Research on the Pros and Cons of the Mouse Model of Alzheimer's Disease

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ABSTRACT

Alzheimer's disease (AD) is the biggest cause of dementia, and the animal model has always been a high research priority in treating AD. This paper introduces three generations of AD mouse models by summarizing their merits and limitations in order to facilitate the understanding of these animal models and design more precise experiments in the future. Conclusions can be drawn that the over-expression paradigm of the first generation may cause extra phenotypes which are unrelated to AD, while the second generation overcame this problem but it is still unsuitable to be used in preclinical immunotherapy studies because of the discrete affinity for anti-A β antibodies. Finally, the third generation was introduced to the community. Without the Arctic mutation, this generation could also accumulate wild-type human A β quickly.

Keywords: Alzheimer's disease, Mouse model, Generation, Over-expression, Transgenic, Knock-in

1. INTRODUCTION

As a growing health concern, AD is the commonest neurodegeneration. The global prevalence of AD is predicted to reach a higher level in the coming decades. However, this disease has not received any particular effective treatment. Therefore, relevant research which can help people recapitulate clinical pathology precisely and develop effective medicine is needed urgently. There is evidence revealing that more than 400 treatment candidates did not get treated successfully in clinic although there were also some breakthroughs historically. All these failures can be attributed to various factors, of which the most crucial one is the inappropriate animal model in preclinical studies. Therefore, the research about animal models of preclinical experiments in AD is indispensable.

This paper reviews three generations of mouse models of Alzheimer's disease. The author takes some models which are used frequently as examples to indicate their features and symptoms, and then summarizes the pros and cons of each generation. According to Games, Dora et al., each model has its unique pathology that provides insights into disease mechanisms and interactive features of neuropathologic cascades [8]. With these understandings and experiences, researchers could gradually dissolve the limitations of these models and design the animal models more similar to the molecular mechanism of human disease in the future. With these relevant mechanistic and preclinical studies contributed by mouse models, the treatment development could get benefits indirectly. As a result, it lays a better foundation for the understanding of the treatment and pathological mechanism of AD [8].

2. BACKGROUND AND MANAGEMENT

AD always causes progressive intellectual failure in aged humans, and this disease usually presents memory loss, being involved with multiple cognitive and behavioral parts.

The majority of AD occurs on a sporadic basis, and the familial form of AD (FAD) is extremely rare. FAD is caused by mutations in three genes. They are amyloid precursor protein (APP), presenilin 1 (PS1), and presenilin 2 (PS2). APP is cleaved sequentially by β secretase (BACE1) and γ -secretase enzyme in the brain and generates some soluble β -amyloid (A β) fragments. The protein γ -secretase is composed complicatedly of PS1 or PS2, nicastrin, anterior pharynx defective-1 (Aph1), and presenilin enhancer 2 (PEN2). As a result, most mutations of FAD lead to the A β 42 which has neurotoxicity release by influencing the γ -secretase of proteolytic processing. AD is not an inevitable disease for old people. The risk of individuals developing AD may increase or reduce by some lifestyle factors. Although there is no effective drug for AD, several articles about the benefits of physical activity and exercise have been written. According to the articles, it is believed that doing exercise can help the elders maintain cognition. Multimodal interventions included an active lifestyle could be an effective way for the old [11].

AD is regarded as an ideal disease for animal modeling. The pathological hallmarks of AD, such as amyloid plaques and neurofibrillary tangles (NFTs), are well recognized. Besides, well-defined pathological behavior such as the loss of cognitive can also help researchers improve the animal model.

3. THE FIRST GENERATION OF MOUSE MODELS

3.1 Transgenic models based on overexpression of APP

The amyloid hypothesis states that the reason for AD pathological process is that the A β production and A β clearance are imbalanced. Based on this hypothesis, the transgenic model has been gradually developed. After confirming the FAD mutations, some researchers started to do research on AD models, and the over-expression of transgenes that contain these mutations was an important part for their research [2]. The first generation mouse model of AD uses various promoters to over-express APP with or without FAD mutations [1]. In this topic, the author would take PDAPP and Tg2576 as instances to review this generation of the mouse models of AD.

3.2 Examples and features of the first generation models

Games et al. applied platelet derived growth factor- β (PDGF- β) promoter and generated PDAPP mice. The PDGF- β promoter is highly expressed in the central nervous system, and can drive a human APP transgene with a FAD associated mutation (V717F) [3]. Compared to the APP level of endogenous mice, these transgenic mice displayed an 18-fold elevation of APP RNA and a 10-fold elevation of human APP protein approximately [2]. Human A β peptides (as shown in Figure 1) of this model also proportionately increased. Besides, these mice progressively developed many pathological hallmarks of AD [3]. Furthermore, PDAPP mice play a primary role for APP/A β in the genesis of AD.



