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Molecular Targets for the Treatment of Alzheimer's Disease

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Alzheimer's disease (AD) is regarded as a prototype of the neurodegenerative disorder characterized by progressive memory impairment and multiple cognitive deficits in mid to late life. Its pathological hallmarks consist of neuritic plaques and neurofibrillary tangles in the cerebral cortex, accompanied by neuronal loss. These neuropathological findings are prominent in the temporal neocortex and hippocampus. There are a small proportion of AD cases (~10%) that appear to be transmitted as pure autosomal dominant. Mendelian traits with age-dependent but high penetrance. Molecular genetic studies on pedigrees with the latter type of familial Alzheimer's disease (FAD) with molecular genetic tools have led to the discovery of four different genetic loci associated with inherited susceptibility to AD. It is generally suggested that late-onset AD is caused by a complex set of genetic and environmental factors, such as diet, blood pressure, education, social interaction, and others. In this communication, some of the known risk factors relevant to etiopathogenesis of Alzheimer's disease to date will be briefly reviewed.

Key Words: *Alzheimer's disease, Etiopathogenesis, Amyloid cascade hypothesis, Risk factors, Therapeutic targets*

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder clinically characterized by the inexorable decline of cognitive function, alterations in judgment, perception and personality, and ultimately the loss of essential qualities that define a human existence. The definite diagnosis of AD is made by postmortem analysis of brains of patients with dementia. Intracellular neurofibrillary tangles (NFT) containing hyperphosphorylated tau protein and apolipoprotein E and extracellular senile (neuritic) plaques containing a variety of proteins, including β-amyloid (Aβ), α-synuclein, ubiquitin, apolipoprotein E, presenilins and α1-antichymotrypsin, are considered neuropathological hallmarks of AD[1, 2].

Currently, AD is not only the leading cause of senile dementia, but it is also the most prevalent neurodegenerative disease worldwide, and is subsequently, an increasingly threatening national health problem both abroad and in this country. Despite the fact that AD affects up to 15% of people over the age of 65 and nearly half of all individuals by the age of 85 [3, 4], therapeutic management of the disease is primarily targeted toward palliative treatment of symptoms rather than forestalling the progression of the disease. The major obstacle in managing the disease and designing a rationale for therapeutic targets is our incomplete understanding of the etiopathogenesis of the disease. Several hypotheses, mainly focusing on these hallmarks, have been proposed in an attempt to explain the pathogenesis of AD including theories involving amyloid deposition, tau phosphorylation, oxidative stress, metal ion dysregulation and inflammation. Unfortunately, despite strong evidence that these aspects are associated with AD and almost certainly play a role in the disease process, none of these theories is sufficient to explain the whole spectrum of abnormalities found in AD.

Cholinergic hypothesis

The first clinical signs of AD are impairments of memory and other cognitive function, such as language and visuospatial function, some of which can be explained by loss of cholinergic neurons in the basal forebrain. This loss contributes to the symptom development of AD. The acetylcholine (ACh) neurons in basal forebrain, which provide major inputs to the hippocampus and neocortex, are among the most precociously and severely affected in AD[5, 6]. It is not yet clear, however, whether the extensive loss of neurons and pre-synaptic terminals observed in AD is one of the primary features of this disease or a consequence of the Aβ, pathology (Fig. 1).

The major hypotheses of mechanisms of cholinergic neurodegeneration in AD that have been pursued include: (1)

