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

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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 are plaques and tangles formed 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

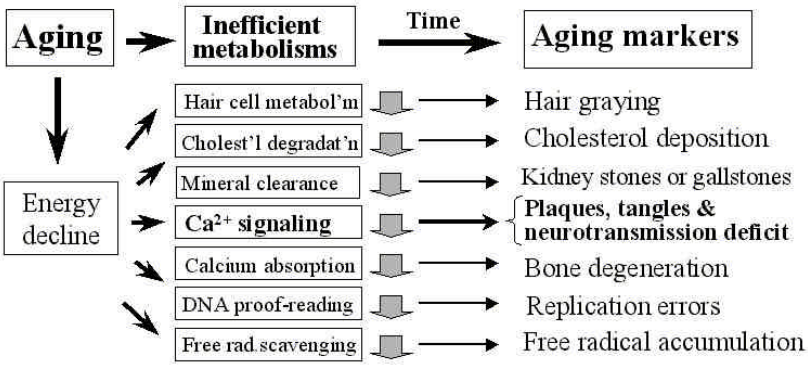

Figure 1. Where do these aging markers come from? They have the same origin (aging), but differ only in the individual biochemical pathways that underlie their formation. These pathways will become inefficient during aging thus aging markers will appear in essentially everyone and at about the same time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

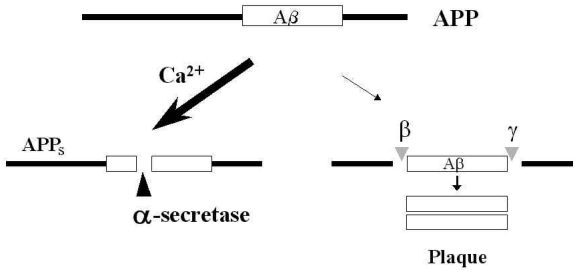

Figure 2. Two pathways in APP processing. The two pathways compete for the same APP pool. Note that: (a) Aβ comes from intact APP, so no Aβ can be overly generated unless more APP has escaped α-secretase attack; (b) α-processing overwhelms β-/?-pathway; and (c) α-pathway is a regulated process, so it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

in DNA replication/transcription and accumulation of free radicals. Because these events occur *normally* throughout life but not *accumulated* in young people (constantly corrected or removed by proof-reading and scavenge systems)(23), their age-related accumulation in most cases can also be traced to the inefficiency in DNA normal proof-reading and free radical normal scavenging, respectively (Figure 1).

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 Aβ, the core of amyloid plaques.

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

Of the three scenarios, "APP gene over-expression" 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 "β/?-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 Aß. However, few, if any, of these studies have provided a necessary rationale: why and how can ß/?-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, Aβ 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 Aβ over-production? 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, Aβ 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

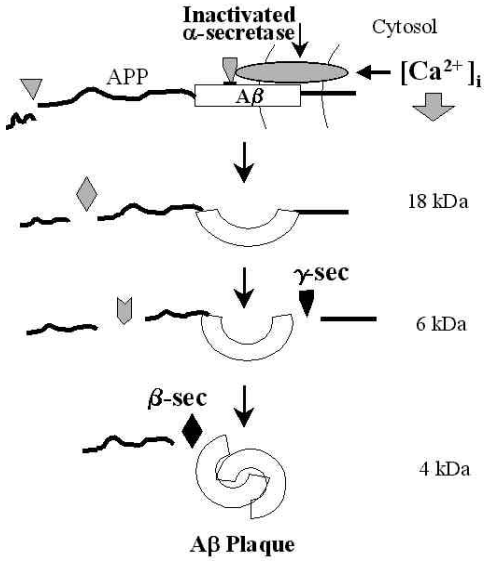

Figure 3. A "progressive trimming" model for plaque formation. Upon inactivation of α-secretase, APP will accumulate and be attacked by other proteases including β-/?-secretases, which will progressively trim APP to its core. In the end, APP will become uniform Aβ aggregates.

