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Review

Transgenic models of Alzheimer's disease: Better utilization of existing models through viral transgenesis ☆

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ABSTRACT

Animal models have been used for decades in the Alzheimer's disease (AD) research field and have been crucial for the advancement of our understanding of the disease. Most models are based on familial AD mutations of genes involved in the amyloidogenic process, such as the amyloid precursor protein (*APP*) and presenilin 1 (*PS1*). Some models also incorporate mutations in tau (*MAPT*) known to cause frontotemporal dementia, a neurodegenerative disease that shares some elements of neuropathology with AD. While these models are complex, they fail to display pathology that perfectly recapitulates that of the human disease. Unfortunately, this level of pre-existing complexity creates a barrier to the further modification and improvement of these models. However, as the efficacy and safety of viral vectors improves, their use as an alternative to germline genetic modification is becoming a widely used research tool. In this review we discuss how this approach can be used to better utilize common mouse models in AD research. This article is part of a Special Issue entitled: Animal Models of Disease.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease associated with age-related cognitive dysfunction [1]. There is currently no cure for AD, and treatment regimens only partially mask the symptoms while the disease progresses within the brain. AD is always fatal, with death often a result of infections or complications many patients acquire while bed ridden, such as pneumonia or cardiac arrest [2]. As both the average human lifespan and the global population continue to increase, diseases associated with aging are becoming a greater burden on society [3]. Over 5.4 million people in the United States currently suffer from the condition, and this number is expected to double in the next 20 years. Worldwide, it is believed that as many as 35.6 million individuals suffer from AD. It is expected that the cost associated with AD will approach 200 billion dollars in 2012 in the United States alone and could reach 1.1 trillion dollars by the year 2050 [3].

There has been substantial effort placed into developing animal models as a means to better understand AD and its underlying pathologies. There are two broad forms of AD, familial or early-onset AD (FAD) and sporadic or late-onset AD (LAD). FAD results from point mutations in genes directly related to the production of the amyloid-β (Aβ) peptide, such as the amyloid-β precursor protein (APP) [4,5] or presenilin-1 (PS1) [6]. Although LAD has some degree of heritability, the strongest elements of risk stem from aging, environmental factors and commonly inherited alleles of AD associated genes [7]. A majority of the models created to study AD have involved overexpressing mutant proteins that were first discovered in FAD [8]. The discovery and exploration of these mutations has led to a variety of animal models, which have led to a better understanding of AD pathogenesis.

Most mouse models are typically created by microinjecting complementary DNA (cDNA), containing a transgene of interest into the pronuclei of a large number of zygotes [9]. Resulting embryos are then implanted into pseudopregnant dams for normal gestation. Producing gene targeted mice is a more involved process, although knock-out lines for most genes in the mouse are now available (https://www.komp.org/). Creating a simple modification to the mouse genome is relatively routine, although generating viable animals can take many attempts, and still consumes a significant amount of resources. After the initial genetic modification is made, a new mouse line can be crossed into a pre-existing line that already displays one or more other aspects of the disease pathology. Hence, given sufficient time and funding, one could build increasingly complex models of the disease.

Despite many concerted attempts, none of our existing mouse models are perfect surrogates for AD. This does not mean that these models are without merit. Through the use of mouse models, many of the underlying mechanisms that drive AD pathology and neurodegeneration have been elucidated. Most, but not all, models overexpress proteins involved with

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the disease [3,10]. Some models have predominantly amyloid pathology, others tau pathology, and a few have both. However, for many investigators, the complex interactions between AD and other genetic factors are becoming the main focus of their research. This creates a serious logistical problem. Our pre-existing mouse models are already complex, and often involve multiple genetic modifications to reasonably mimic AD neuropathology. As a simple example, consider a situation with a mouse line harboring mutations in three AD associated genes, *APP*, *PS1* and *MAPT*. If these genes assort independently, and wild type littermate controls are needed for an experiment, then a basic husbandry scheme will only generate about 3% usable animals. Although there are ways that this can be streamlined in practice, three germline modifications represent the practical upper limit as to what can be accommodated in a modern laboratory. Therefore, to advance the field in new directions, and incorporate the influence of novel genetic factors, we will need to use a different approach. As the efficacy and safety of viral vectors improves, their use as an alternative to the direct modification of the mouse germline is currently the best option for investigators to better study the complex disease mechanisms involved in AD. In this short review, we discuss how this approach can be taken advantage of to better utilize common mouse models in AD research.