Figure 1 A β immunohistochemistry highlights the plaques in the frontal cortex (a) and cerebral amyloid angiopathy (CAA) where A β accumulates within blood vessel (b, arrows). An A β cored plaque is shown at higher magnification in (c) showing a central core. In severe CAA A β accumulates within capillaries (d) [17].

And Hsiao et al. adopted a similar method by using a hamster prion (PrP) promoter to generate Tg2576 mice. The PrP promoter can over-express a human APP the Swedish transgene with FAD mutation (K670N/M671L) by widely driving the expression in the nervous system [5]. Unlike the APP level of the endogenous mouse, Tg2576 mice displayed over a 5-fold elevation of human APP. The amyloid deposition of Tg2576 mice and PDAPP mice is age-dependent, and the downstream consequences, thioflavin S-positive plaques, gliosis and dystrophic neurites, are also similar to those found in AD. For the first time, 11 to 13 months age of Tg2576 appeared plaque amyloid clearly. Since then, Tg2576 mouse line became a transgenic AD model that has been researched greatly [2].

Similar to approaches of PDAPP and Tg2576 mice, development has also been achieved in a number of other transgenic lines. These models have some typical features such as amyloid plaques, elevated levels of A β , dystrophic neurites, and gliosis. The methods are also used to commonly model behavioral deficits [8]. However, specific details of amyloid pathology are different depending on the line. PDAPP and Tg2576 mice are also different to some extent. For instance, Tg2576 mice are famous for their giant plaques [10], and they display more vascular amyloid deposition [9], but these phenomena are commonly absent in PDAPP mice [2].

3.3 Merits and limitations

First-generation mouse models are useful in AD research. These APP over-expressing mice recap cerebral A β accumulation in some aspects and exhibit pathological hallmarks of amyloid. In some conditions, subsequent processes of A β deposition in these mice, such as the loss of synaptic markers, the accumulation of BACE1 and hyperphosphorylated tau [6], are similar to those in AD. Besides, as a strategy of treatment for AD, APP-overexpressing mice are also available in validating and assessing γ -secretase inhibition and BACE1[1]. Another application used as a therapeutic strategy of AD is the preclinical treatment of anti-A β antibody aducanumab, which can reduce soluble and insoluble A β by a dose-dependent manner in Tg2576 [7].

Although first-generation mice have been used in many studies, they have many limitations [1]. Firstly, APP over-expression results in the overproduction of A β . This process leads to the difficulty in distinguishing the functional effects of additional A β and of other overproduced fragments. Secondly, the overt disease onset is quite later than A β pathology accumulation in humans, while in these models cognitive impairment often precedes the A β arising. In addition, the early lethality of APP23 and Tg2576 mice is also unreasonable. Therefore, there is a perspective states that some phenotypes might not be a part of normal disease pathology, they are the result of APP- or APP/PSoverexpression. Thirdly, there is no neurofibrilaries (NTFs), a feature of preclinical, in these models. Fourthly, because of the different mouse strains, promoters, and transgene constructs, it is extremely hard to standardize phenotypes for different models.

4. THE SECOND GENERATION OF MOUSE MODELS

4.1 Single APP knock-in mouse models

APP over-expressing mouse models contain intrinsic problems that may induce artificial phenotypes. To overcome these problems, Saito et al. created new models which can overproduce A β 42, but do not over-express APP [12]. Saito et al. developed single App knock-in mouse models harboring the Swedish (KM670/671NL) and Beyreuther/Iberian(I716F) mutations with or without the E693G Arctic mutation (App^{NL-G-F} and App^{NL-F} mice, respectively) [4]. These models are the second generation of Alzheimer's disease mouse models. In this topic, the author would take App^{NL-F} and App^{NL-G-F} mice as instances for review.

4.2 Examples and features of the secondgeneration models

Second-generation models were manipulated by a knock-in strategy. Saito et al. changed three amino acids, introduced mutations, created respective lines, and eventually humanized the murine A β sequence. The total amount of A β 40 and A β 42 is elevated by the Swedish mutation, and the Beyreuther/Iberian mutation increases the ratio of A β 42 to A β 40. So App^{NL-F} mice could exhibit an increasing A β 42 production and a high Ab42/Ab40 ratio while the expression levels of APP or other fragments are not alert. Because of the increase in C-terminal fragment β (CTF- β) and decrease of C-terminal fragment α (CTF- α), it remains the same for the entire amount of CTF in App knock-in mice [12] [13].

App^{NL/NL} mice only carry the Swedish mutation. Compared to App^{NL-F/NL-F} mice, they are negative controls [15], because Swedish mutations facilitate β cleavage of APP. It is confirmed that the amount of CTF- β and sAPPb has nothing to do with a mouse's pathology or cognitive function by examining AppNL mice [12] [13].