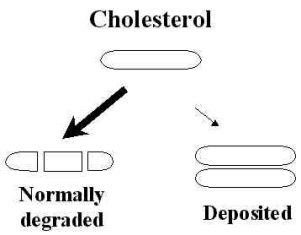

Figure 4. Two pathways in cholesterol catabolism. Cholesterol, like any other macromolecules, will end up in one of the two pathways which compete for the same pool. Thus, (a) the deposited cholesterol must come from the reduced normal degradation; and (b) promoting the latter will reduce the former.

differs from the latter by its natural, slow, and insidious features.

Because these insults all enhance Aβ in a way similar to aging, their mechanism of actions should also be similar to that of aging. Thus, if β-/?-secretases were really overactivated by aging, then all these other insults would be expected to "overactivate" β-/?-secretases as well. This concept is perhaps inconsistent with the apparent *loss of function* caused by these insults to the body.

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 (APP_s) 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 APP_s.

Slowdown of APP_s secretion will result in some APP unclipped and accumulated, and this, in turn, will allow other proteases, including β/?-secretases, to attack it. As proposed in Figure 3, the N-terminal portion of APP is extracellular, so many proteases there will act on it non-specifically to generate Aß-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 (Aß), which is aggregating and resistant to further attacks. So, in the end of this "progressive trimming", APP will become uniform Aß aggregates, and remain as such until after the person's death. Thus, postmortem examination of the plaques will find mainly the uniform Aß (Figure 3, bottom). This, at first glance, would convey an impression that a specific ß-secretase has "selectively" and "actively" cleaved Aß 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 Aß 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 APP_s 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 APP_s, but not Aβ, indicates that the activities of 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 APP_s is much more abundant than Aβ, it would not be unexpected that minor changes in APP_s 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

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

	Reagent	on APP_s	on Ca^{2+}
1	NGF	↑	↑
	EGF	↑	↑
	FGF	↑	↑
2	Glutamate	↑	↑
	Acetylcholine	↑	↑
	Serotonin	↑	↑
	AMPA	↑	↑
3	Carbachol	↑	↑
	Bradykinin	↑	↑
	Thapsigargin	↑	↑
	Electr.depolarizn.	↑	↑
	Thrombin	↑	↑
	Phorbol ester	↑	↑
	Estrogen	↑	↑
	Vasopressin	↑	↑
	Ca^{2+} ionophore	↑	↑

4	Atropine		
	Cholera toxin	↓	↓
	NDGA	↓	↓
	Brefeldin A	↓	↓
	Foskolin	↓	↓

* 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, Aβ genesis would be a *by-product* of the inefficient APP processing, therefore not an essential part of life. But, both APP_s and Aβ 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_s, growth factors, hormones, ATP, etc.), whereas those due to inefficiency will *increase* (Aß, deposited cholesterol, gallstones, cataracts, glucose in the urine, etc.).

If Aß 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 Aβ levels in aging can be explained by naturally inactivated a-secretase, but not necessarily by its "overactivated" ß/?-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_s, but also *reduce* Aß.

[c] Studying Aß origin by focusing exclusively on ß/?-secretases 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 Aß deposition, a *physiological* process, to *pathological* events such as cancer growth or HIV proliferation.

[d] Inhibiting ß/?-secretases, 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 Aß 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_s 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-

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 *increases* 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 Ca2+ channel (59), it is conceivable that presenilins act as Ca2+ 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 Ca2+ channeling ability. This will reduce Ca2+/a-secretase activity leading to the same (but more severe) amyloid deposition as in aging (no extra intact APP, no Aβ can be overproduced; Aβ42 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,