2. Hallmarks of AD

AD neuropathology is characterized by two hallmarks: plaques (either diffuse or neuritic) comprised of the Aβ peptide, and neurofibrillary tangles (NFTs) comprised of hyper-phosphorylated tau in the hippocampus and other cortical regions [1].

2.1. Amyloid pathology

The Aβ peptide is formed when APP (a membrane protein with 4 isoforms ranging from 695 to 770 amino acids in length) [11], is cleaved by two enzymatic activities. Under non-amyloidogenic conditions, α-secretase cleaves APP within the Aβ region, followed by γ-secretase cleavage, producing the APP intracellular domain (AICD), the soluble APP fragment α (sAPPα), and the p3 fragment [12]. In the amyloidogenic pathway APP is first cleaved by β-secretase. This is again followed by γ-secretase cleavage, releasing the AICD, sAPPβ, and the Aβ peptide (Fig. 1) [13]. Aβ peptides are 36–43 amino acids long, the most common being $A\beta_{40}$, which represents about 80% of the total pool. A less common (<10%) form of the peptide, $A\beta_{42}$, is considered to be more pathogenic, having a high tendency to aggregate [14,15]. Aggregation creates oligomers, fibrils, filaments and, ultimately, plaques [16]. These plaques form in the cortex and hippocampus [17], and the neuritic form is surrounded by dystrophic neurites and activated glia [18], and are likely an ongoing source of neuroinflammation [19,20]. Although plaques are directly connected to the disease process, recent evidence suggests that diffusible Aβ oligomers may play a greater role in driving neurodegeneration [21,22].

Under normal conditions, ~90% of APP processing is performed within the non-amyloidogenic pathway and ~10% by the amyloidogenic pathway [23]. It is not known what causes the shift to increased APP processing by the amyloidogenic pathway in AD [24]. One hypothesis suggests that increased cholesterol levels result in lipid raft formation, which has been suggested to be the physiological site of β-secretase and γ-secretase activity [25–27]. β-Secretase is the rate-limiting step in Aβ generation and β-secretase levels are thought to increase throughout the progression of AD [23,28]. In other cases, mutations in AD related proteins (APP, PS1, PS2); which are typical in cases of FAD, can enhance Aβ generation by increasing available substrate and preferential cleavage sites of APP [8,29,30]. Other findings point to a decrease in Aβ clearance in the brain, resulting in a gradual accumulation during aging [31].

2.2. Tau pathology

It is likely that a major portion of the neurodegeneration observed in AD and other tauopathies, diseases involving the pathological aggregation of the tau protein, results from tau pathology. For example, the number of NFTs correlates with clinical disease severity [32,33]. The amyloid cascade hypothesis suggests tangle formation is driven at some level by Aβ [34–38]. This hypothesis suggests that Aβ is the causative factor in AD pathology, resulting in alterations in the equilibrium of tau phosphorylation and dephosphorylation, ultimately leading to tangle formation, neuronal dysfunction, and cell death [38–40] (Fig. 3).

Tau is a microtubule-stabilizing protein present in the cytoplasm of axons but generally absent in the dendrites [41]. Tau acts on tubulin to promote polymerization of the microtubule, paving the way for axonal transport [42]. The *MAPT* gene, which encodes the tau protein, resides on chromosome 17 and is comprised of 16 exons [43]. *MAPT* has four domains: N-terminal, C-terminal, microtubule binding domain (MBD), and a proline-rich domain [44,45]. The N-terminal and MBD regions are alternatively spliced to include or exclude exons 2, 3 and 10, resulting in 6 possible isoforms of the tau protein [46]. The resulting isoforms contain either 3 or 4 microtubule binding domains (3R and 4R respectively) and contain 0, 1 or 2 N-terminal domains (0N, 1N or 2N respectively) separated by a proline rich domain [47]. *MAPT* also contains two haplogroups, H1 and H2, where increased H1 levels correlate with an increased risk of tauopathy [47].