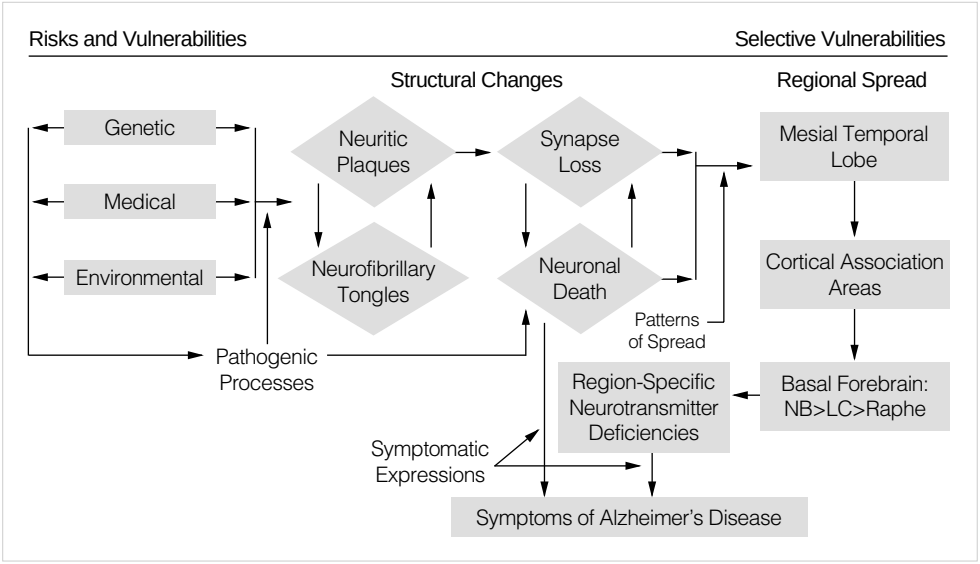

Fig. 1. Cholinergic deficiency in Alzheimer's disease: a pathogenic model. NB, nucleus basalis of Meynert; LC, locus ceruleus.

Table 1. Genetics of Alzheimer's disease

Familial, early onset, autosomal dominant		
Chromosome 21q21.3, β-amyloid precursor protein (APP)	Amino acid substitution 'point' mutation	Various mutations; responsible for Aβ; 2-3% of familial Alzheimer's disease (FAD) Increase in Aβ (Aβ_{42}/Aβ_{40} ratio)
Chromosome 14q24.3, Presenilin 1 (PS-1, S182)	Missense mutation in a transmembrane protein (function unknown)	70% of early-onset FAD; age of onset 30-60y; rapid course; seizures and myoclonus; Increase in Aβ (Aβ_{42}/Aβ_{40} ratio); essential for γ-secretase activity
Chromosome 1q31-42, Presenilin 2 (PS-2, STM-2)	Same as above 67% homologous with PS-1	Germans from the Volga River region (Russia) Increase in Aβ (Aβ_{42}/Aβ_{40} ratio); essential for γ-secretase activity
Familial or sporadic, late onset		
Chromosome 19q13.32, apolipoprotein E (APOE)	Effects on amyloid and tau; cholesterol internalization; 29% lifetime risk	A susceptibility factor; Three polymorphisms; Increased lifetime risk (~29%) with the APOE ε4 allele Increase in Aβ aggregation

excitotoxicity; (2) growth factor deprivation; (3) oxidative stress; (4) inflammation; (5) mitochondrial dysfunction; and (6) amyloid toxicity. Of course, each hypothesis can incorporate a various array of sub-mechanisms (e.g., gliosis, nitric oxide excess, calcium dysregulation), and each mechanism may border, overlap, or intersect with one or more of the others.

Genetic conditions and AD (Table 1)

To date, four genes have been established to be associated with AD phenotypes, including the amyloid precursor protein gene, apolipoprotein E (*ApoE*) gene, and presenilin 1 (*PS-1*) and presenilin 2 (*PS-2*) genes[7]. The majority of familial AD cases are associated with *PS-1* mutations, and the majority of sporadic cases are related to ApoE-ε4[8, 9]. It has become clear that genetic and environmental factors are involved in the pathophysiology of this disease, but it remains unclear how these factors combine and ultimately lead to the neurodegenerative process in AD[10].