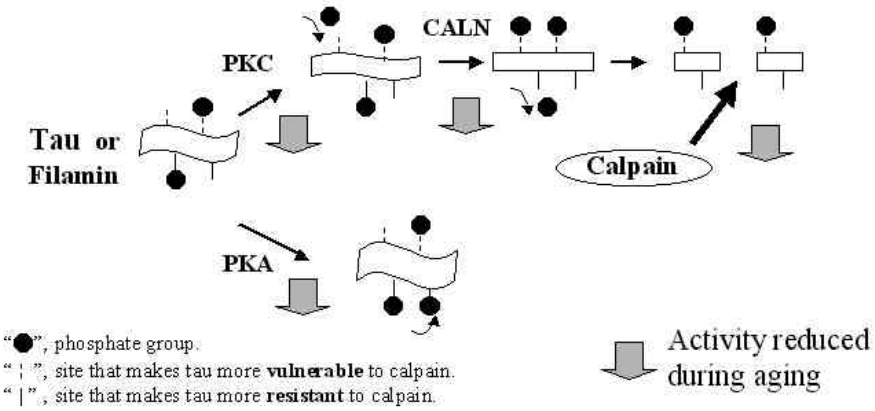

Figure 5. Tau normal metabolism and changes during aging. Physiologically, tau is degraded by calpain in cooperation with PKC and calcineurin (CALN) in response to Ca^{2+} signaling. But during aging, these enzymes will become inefficient, so some tau will stay unclipped and accumulate over time. PKA, 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 Ca2+-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 Ca2+ 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 Ca2+ signals in nerve cells throughout life. But, this process will profoundly change during aging process when basic metabolisms including Ca2+ signaling will decline. This, in turn, will inactivate various Ca2+-dependent enzymes including calpain and calcineurin (81-84), resulting in apparent hyperphsophorylation 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).

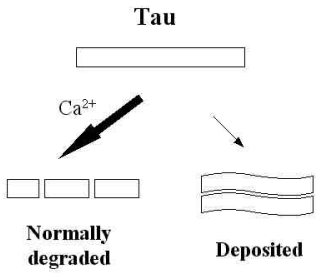

Figure 6. Two pathways in tau metabolism. Like any other proteins, tau will end up in one of the two outcomes which compete for the same pool. Thus, promoting normal degradation will reduce its deposition.

This model emphasizes three points. First, tangle formation is the result of inefficient catabolism of tau (Figure 6), a natural event during aging thus similar to Aß 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 Aß 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 Aß 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 neuro-transmission 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

Table 2. Which rationales are more reasonable?

Common Rationale	Alternative Rationale
Plaques and tangles are "hallmarks of AD"	No aging, no plaques/tangles; during aging, everyone has them. So, they are hallmarks of aging
As hallmarks of AD, they mark AD brains only, like HIV is to AIDS	As aging markers, they mark AD but also non-AD brains, fundamentally different from HIV is to AIDS
As hallmarks of AD, the only question would be: what they do in the AD brain and how they kill cells there?	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?
As hallmarks of a disease, they themselves should be "disease processes", thus must be caused by pathogens/ metabolic mistakes	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
Many pathological insults cause APP and tau metabolic abnormalities in vitro, so they all can be the potential causes for AD	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
Aβ is generated by the actions of β/?-secretases, so inhibiting them will reduce Aβ	Every Aβ must come from an intact APP, so a key issue is: why is more intact APP available during aging?
Aβ is an abnormal product, so we need to inhibit "abnormal" β/?-secretases	If so, then we also need to inhibit "abnormal" cholesterol "deposit-ase", mineral "precipit-ase" or hair "gray-ase"
Trisomy and gene mutations cause plaques and tangles in Down's and familial AD, so similar mistakes may exist in sporadic AD	Rare genetic defects cause cholesterol deposits and cataracts in juveniles, but can the same genetic defects account for senile cholesterol plaques and senile cataracts?
Plaques and tangles are the earliest changes in the brain, so they are the triggers for neurodegeneration	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?
The most successful drugs are all inhibitors of something (penicillin, aspirin, HIV inhibitor), so AD also needs inhibitors	These drugs inhibit pathogens in conventional diseases, but senile disorders originate from aging and generally need activators for the inefficient metabolisms

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.