The tau protein undergoes a variety of post-translational modifications [48–50], including cross-linking [51], glycosylation [52], nitration [53], and phosphorylation [54]. The large number of modifications suggests that tau is highly-regulated by complex pathways. The primary cause of tau dysfunction is due to phosphorylation of tau by enzymes such as GSK3β [55], AKT [56], or CDK5 [57], along with many other kinases [47,58]. Phosphorylation of tau occurs at approximately 45 sites, at serine and threonine residues [59–61]. The MBDs of tau confer a positive charge to the protein, allowing it to stably bind the negatively charged microtubules [43]. In the disease state tau becomes hyperphosphorylated, resulting in the neutralization of its net positive charge (Fig. 2). This causes tau to dissociate from the microtubules and aggregate to form paired helical filaments (PHFs) with other hyperphosphorylated tau molecules [60,62,63]. Upon the dissociation of tau, the microtubule network becomes destabilized and axonal transport may be disrupted [64]. Over time, PHFs will accumulate to form neurofibrillary tangles, primarily in pyramidal neurons [65].

3. Viral transgenesis

Viral gene therapy was once thought to be the pinnacle for the treatment of diseases and the future of medicine [66]. Unfortunately, the safety of such technologies was brought into question after the fatality of a patient in a clinical trial using adenoviral vectors [67]. Although the future of therapeutic gene delivery is still debatable, the use of viral vectors to alter gene expression in animals has expanded significantly. It is through viral transgenesis that existing AD mouse models can be better utilized, reducing monetary cost and time investment as well as allowing rapid, large scale screens of potentially AD related genes. Adenoviruses have been useful tools in some fields due to their high efficiency and ease of production, but offer only transient expression and can trigger a significant immune response, reducing their use in brain research [68–71]. Despite a large field of useful viruses, there are now two main viral technologies used in somatic transgenesis: lentivirus and adeno-associated virus (AAV). Here we will provide a brief overview on the benefits of each.

3.1. Lentiviruses

Lentiviruses have become popular in somatic gene modification due to their ability to integrate transgenes into the host genome,

