<td>As hallmarks of a disease, they themselves should be “disease processes”, thus must be caused by pathogens/metabolic mistakes</td>
<td>As aging markers, they are perfectly normal in elderly. Though deposited faster in AD, the deposition process is still a natural event and can be accelerated by risk factors</td>
</tr>
<tr>
<td>Many pathological insults cause APP and tau metabolic abnormalities in vitro, so they all can be the potential causes for AD</td>
<td>Although many insults can lead to the same lesions, there is only one reason for plaque/tangle formation in most AD patients: aging while they were normal</td>
</tr>
<tr>
<td>Aβ is generated by the actions of β/γ-secretases, so inhibiting them will reduce Aβ</td>
<td>Every Aβ must come from an intact APP, so a key issue is: why is more intact APP available during aging?</td>
</tr>
<tr>
<td>Aβ is an abnormal product, so we need to inhibit “abnormal” β/γ-secretases</td>
<td>If so, then we also need to inhibit “abnormal” cholesterol “deposit-ase”, mineral “precipit-ase” or hair “gray-ase”</td>
</tr>
<tr>
<td>Trisomy and gene mutations cause plaques and tangles in Down’s and familial AD, so similar mistakes may exist in sporadic AD</td>
<td>Rare genetic defects cause cholesterol deposits and cataracts in juveniles, but can the same genetic defects account for senile cholesterol plaques and senile cataracts?</td>
</tr>
<tr>
<td>Plaques and tangles are the earliest changes in the brain, so they are the triggers for neurodegeneration</td>
<td>They are the earliest visible changes and should be triggered by an even earlier and invisible factor. If so, then which one is the real trigger?</td>
</tr>
<tr>
<td>The most successful drugs are all inhibitors of something (penicillin, aspirin, HIV inhibitor), so AD also needs inhibitors</td>
<td>These drugs inhibit pathogens in conventional diseases, but senile disorders originate from aging and generally need activators for the inefficient metabolisms</td>
</tr>
</tbody>
</table>

different from pathogen-caused conventional diseases by etiologies as well as by intervention strategies (the use of metabolic activators versus inhibitors)(12, 90).

8. CONCLUSIONS

In this study, we have raised questions about some of the common rationales that have been guiding AD research (summarized in Table 2). For example, plaques and tangles are widely viewed as “hallmarks of AD”, but in our opinion, they are better to be considered hallmarks of aging. Conceptual disparities on this issue will largely determine what to look for in our quest and what to expect in the end. Since our model has provoked an intense debate about the medical nature of AD (14-17), it thus awaits the judgement of the medical research community.

9. ACKNOWLEDGEMENTS

Supported by an U.S. Department of the Veterans Affairs Merit Review Program.

10. REFERENCES


Where do plaques and tangles come from?


Send correspondence to: Ming Chen, Ph.D., Neuroscience Research Laboratory, Medical Research Service (151), Bay Pines VA Medical Center, Bay Pines, Florida 33744. Tel: 727-398-6661 ext 4049; Fax: 727-398-9467. E-mail: michen@com1.med.usf.edu

Key words: Aging, Alzheimer disease, Amyloid, Tau, Calpain, Atherosclerosis, Review