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10. REFERENCES

1. Khachaturian, Z.S. Diagnosis of Alzheimer's disease. *Arch. Neurol.* 42, 1097-1105 (1985)
2. Selkoe, D.J. Translating cell biology into therapeutic advances in Alzheimer's disease. *Nature* 399 (Suppl), A23-31 (1999)
3. Price, D.L., Sisodia, S.S., Borchld, D.R. Genetic neurodegenerative diseases: the human illness and transgenic models. *Science* 282, 1079-1083 (1998)
4. Mattson, M. P. Cellular actions of ß-amyloid precursor protein and its solution and fibrillogenic derivatives. *Physiol. Rev.* 77, 1081-1132 (1997)
5. Chen, M., Durr, J., and Fernandez, H.L. Possible role of calpain in normal processing of Alzheimer's beta-amyloid precursor protein in human platelets. *Biochem. Biophys. Res. Commun.* 273, 170-175 (2000)
6. Chen, M. Alzheimer's a-secretase may be a calcium-dependent protease. *FEBS Lett.* 417, 163-167 (1997)
7. Chen, M. The Alzheimer's plaques, tangles and memory deficits may have a common origin. Part I: A calcium deficit hypothesis. *Front. Biosci.* 3, a27-31(1998)
8. Chen, M. The Alzheimer's plaques, tangles and memory deficits may have a common origin. Part II: Therapeutic rationale. *Front. Biosci.* 3, a32-37 (1998)
9. Chen, M., Fernandez, H.L. The Alzheimer's plaques, tangles and memory deficits may have a common origin. Part IV: Can calpain act as a-secretase? *Front. Biosci.* 3, a66-75 (1998)
10. Chen, M., Fernandez H.L. The Alzheimer's plaques, tangles and memory deficits may have a common origin. Part V: Why is Ca^{2+} signal lower in the disease? *Front. Biosci.* 4, a9-15 (1999)