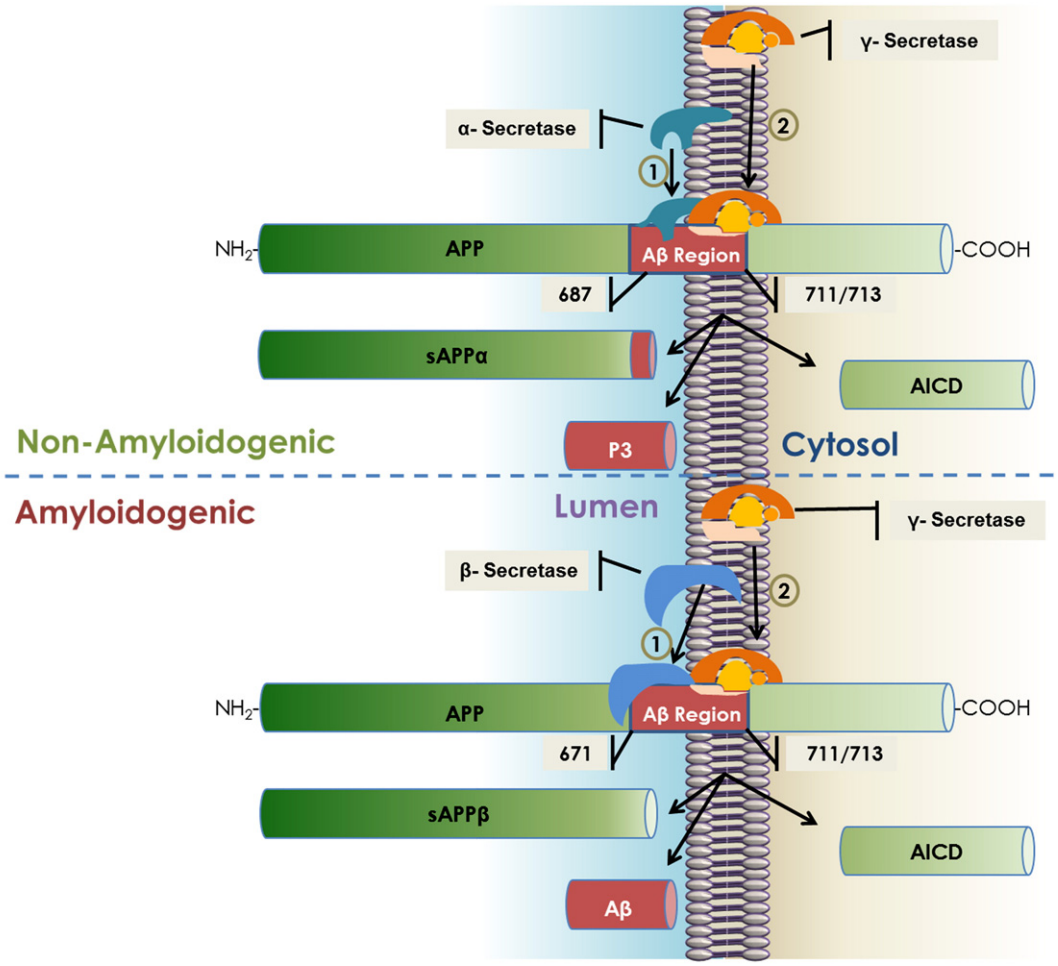

Fig. 1. APP processing and generation of toxic beta-amyloid. APP is a transmembrane protein which can be processed by either the non-amyloidogenic or amyloidogenic pathways. Upper panel: During non-amyloidogenic processing, an enzymatic activity called α-secretase cleaves APP (1) to generate a soluble N-terminal fragment (sAPPα) and an 83 amino acid C-terminal fragment (C83). Next, the γ-secretase complex (2), with PS1 acting as the catalytic subunit, cleaves the C83 fragment within the transmembrane domain, releasing a secreted peptide known as P3. Lower panel: In amyloidogenic processing, an enzymatic activity called β-secretase (1) cleaves APP to generate different soluble N-terminal (sAPPβ) and C-terminal (C99) fragments. Cleavage of the C99 fragment by γ-secretase (2) results in the generation of the Aβ peptide. Processing of APP in either pathway results in the production of an APP intracellular domain (AICD) with poorly defined function.

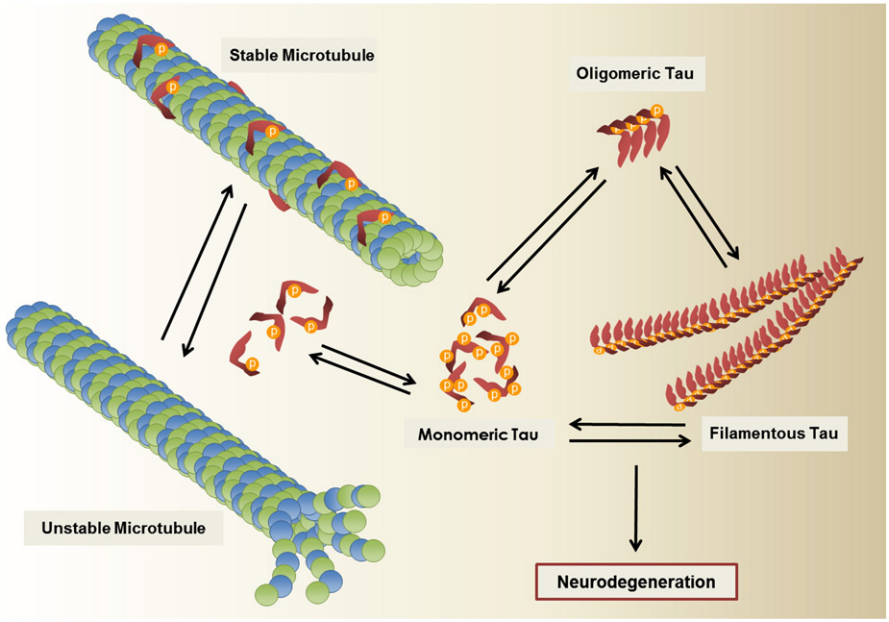

Fig. 2. Tau hyperphosphorylation leads to neurodegeneration. Tau is a cytosolic microtubule stabilizing protein. When tau is hyperphosphorylated, there is an increased tendency for the formation of higher order structures, such as oligomers, filaments, and toxic NFTs.