Amyloid cascade hypothesis

The basic tenet of the current Aβ hypothesis of AD is that the process of Aβ accumulation as amyloid triggers a complex pathological reaction that leads to tau, and in rarer cases synuclein, aggregation, inflammation, oxidative stress, neuronal dysfunction, and ultimately, clinical dementia. The extracellular plaques mainly contain Aβ peptides[11] which are derived by two proteolytic cleavages from the larger amyloid precursor protein (APP). In the first step, a 99-residue C-terminal fragment (C99) is generated by β-secretase cleavage (BACE1). This product is further processed by γ-secretase activity generating Aβ peptides. An alternative cleavage pathway by α- and subsequent γ-cleavage precludes the generation of Aβ and leads to the generation of the 16 amino acids shorter peptide termed p3. Mutations in the APP gene

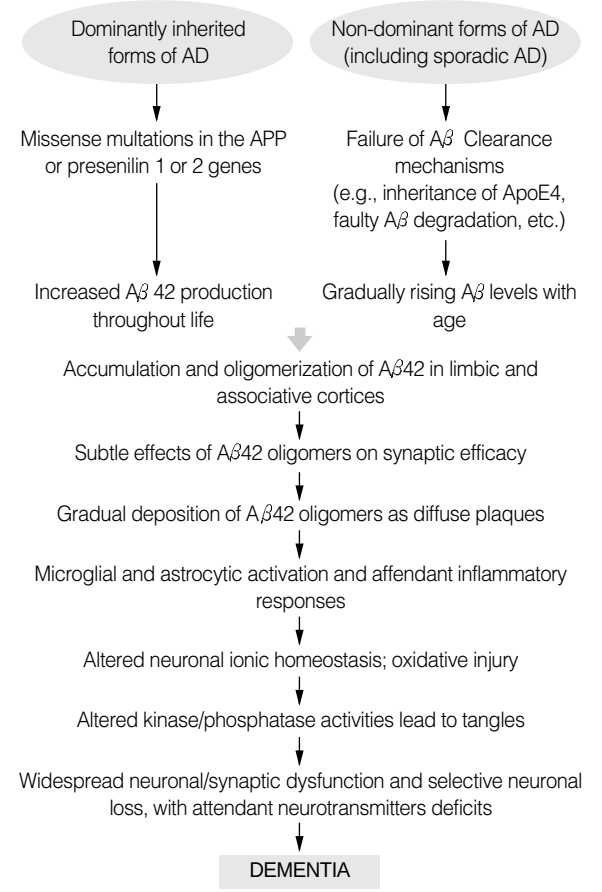

Fig. 2. Sequence of pathogenic steps of Alzheimer's disease: the amyloid hypothesis (from Selkoe[11]).

and the presenilins (PS-1, PS-2) account for most of the familial early onset cases of AD either by enhancing the production of pathological Aβ or the 42-amino acid form, which easily aggregates[11]. The concept of an Aβ cascade has been valid for more than 10 years, and provided a reasonable basis for AD therapeutic strategies (Fig. 2).

Apolipoprotein E and AD

The established genetic risk factor is the E4 isoform for the lipid transport molecule apolipoprotein E (*APOE*: gene; ApoE: protein)[8, 12, 13] which is also a risk factor for coronary atherosclerosis[14]. The ε4 allele for *APOE* has sometimes been implicated in vascular dementia (VaD) and stroke[15], the second most common form of senile dementia. Although the role of apoE in the scavenging of β-amyloid in the brain is well documented, the exact mechanism by which cholesterol directly alters β-amyloid production is not entirely clear. Cholesterol exerts numerous effects on the APP secretase function (in addition to those reported on the apoE-LDL-receptor pathway). Increases in intracellular cholesterol concentrations lead to inhibition of α-secretase activity but stimulate β- and γ-secretase activities. Cholesterol-lowering agents would act indirectly to prevent the effect of vascular risk factors, such as circulating levels of cholesterol or atherosclerotic plaque deposition, from modulating the age of onset in AD. Although it remains difficult to determine how cholesterol-lowering agents affect the pathophysiology of AD, recent findings showing the presence of polymorphic genetic variants in the *HMGR* gene[16] provide us with a possible explanation of the molecular basis and a target for the beneficial effect against AD.