11. Chen, M. and Fernandez, H.L. Ca2+signaling down-regulation in aging and Alzheimer's disease: Why is Ca2+ so difficult to measure? *Cell Calcium* 26, 149-153 (1999)
12. Chen, M., Fernandez, H.L. Revisiting Alzheimer's disease from a new perspective: Can "risk factors" play a key role? *J. Alzheimer Dis.* 2, 97-108 (2000)
13. Chen, M., Fernandez, H.L. How important are risk factors in Alzheimer's disease? *J. Alzheimer Dis.* 2, 119-121 (2000)
14. Mattson, M.P. Risk Factors and Mechanisms of Alzheimer's Disease Pathogenesis: Obviously and Obviously Not. *J. Alzheimer Dis.* 2, 109-112 (2000)
15. Price, J.L., Phil, D., Rubin, E.H., Morris, J.C. Comments on Revisiting Alzheimer's disease from a new prospective: can "risk factors" play a key role? *J. Alzheimer Dis.* 2, 113-114 (2000)
16. Khachaturian, Z.S. Aging: a cause or a risk for AD? *J. Alzheimer Dis.* 2, 115-116 (2000)
17. Ghanbari, H. Risk factors versus Alzheimer's disease or symptoms associated with Alzheimer's disease. *J. Alzheimer Dis.* 2, 117 (2000)
18. Cassel, C.K., Cohen, H.J., Larson, E.B., Meier, D.E., Resnick, N.M., Rubenstein, L.Z., Sorenson, L.B. (eds) *Geriatric Medicine.* 3th edn. Springer, New York (1997)
19. Ricklefs, R.E., and Finch, C.E. *Aging: a natural history*. Scientific American Library, New York (1995)
20. Beal, M.F. Energetics in the pathogenesis of neurodegenerative diseases. *Trend Neurosci.* 23, 298-304 (2000)
21. Hoyer, S. Risk factors for Alzheimer's disease during aging. Impacts of glucose/energy metabolism. *J. Neural. Transm.* 54 (Suppl), 187-194 (1998)
22. de la Torre, J.C. Cerebral hypoperfusion, capillary degeneration, and development of Alzheimer disease. *Ann. N.Y. Acad. Sci.* 903, 424-436 (2000)
23. Albert, B., Bray, D., Lewis, J., Raff, M., Roberts, K., Watson, J.D. *Molecular Biology of the Cell* 3rd edn. Garland Co., New York (1994)
24. Cordell, B. beta-Amyloid formation as a potential therapeutic target for Alzheimer's disease. *Ann Rev Pharmacol. Toxicol.* 34, 69-89 (1994)
25. Selkoe, D.J. Normal and abnormal biology of the beta-amyloid precursor protein. *Ann. Rev. Neurosci.* 17, 489-517 (1994)
26. Vassar, R., Bennett, B.D., Babu-Khan, S. *et al.* Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* 286, 735-741(1999)
27. Sinha, S., Anderson, J.P., Barbour, R. *et al.* Purification of cloning of amyloid precursor protein beta-secretase from human brain. *Nautre* 402, 537-540 (1999)
28. Yan, R., Bienkowski, M.J., Shuck, M.E. *et al.* Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity. *Nature* 402, 533-537 (1999)
29. Hussain, I., Powell, D., Howlett, D.R. *et al.* Identification of a novel aspartic protease (Asp 2) as beta-secretase. *Mol. Cell Neurosci*. 14, 419-427 (1999)
30. Selkoe, D.J., Wolfe, M.S. In search of gamma-secretase: presenilin at the cutting edge. *Proc. Natl. Acad. Sci.USA.* 97, 5690-5692 . (2000)
31. Greenfield, J.P., Tsai, J., Gouras, G.K., Hai, B., Thinakaran, G. *et al*. Endoplasmic reticulum and trans-Golgi network generate distinct populations of Alzheimer beta-amyloid peptides. *Proc. Natl. Acad. Sci. USA.*. 96, 742-747 (1999)
32. Roberts, G.W., Gentleman, S.M., Lynch, A., Graham, D.I. ßA4 amyloid protein deposition in brain after head injury. *Lancet* 338, 1422-1423 (1991)
33. Jendroska, K., Hoffmann, O.M., Patt, S. Amyloid beta peptide and precursor protein (APP) in mild and severe brain ischemia. *Ann. N. Y. Acad. Sci.* 826, 401-405 (1997)
34. Pluta, R., Barcikowska, M., Misicka, A., Lipkowski, A.W., Spisacka, S. Januszewski, S. Ischemic rats as a model in the study of the neurobiological role of human beta-amyloid peptide. Time-dependent disappearing diffuse amyloid plaques in brain. *Neuroreport* 10, 3615-3619 (1999)