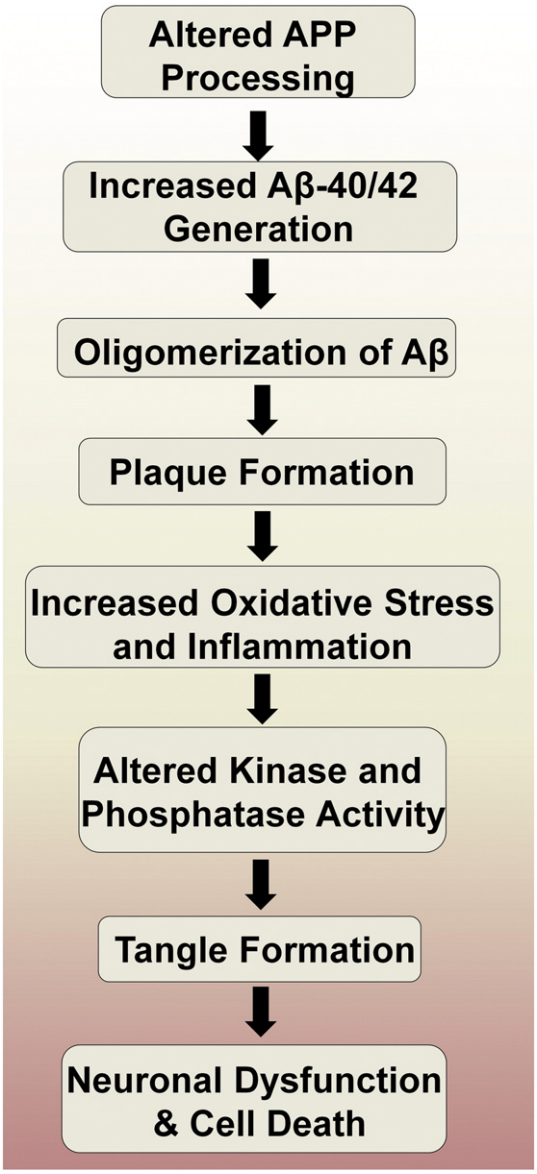

Fig. 3. A simplified overview of the amyloid cascade hypothesis. One version of the amyloid cascade hypothesis proposes that the increase in Aβ biosynthesis, resulting from alterations in APP processing, drives AD pathogenesis. Aggregation of Aβ results in the formation of neuritic plaques, increased oxidative stress, and NFT formation, resulting in neurodegeneration. Other variations are possible. For example, altered clearance of Aβ likely plays a significant role in disease progression.

resulting in long lasting expression. From the *Retroviridae* family, these enveloped retroviruses utilize single-stranded RNA to transfer genetic material and encode for reverse transcriptase and integrase in order to integrate into host cells [72]. It is the ability of lentiviruses to transduce non-dividing cells which make them a popular tool for somatic brain transgenesis, as cells such as neurons are mitotically inactive [73]. They also offer high transduction efficiencies in many cell types, and display long-lasting transgene expression, due to genomic integration [74]. Despite the ability of lentiviruses to integrate into the host's genome, expression does not always follow. Many early lentivirally-transduced animals would lack transgene expression due to the host's antiviral immune response [75]. Upon integration into the host genome, border regions between host and transgene sequences become methylated [76]. This methylation, which silences gene expression, is a part of the cell's own innate immune response, due to the detection of viral promoter and enhancer regions along these border regions. Modern lentivirus systems avoid this gene silencing mechanism by having portions of their 3′ long-terminal repeats deleted, characterizing these viruses as self-inactivating [77–79]. In addition, modern lentiviruses have been pseudotyped to express mixed virions by combining different retroviral systems, resulting in lentiviral systems which can effectively transduce many different cell types [80]. These recent advancements in lentiviral technology make them an advantageous system to use in somatic transgenesis.

One major drawback to lentiviral systems is that some are derived from viruses which infect humans such as the human immunodeficiency virus (HIV) which raises concerns about potential adverse effects which may result from accidental infection [81]. A majority of health concerns posed by using a modified HIV have been eliminated by the removal of genes required for viral replication. In addition, previous generations of retroviruses would integrate into oncogenes, such as *LMO2*, increasing the risk for tumorigenesis [82,83]. Many of these concerns have been assuaged by studies indicating that tumorigenesis is not induced by lentiviruses *in vivo* or *in vitro* [84]. Another limitation to lentiviral systems is that their packaging size is limited to approximately 8.5 kb to retain optimal transduction efficiency, which must include the promoter and *cis*-acting elements leaving less room for the transgene itself [81].

Lentiviruses have also been shown to be useful in generating stable transgenic lines. Injecting lentivirus into embryos can generate mice with the transgene of interest in germline cells, allowing the transgene to be passed to subsequent generations [85]. Tissue specificity has been achieved by inserting specific promoter sequences, such as the synapsin-1 neuron specific promoter, into the lentiviral sequence [86]. As the efficiency and safety of lentiviral systems continue to improve, they are poised to become one of the dominant systems of viral somatic transgenesis in translational research, and will expand the ability of researchers to utilize animal systems as research models.