In App^{NL-F} mice, high levels of A β 42 lead to the pathological A β deposition in hippocampus and the cerebral cortex, accompanying enhanced neuroinflammation [1]. In App^{NL-F/NL-F} mice, initial deposition of A β is detected on the 6th month, and memory dysfunction develops on the 18th month.

In APP-Tg mice, the amyloid plaques consist of A β 1 – 40 predominately. They are unphysiologically giant in contrast to authentic AD patients. However, in App^{NL-} ^F mice, the amyloid plaques are mainly composed of pathogenic A β 1/3pE-42. This manner is highly similar to those observed in human AD patients [14]. Besides, as for the reduction of mushroom spines in AD brain indicated by some research, the loss of hippocampal mushroom spines in App^{NL-F} mice is also demonstrated.

App^{NL-G-F} mouse models, whose A β is more oligomerization/fibrillization-prone, show 3 times greater AD pathology and cognitive abnormalities in comparison to App^{NL-F} mice [1].

4.3 Merits and limitations

Second-generation mouse models used in several basic findings are contributed to the basic biology of AD. Hama et al. developed ScaleS, a new sorbitol-based optical clearing method. It can be combined with the conventional model and. compared with immunohistochemistry, it can increase brain volumes of an analysis of the A β burden degree. There are many techniques that prove the second-generation mouse models' capability of evaluating the impact of new therapeutic goals of A β pathology [1]. In addition, there is a hypothesis suggesting that the new Alzheimer candidate gene PLD3 is involved in APP processing. And the App knock-in mice are also used to challenge this hypothesis.

The second-generation mice have some progress in pathology, but they still have some limitations. Firstly, like the first-generation mice, these mice also cannot exhibit neurodegeneration or tau pathology. As a result, App knock-in mice are considered as a preclinical model. Secondly, because APP gene is a murine sequence, except the part of intron 15 – 17, APP in mice may behave differently from it in humans. For example, KPI domain-containing APP variants are expressed in human brains, but not in mouse brains [12].



5. THE THIRD GENERATION OF MOUSE MODELS

5.1 APP knock-in mouse models without the Arctic mutation

Out of all kinds of App knock-in mice, App^{NL-G-F} mice exhibit the most rapid pathology. However, because of the ability of Arctic mutation that rending A β resistant to proteolytic degradation and prone to aggregation, Arctic mutation is unfit for investigating A β metabolism and clearance. Furthermore, due to its discrete affinity for anti-A β antibodies, preclinical immunotherapy research is unsuitable. Therefore, before disease-modifying strategies targeting mechanisms upstream of A β deposition should be developed. The third generation which has the same speed as App^{NL-G-F} mice in accumulating wild-type human A β without the Arctic mutation is a prerequisite [4]. In this topic, the author would take App^{NL-F}Psen1^{P117L} mice as an instance to review this generation of mouse models of AD.

5.2 Examples and features of the third generation models

A pathogenic mutation (P117L) had been introduced in mutant PSEN1 knock-in mice, and Saito et al. used the mice to generate a third-generation model. It shows an early accumulation of wild-type human A β by crossbreeding the App^{NL-F} line with the Psen1^{P117L/WT} line. App^{NL-F}Psen1^{P117L} mice showed a larger number of cored plaques in the cortex and hippocampus and more gliosis in the hippocampus than they were seen in App^{NL-G-F} mice.

Flood et al. made a combination of App and Psen1 mutations, thus generating double knock-in mice. They harbored the Swedish mutations in the App gene and the P264L/P264L mutation in the Psen1 gene [4]. Li et al. used the Swedish, Dutch, and London mutations and generated a model where cerebral amyloid angiopathy (CAA) was carried [16].

5.3 Merits and limitations

In many cases, App^{NL-F}Psen1^{P117L} mice are applicable to examine the roles of hippocampal neuroinflammation in AD etiology. However, these mice may not be adequate for researching BACE1, γ -secretases, and their modifiers. The reason for this is that the β - and γ -secretases will catalyze cleavages, and these cleavages will be factitiously changed by the mutations [4].

6. CONCLUSION

Through reviewing and summarizing three generations of mouse models of AD, it can be concluded that the first-generation over-expression transgenic mouse models advanced the understanding of AD pathology; the second-generation single knock-in mouse models dissolved the additional phenotypes which are unrelated to AD in first-generation models; the third-generation APP knock-in mouse models overcame some limitations of Arctic mutation. These lines have solved several previous limitations and pointed the way for future models. Mutant mice are expected due to their capability of examining catabolism and clearance of A β [4]. Preclinical research such as immunotherapy may achieve its development in re-experimenting new models for the identification of treatment candidates.

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