35. Gabuzda, D., Busciglio, J., Chen, L.B., Matsudaira, P. and Yankner, B.A. Inhibition of energy metabolism alters the processing of amyloid precursor protein and induces a potentially amyloidogenic derivative. *J. Biol. Chem.* 269, 13623-13628 (1994)
36. Galli, C., Piccini, A., Ciotti, M.T., Castellani, L., Calissano, P. *et al.* Increased amyloidogenic secretion in cerebellar granule cells undergoing apoptosis. *Proc. Natl. Acad. Sci. USA.* 95, 1247-1252 (1998)
37. Candy, J.M., McArthur, F.K., Oakley, A.E., Taylor, G.A., Chen, C.P.L.-H. *et al.* Aluminum accumulation in relation to senile plaque and neurofibrillary tangle formation in the brain of patients with renal failure. *J. Neurochem.* 107, 210-218 (1992)
38. Olivieri, G., Brack, C., Muller-Spahn, F., Stahelin, H.B., Herrmann, M. *et al.* Mercury induces cell cytotoxicity and oxidative stress and increases beta-amyloid secretion and tau phosphorylation in SHSY5Y neuroblastoma cells. *J. Neurochem.* 74, 231-236 (2000)
39. Golde, T.E., Estus, S., Younkin, L.H., Selkoe, D.J, Younkin, S.G. Processing of the amyloid protein precursor to potentially amyloidogenic derivatives. *Science* 255, 728-730 (1992)
40. Buxbaum J.D., Ruefli, A.A., Parker, C.A., Cypess, A.M., and Greengard, P. Calcium regulates processing of the Alzheimer amyloid protein precursor in a protein kinase C-independent manner. *Proc. Natl. Acad. Sci. USA.* 91, 4489-4493 (1994)
41. Skovronsky, D.M., Moore, D.B., Milla, M.E., Doms, R.W., Lee, V.M.-Y. Protein kinase C-dependent alpha-secretase competes with beta-secreatse for cleavage of amyloid-beta precursor protein in the trans-Golgi network. *J. Biol. Chem.* 275, 2568-2575 (2000)
42. Xu, H., Gouras, G.K., Greenfield, J.P., Vincent, B., Naslund, J. *et al.* Estrogen reduces neuronal generation of Alzheimer beta-amyloid peptides. *Nature Med.* 4, 447-451 (1998)
43. Van Nostrand, W.E., Wagner, S.L., Shankle, W.R., Farrow, J.S., Dick, M. *et al.* Decreased levels of soluble amyloid-beta protein precusor in cerebrospinal fluid of live Alzheimer's disease patient. *Proc. Natl. Acad. Sci. USA.* 89, 2551-2555 (1992)
44. Lannfelt, L., Basun, H., Wahlund, L.-O., Rowe, B.A., Wagner, S.L. Decreased alpha-secretase-cleaved amyloid precursor protein as a diagnostic marker for Alzheimer's disease. *Nature Med.* 1, 829-832 (1995)
45. Dyrks, T., Monning, U., Beyreuther, K., Turner, J. Amyloid precursor protein secretion and beta A4 amyloid generation are not mutually exclusive. *FEBS Lett.* 349, 210-214 (1994)
46. Zhang, W., Espinoza, D., Hines, V., Innis, M., Mehta, P., and Miller, D. L. Characterization of beta-amyloid peptide precursor processing by the yeast Yap3 and Mkc7 proteases. *Biochim. Biophys. Acta* 1359, 110-122 (1997)
47. Komano, H., Seeger, M., Gandy, S., Wang, G. T., Krafft, G. A., and Fuller, R. S. Involvement of cell surface glycosyl-phosphatidylinositol-linked aspartyl proteases in alpha-secretase-type cleavage and ectodomain solubilization of human Alzheimer beta-amyloid precursor protein in yeast. *J. Biol. Chem.* 273, 31648-31651(1998)
48. Buxbaum, J. D., Liu, K. N., Slack, J. L., Stocking, K. L., Peschon, J. J., Johnson, R. S., Castner, B. J., Cerretti, D. P., and Black, R. A. Evidence that tumor necrosis factor alpha converting enzyme is involved in regulated alpha-secretase cleavage of the Alzheimer amyloid protein precursor. *J. Biol. Chem.* 273, 27765-27767 (1998)
49. Lammich, S., Kojro, E., Postina, R., Gilbert, S., Pfeiffer, R. *et al.* Constitutive and regulated alpha-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. *Proc. Natl. Acad. Sci. USA.* 96, 3922-3927 (1999)
50. Buxbaum, J.D., Gandy, S.E., Cicchetti, P., Ehrhich, M.D., Czernik, A.J. *et al.* Processing of Alzheimer beta/A4 amyloid precursor protein: modulation by agents that regulate protein