3.2. Adeno-associated viruses

In recent years, recombinant AAVs (rAAVs) have come into their own with new technological breakthroughs, correcting many of the previous deficiencies, and conferring greater therapeutic potential [87,88]. AAVs are small non-enveloped parvoviruses which use single-stranded DNA as their genetic material [89,90]. Upon infection, viral single-stranded DNA is taken up into the nucleus, converted to double stranded DNA, and is expressed throughout the life of the cell. These viruses are dependent on other viruses, such as adenovirus or herpes simplex virus to achieve proper infection [91,92]. Wild type AAV has the ability to integrate into a specific site of chromosome-19 [93,94]; this feature has been removed in modern rAAVs [95].

Currently, ten different serotypes of AAVs have been well described [96,97]. The primary difference between serotypes lies in the capsid amino acid sequence, which alters the tropism of each serotype for different cell types [98,99]. The first broadly studied serotype was AAV2, which is considered to be one of the least efficient AAVs in the CNS as it transduces far fewer cells than other serotypes [100,101]. Serotypes 1, 4, and 5 have shown much greater levels of transduction than AAV2 [101]. Interestingly, AAV4 has shown an affinity to transduce ependymal cells [101]. Overall, analysis of each serotype showed that AAV8 has the highest transduction efficiency in the CNS [102]. However, this serotype may be overly efficacious as studies have shown that GFP delivery *via* AAV8 can produce an overabundance of GFP protein, resulting in neurotoxicity [103]. Different serotypes have a varying degree of specificity within the CNS in regards to transduction efficiency. It has been shown that serotype 9 transduces the hippocampus at higher levels than serotypes 7, 8 and rh10 [97,104]. Serotype 9 also holds greater promise as it has been shown to cross the blood–brain barrier in the absence of permeablizing reagents and it also preferentially transduces neurons and astrocytes [105,106]. Similar to serotype 9, serotype sh10 has

displayed high transduction efficiencies with a small range of spread from the site of injection [97].

These vectors have several advantages. First, rAAVs are particularly useful in neurological studies as transgene expression occurs in dividing and quiescent cells, such as neurons [107]. Not only do these rAAVs have the ability to transduce non-dividing cells, but also the transgene expression in these cells is long lasting with the appropriate promoter [108]. Second, AAVs pose little infective risk to humans [109]. It is postulated that 85% of the human population is infected by AAVs, yet no associated pathology has been observed and does not induce a significant immune response [110,111]. Finally, the replicative ability of these vectors has been eliminated, which reduces the likelihood of unintended infection. After it was discovered that two inverted terminal repeats facilitate replication of these viruses, they were excised, resulting in a replication deficient rAAV system [73]. rAAV production relies upon *trans* expression of genes, typically packaged into plasmids, derived from adenovirus and the *rep* and *cap* genes from the deleted intronic regions [112,113].

There are disadvantages to utilizing AAVs in transgenesis. The main limitation of AAV systems is that the packaging size is generally limited to 4.7 kb [114] However, there are some exceptions to this limitation. It has been shown that AAV5 vectors may carry up to 8.9 kb [115]. This small packaging size severely limits the practicality of using AAVs for transgenesis. Another drawback of AAVs when compared to lentiviruses is the lack of genomic integration. Though episomal expression can be long lasting, gene products will not replicate with the genome, resulting in a gradual loss of expression in dividing cells. In neurons, however, this disadvantage is negligible.

4. Current models of AD

Alzheimer's disease remains a uniquely human disorder. Although non-human primates display some resemblances to humans when it comes to amyloid deposition and fibrillary lesions, both pathologies do not occur in the same species. For example, macaques develop amyloid plaques with a slightly different structure than those found in humans and tend to have a greater $A\beta_{42}$ composition [116,117]. Other species, such as baboons, have neurofibrillary tangles that have some similarity to those seen in AD, but still lack the complete AD phenotype [118–120]. Outside of non-human primates, canines have shown some potential as a model of amyloid pathology. The human and canine APP sequences are highly similar, and canines do develop diffuse amyloid plaques similar to those seen in early AD cases [121,122]. Canines, however, do not develop the tangle pathology seen in the human AD brain [123]. Although there is some appeal to using natural models of AD, a major obvious pitfall is their relatively long lifespan. Since AD pathology is age-related, this can make longitudinal aging studies in these species untenable [124,125], and these species are not easily genetically modified to incorporate mutations that might accelerate the pathology.