Presenilins and AD

The presenilins are multispanning membrane proteins (presenilin 1, PS-1 and presenilin 2, PS-2) first identified for their genetic association with AD. They are essential components of a multiprotein protease complex implicated in regulated intramembrane proteolysis of several type 1 membrane proteins including the amyloid precursor protein (APP) and developmentally important Notch receptors[17]. Previous studies indicate presenilin proteins form enzymatically active high molecular mass complexes (γ-secretase) consisting of heterodimers of N- and C-terminal fragments in association with nicastrin, presenilin enhancer-2 (PEN-2) and anterior pharynx defective-1 (APH-1) proteins. The variety of identified substrates indicates a critical role for PS in cell metabolism involving controlled cleavage of protein transmembrane domains and signal transduction. The role of PS in γ-secretase activity is compelling and includes lack of Aβ amyloid peptide generation and Notch signalling in PS double knockout cells, cofractionation of activity with PS, abolition of activity with mutation of conserved aspartates in transmembrane domains six and seven, binding of γ-secretase aspartyl protease inhibitors to PS, as well as identification of homologues with protease-associated domains[18]. Following a primary cleavage event that causes ectodomain shedding, PS-dependent 'γ-secretase' proteolysis results in generation of a membrane spanning stub (such as Aβ peptide) and a cytoplasmic fragment such as the APP intracellular domain or the Notch intracellular domain which translocates to the nucleus for regulation of gene expression[17]. All presenilin mutations cause a dominant gain of function and

may induce AD by enhancing $A\beta_{42}$ production, thus promoting cerebral β-amyloidosis.

Estrogen and AD

Epidemiological data showing a predisposition of women to develop AD led many researchers to investigate the role of sex steroids, namely estrogen, in disease pathogenesis. Although there is circumstantial support for the role of estrogen, the unexpected results of the Women's Health Initiative (WHI) Memory Study, which reported an increase in the risk for probable dementia and impaired cognitive performance in postmenopausal women treated with a combination of estrogen and progestin, have raised serious questions regarding the protective effects of estrogen. Although explanations for these surprising results vary greatly, the WHI Memory Study cannot be correctly interpreted without a complete investigation of the effects of the other hormones of the hypothalamic-pituitary-gonadal (HPG) axis on the aging brain. Certain hormones of the HPG axis, namely, the gonadotropins (luteinizing hormone and follicle-stimulating hormone), are not only involved in regulating reproductive function via a complex feedback loop but are also known to cross the blood-brain barrier. Webber et al. propose that the increase in gonadotropin concentrations, and not the decrease in steroid hormone (e.g., estrogen) production following menopause/andropause, is a potentially primary causative factor for the development of AD[19].

Mitochondria, Oxidative stress, and AD

Accumulating evidence supports the hypothesis that oxidative stress generated by various mechanisms may be among the major risk factors that initiate and promote neurodegeneration. Compared with other tissues, the central nervous system may be particularly susceptible to oxidative damage. Many authors suggest that an imbalance between the generation of free radicals and antioxidants may be involved in the pathogenesis of most neurodegenerative diseases. The fact that age is the most important risk factor of sporadic AD provides considerable support for the free radical hypothesis. Many considerations suggest that free radicals and consequently mitochondrial dysfunction are involved in age-related pathologies of AD[20]. It seems very likely that oxidative damage and defective mitochondrial function are the earliest events in AD[21]. However, oxidative stress could be a necessary, but insufficient factor such that the development of disease is dependent upon an additional factors for the onset of occult pathogenesis.