phosphorylation. *Proc. Natl. Acad. Sci. USA.* 87, 6003-6006 (1990)
51. McGowan, E. B., Yeo, K. T., and Detwiler, T. C. The action of calcium-dependent protease on platelet surface glycoproteins. *Arch. Biochem. Biophys.* 227, 287-301 (1983)
52. Schmaier, A. H., Bradford, H. N., Lundberg, D., Farber, A., and Colman, R. W. Membrane expression of platelet calpain. *Blood* 75, 1273-1281 (1990)
53. Li, Q. X., Evin, G., Small, D. H., Multhaup, G., Beyreuther, K., and Masters, C. L. Proteolytic processing of Alzheimer's disease beta A4 amyloid precursor protein in human platelets. *J. Biol. Chem.* 270, 14140-14147 (1995)
54. Nishizuka, Y. Studies and perspectives of protein kinase C. *Science* 233, 305-312 (1986)
55. Hong, D.H., Huan, J., Ou, B.R., Yeh, J.Y., Saido, T.C., *et al.* Protein kinase C isoforms in muscle cells and regulation by phorbol ester and calpain. *Biochim. Biophys. Acta* 1267, 45-54 (1995)
56. Khachaturian, Z.S. Calcium hypothesis of Alzheimer's disease and brain aging. *Ann. N. Y. Acad. Sci.* 747, 1-11 (1994)
57. Berridge, M.J. The AM and FM of calcium signalling. *Nature* 386, 759-760 (1997)
58. Putney, Jr., J.W. Calcium signaling: up, down, up, down….what's the point? *Science* 279, 191-192 (1998)
59. Levy-Lahad, E., Wasco, W., Poorkaj, P., Romano, D.M., Oshima, J. *et al.* Candidat gene for the chromosome 1 familial Alzheimer's disease locus. *Science* 269, 973-977 (1995)
60. Kelley, W.N. *et al.* (eds) *Textbook of Internal Medicine.* 2nd edn. J.B. Lippincott. Philadelphia (1992)
61. Cotran, R.S., Kumar, V., and Collins, T. *Pathologic Basis of Disease.* 6 edn. W.B. Saunders Co., Philadelphia (1999)
62. Leissring, M.A., Parker, I., LaFerla, F.M. Presenilin-2 mutations modulate amplitude and kinetics of inositol 1,4,5-trisphosphate-mediated calcium signals. *J. Biol. Chem.* 274, 32535-32538 (1999)
63. Goedert, M. Tau protein and neurofibrillary pathology of Alzheimer's disease. *Trends Neurosci.* 16, 160-165 (1993)
64. Godemann, R., Biernat, J., Mandelkow, E., Mandelkow, E.M. Phosphorylation of tau protein by recombinant GSK-3beta: pronounced phosphorylation at select Ser/Thr-Pro motifs but no phosphorylation at Ser262 in the repeat domain. *FEBS Lett.* 454, 157-164 (1999)
65. Iqbal, K., Grundke-Iqbal, I. Molecular mechanism of Alzheimer's neurofibrillary degeneration and therapeutic intervention. *Ann. N. Y. Acad. Sci.* (1996) 777, 132-138
66. Suzuki K, Sorimachi H, Yoshizawa T, Kinbara K, Ishiura S. Calpain: novel family members, activation, and physiologic function. *Biol. Chem. Hoppe-Seyler* 376, 523-529 (1995)
67. Siman, R., Baudry, M., Lynch, G. Brain fodrin: substrate for calpain I, an endogenous calcium-activated protease. *Proc. Natl. Acad. Sci. USA.* 81, 3572-3576 (1984)
68. Nixon, R.A. Calcium-activated neutral proteinases as regulators of cellular function. Implication for Alzhimer's disease pathogenesis. *Ann. N. Y. Acad. Sci.* 568, 198-208 (1989)
69. Chen, M., Stracher, A. In situ phosphorylation of platelet actin-binding protein by cAMP-dependent protein kinase stabilizes it against proteolysis by calpain. *J. Biol. Chem.* 264, 14282-14289 (1989)
70. Lynch, G., Baudry, M. Brain spectrin, calpain and long-term changes in synaptic efficacy. *Brain Res. Bull.* 18, 809-815 (1987)
71. Kandel, E.R., Schwartz, J.H., Jessell, T.M. *Principles of Neural Science* 3rd edn. Simon & Schuester, New York (1991)