The last two decades have seen many investigators undertake the task of developing a mouse model that recapitulates the key features of AD. Although these models have had a fair amount of success, none can be described as being a complete model of AD [126]. In spite of a general success in elucidating much of the underlying pathobiology of the disease, the incomplete nature of these models is likely at least partially responsible for our ongoing failures to develop clinically useful AD therapeutics that target the disease mechanism. Other explanations are possible. For example, there may be fundamental characteristics of human neurons which make them more readily damaged and prone to degeneration. Second, our reliance on FAD mutations driven may not be completely relevant to sporadic AD. In addition, these transgenes are often inappropriately expressed to levels not seen in AD and may generate pathology in brain regions which do not accurately resemble the disease state. Finally, it could simply be due to the fact that the biochemical processes involved in AD pathogenesis do not have adequate time to generate pathology in animal models due to their shorter lifespan. Despite the fact that many genetic modifications promote the expression of specific AD related genes, other enzymes and/or processes involved in the disease still function at base rates, possibly preventing full development of pathology.

Rodents remain the most commonly-used animal models of AD. The genomes of the rat and mouse have been well described, making genetic manipulation easier, and the cost of maintaining these animals is low compared to larger animals such as non-human primates and canines. Unfortunately, neither rats nor mice naturally develop AD pathology [127]. However, this can be overcome by the introduction of human mutations which can drive similar phenotypes in rodent brain. There are a plethora of transgenic AD models described in the literature, and we will briefly cover the more notable and popular models to give a sense of what options are available. This section is intended to be an overview, not an exhaustive review of the AD mouse model literature. There are many excellent recent reviews on this topic: [3,128–130]. A summary of all models discussed herein may be found in Table 1.

4.1. Mouse models of amyloid pathology

Nearly all mouse models of AD rely upon FAD mutations in the *APP* gene. Three major isoforms of APP expressed the 695, 751 and 770 length isoforms [131]. Of these, APP_{695} is the isoform most commonly expressed in neurons, whereas isoforms APP_{751} and APP_{770} can be found in both neurons and glia [131]. The PDAPP mouse was the first AD transgenic mouse created, and contained the APP^{V717F} (Indiana) mutation driven by the platelet-derived growth factor β promoter on a C57B6 × DBA2 background [4]. This model generates high levels of human $A\beta_{40}$ and $A\beta_{42}$, but the V717F mutation causes an increase in the $A\beta_{42:40}$ ratio, due to an increase in the amount of the more pathogenic $A\beta_{42}$. Plaque deposition in the neocortex is apparent starting at three months of age in homozygotes and six months in heterozygotes. There is significant amyloid deposition in these animals and slight cognitive deficits are observed [132]. Neurons in the locus coeruleus decrease in size, despite the fact that there is no neuronal loss in the cortex or hippocampus as seen in AD [133]. Not only was this the first AD mouse model, but it was one of the first mouse lines used in immunotherapy studies which showed increased amyloid clearance in the brain [134–136].

Not long after the PDAPP line was generated, multiple new models were developed utilizing the FAD Swedish $APP^{K670N/M641L}$ double mutation. The Tg2576 model uses the Swedish double mutation within APP_{695} [5] under the hamster prion protein promoter (PrP) [5]. Levels of insoluble amyloid can be detected within the hippocampus between 6 and 10 months of age [137]. Though it has been reported that plaques may appear as early as 9 months of age, most have reported plaques beginning to appear at 13 months [5,137]. This was the first transgenic AD mouse line reported to display cognitive deficits, despite a lack of neuronal degeneration [138]. Interestingly, these mice develop amyloid deposits in the spine, which results in motor dysfunction [139]. This model has contributed greatly to the AD field. It has been used as the focus of many immunotherapy studies [140,141] and pharmaceutical trials [142], and provided insights into the basic mechanisms of disease [143–145].

The APP23 model takes advantage of the Swedish mutation within the human APP_{751} isoform, under the murine thymocyte differentiation antigen 1 (Thy1) promoter [146]. These mice have extensive plaque development in the neocortex and hippocampus. This was one of the first lines reported to develop cerebral amyloid angiopathy (CAA), an accumulation of amyloid protein within the vascular walls [147,148]. In addition, there are a variety of behavioral and cognitive abnormalities in these mice that occur before much of the amyloid pathology develops [149]. The APP23 line displays neurodegeneration, uncommon in

Table 1
Transgenic murine models of AD.