Metal ions and AD

Oxidation reactions are catalyzed by transition metals such as iron and copper and, as such, the likelihood that an oxidation reaction will take place is probably increased by the regional concentrations of transition metals. Substantial studies show that the metabolism of iron is involved in AD and that the concentration of iron in the brain of AD patients is elevated. Aluminum has also received attention in AD, although a role has never been convincingly demonstrated. Nonetheless, aluminum has been found in high concentrations in both senile plaques and intraneuronal neurofibrillary tangles in the brains of subjects with AD, which suggests that this metal may be involved in the etiopathology of AD[22]. Aluminum, unlike transition metal ions, is unable to redox cycle in electron transfer reactions due to a fixed oxidation state of 3+ in biological systems, but growing evidence suggests that it can act synergistically with iron to increase free radical damage [23]. Recent study shows that accumulated aluminum in the central nervous system modulates amyloid-β formation and deposition. Strong evidence also shows that copper are also implicated in the development of AD[24]. In the AD brain, the concentration of zinc is significantly elevated in senile plaques and the concentration of copper is elevated in the rim of senile plaques. Overall, these studies indicate that the environmental conditions in AD, exacerbated by imbalances in several metals, has the potential for catalyzing and stimulating free radical formation and enhancing neuron degeneration.

Vascular risk factors and AD

Epidemiological and neuropathological studies have suggested that there is an association between common AD and several vascular risk factors, such as hypertension, inheritance of the allele encoding apolipoprotein-E-ε4, myocardial infarctions, diabetes mellitus, ischemic white-matter lesions, generalized atherosclerosis[25] and, most recently, high consumption of animal fat. Long-term high blood pressure starting in middle age can cause severe atherosclerosis and large-artery stiffness in later life. Neuropathological and epidemiological studies have directly and indirectly linked atheroscle-

rotic burden in the brain to AD pathological changes[26]. Furthermore, high blood pressure is a major risk factor for stroke and white-matter lesions, which could promote clinical expressions of AD and dementia[27]. Thus, an atherosclerotic process may be involved in the pathogenesis, progression, and clinical presentation of dementia, including both AD and VaD. Proper antihypertensive treatment may reduce dementia risk and slow down disease progression by improving focal cerebral perfusion and inhibiting angiogenesis of brain endothelial cells.

OTHER MISCELLANEOUS MECHANISMS

Cell cycle re-entry and AD

Attempted cell cycle induction also appears to be an important factor in neuronal cell loss during AD. In pathological specimens of brains from Alzheimer's patients, the cell cycle regulators P16 and CDK4 have increased expression in regions such as the hippocampus. Increasing evidence suggests that cell-cycle proteins can be reactivated and contribute to cell demise (Fig. 3). For example, the Cdk1-cyclin B1 complex can be reactivated in AD[28].

Neuroinflammation and AD

Several lines of evidence indicate that microglial activation may be involved in the pathogenesis of Alzheimer's disease. In addition to assisting with the removal of injured cells and cellular debris, microglia may sometimes aggravate a cellular insult. Microglia may lead to cellular damage in AD not only through the generation of ROS products, but also through the production of cytokines and the demise of neighboring neurons[29]. Microglia promote the production of pro-inflammatory and cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1β, free radicals such as NO and superoxide, and fatty acid metabolites such as eicosanoids that can precipitate cell death.

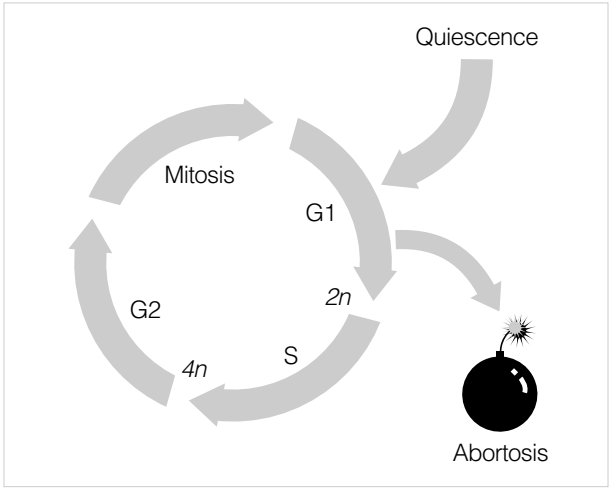

Fig. 3. In Alzheimer's disease the vulnerable neuron enters the cell cycle and then is unable to progress through it with loss of the synchronous nature of the cycle and concomitant dysfunction and neuronal loss. This phenomenon is termed as abortosis (adapted from Raina[28]).

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