72. Pontremoli, S., Melloni, E., Michetti, M., Sparatore, B., Salamino, F., Sacco, O., Horecker, B.L. Phosphorylation by protein kinase C of a 20-kDa cytoskeletal polypeptide enchances its susceptibility to digestion by calpain. *Proc. Natl. Acad. Sci. USA.* 84, 398-401(1987)
73. Pontremoli, S., Melloni, E., Michetti, M., Sparatore, B., Salamino, F., Sacco, O., Horecker, B.L. Phosphorylation and proteolytic modification of specific cytoskeletal proteins in human neutrophils stimulated by phorbol 12-myristate 13-acetate. *Proc. Natl. Acad. Sci. USA.* 84, 3604-3608 (1987)
74. Nixon, R.A., Sihag, R.K. Neurofilament phosphorylation: a new look at regulation and function. *Trends Neurosci*. 14, 501-506 (1991)
75. Litersky, J.M., Johnson, G.V. Phosphorylation by cAMP-dependent protein kinase inhibits the degradation of tau by calpain. *J. Biol. Chem*. 267, 1563-1568 (1992)
76. Chen, M. Regulation of platelet cytoskeleton: a phosphorylation-dephosphoarylation mechanism. Ph.D. Dissertation. State University of New York, USA (1989)
77. Johnson, G.V.W., Jope, R.S., Binder, L.I. Proteolysis of tau by calpain. *Biochem. Biophys. Res. Commun.* 163, 1505-1511(1989)
78. Mercken, M., Grynspan, F., Nixon, R.A. Differential sensitivity to proteolysis by brain calpain of adult human tau, fetal tau and PHF-tau. *FEBS Lett.* 368, 10-14 (1995)
79. Litersky, J.M., Johnson, G.V. Phosphorylation of tau in situ: inhibition of calcium-dependent proteolysis. *J. Neuochem.* 65, 903-911 (1995)
80. Yang, L.S, Ksiezak-Reding, H. Calpain-induced proteolysis of normal human tau and tau associated with paired helical filaments. Eur. J. Biochem. 233, 9-17 (1995)
81. Gong, C.X., Singh, T.J., Grundke-Iqbal, I., Iqbal, K. Alzheimer's disease abnormally phosphoarylated tau is dephosphorylated by protein phosphatase-2B (calcineurin). *J. Neurochem*. 62, 803-806 (1994)
82. Ladner, C.J., Czech, J., Maurice, J., Lorens, S.A., Lee, J.M. Reduction of calcineurin enzymatic activity in Alzheimer's disease: correlation with neuropathologic changes. *J. Neuropathol. Exp. Neurol.* 55, 924-931 (1996)
83. Kayyali, U.S., Zhang, W., Yee, A.G., Seidman, J.G., Potter, H. Cytoskeletal changes in the brain of mice lacking calcineurin alpha. *J. Neurochem*. 68, 1668-1678 (1997)
84. Trojanowski, J.Q., Lee, V.M.-Y. Phosphorylation of paired helical filament tau in Alzheimer's disease neurofibrillary lesions: focusing on phosphatases. *FASEB, J.* 9, 1570-1576 . (1995)
85. Chen, M., Fernandez, H.L. Postpone Alzheimer's disease by targeting Ca2+ signaling: important theoretical issues (manuscript submitted)
86. Banner, C. Toward a molecular etiology of Alzheimer's disease. *Intl. Psychogeriatr.* 2, 135-147 (1990)
87. Smith, D.E., Roberts, J., Gage, F.H., Tuszynski, M.H. Age-associated neuronal atrophy occurs in the primate brain and is reversible by growth factor gene therapy. *Proc. Natl. Acad. Sci. USA.* 96, 10893-10898 (1999)
88. Tang, Y-P., Shimizu, E., Dube, G.R., Rampon, C., Kerchner, G.A., Zhuo, M., Liu, G., Tsien, J.Z. Genetic enhancement of learning and memory in mice. *Nature* 401, 63-69 (1999)
89. Fratiglioni, L., Wang, H.X., Ericsson, K., Maytan, M., Winblad, B. Influence of social network on occurrence of dementia: a community-based longitudinal study. *Lancet* 355, 1315-1319 (2000)
90. Chen, M., Fernandez, H.L. Alzheimer's disease research 25 years later: guiding concepts revisited (manuscript submitted)

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