Model	Background	Transgene/mutation	Promoter	Pathology	References
PDAPP	C57B6, DBA2	APP^{V717F}	PDGF-β	Increased Aβ42 levels. Amyloid deposition by 6 months, no neuronal loss, memory deficits.	[4,131–136]
Tg2576	C57B6, SJL	$APP^{K670N/M671L}$	Hamster PrP	Amyloid plaques seen by 9 months of age, memory deficits, spinal amyloid deposition.	[5,137–145]
APP23	C57B6/DBA/2	$huAPP751^{K670N/M671L}$	Thy1.2	Amyloid deposition by 6 months, motor and cognitive impairment, cerebral amyloid angiopathy.	[146–150]
CRND8	C3H/C57B6	$APP^{K670N/M671L/V717F}$	Hamster PrP	Dense amyloid plaques seen by 5 months, cognitive deficits develop with pathology.	[151,152]
PSAPP	C3H/B6	$APP^{K670N/M671L}$, $PS1^{M146L}$	Hamster PrP	Amyloid plaques seen by 7 months, motor dysfunction by 13 months.	[6,153,154]
APP-PSEN1dE9	C57B6/C3H	$APP^{K670N/M671L}$, PS1ΔE9	Hamster PrP	Amyloid deposition beginning around 6 months, cortical angiopathy and reduced LTP.	[8,155–157]
APP/PS1	CD-1/129	$APP^{K670N/M671L}$, $PS1^{P264L/P264L}$	Knock-in	Amyloid deposition and plaque formation by 6 months of age.	[158–161]
5xFAD	C57B6/SJL	$APP^{K670N/M671L,I716V,V717I}$, $PS1^{M146V,\ L286V}$	Thy1	Massive cerebral amyloid deposition around 2 months, neuronal loss and cognitive impairments.	[162,163]
JNPL3	C57/BL	Tau^{P301L}	Hamster PrP	NFT formation by 6.5 months, behavioral dysfunction.	[164–166]
hTau	129S4/SvJae	Tau^{GFP} (KO), huTau	MAPT	PHF deposition by 9 months, behavioral abnormalities.	[167–169]
3xTg-AD	C57B6	$APP^{K670N/M671L}$, Tau^{P301L}, $PS1^{M146V}$	Thy1, (PS1 knock-in)	Extracellular amyloid detected by 6 months, and PHFs by 15 months.	[34,170–176]

many animal models of amyloid deposition [150]. These findings suggest there must be some other mechanism involved in the neurodegeneration observed in these animals.

The CRND8 transgenic line incorporates two different APP mutations, the Swedish APP double mutation and the APP^{V717F} mutation in APP_{695}. Essentially, this model represented the logical next step of mouse model development, by incorporating the mutations of two other successful lines, the Tg2576 and the PDAPP lines, into a single model. These mice rapidly develop amyloid pathology, showing signs of amyloid deposition by nine weeks of age, and dense plaque formation by five months [151]. These mice also display impaired fear conditioning response, which correlate to the plaque load [152]. Due to the rapid onset of neuropathy this line exhibits a decreased lifespan with a 50% mortality rate by three months of age [151].

Other common amyloid models combine several FAD mutations into one line, by combining an *APP* mutation with an FAD *PS1* mutant. These models display a more rapid rate of pathogenesis compared to monogenic models. The PSAPP line was one of the earlier bigenic AD models. This line is a hybrid of two previously established transgenics, the aforementioned Tg2576 line and another line carrying the $PS1^{M146L}$ mutation [6,153]. These mice have more rapid amyloid deposition than Tg2576 mice alone, with greater levels of $Aβ_{42}$ seen in deposits, and suffer from hyperactivity and cognitive deficits as determined by Y-maze testing [6,153]. Deposits are visible beginning around nine months of age and levels gradually increase until about 12 months [154]. Deposits are also detected in the vasculature [154]. This line provided interesting insights about the connection between APP and PS1, as the addition of the PS1 mutation increased Aβ levels beyond what is seen in the singly transgenic Tg2576 line containing only the Swedish mutation [154].

The APP/PS1ΔE9 line is another model that combines APP and PS1 mutations to generate a model that displays extensive amyloid pathology. This line contains the Swedish APP mutation and a deletion of exon nine from PS1 [8,155]. Approximately equal proportions of $Aβ_{40}$ and $Aβ_{42}$ are seen in this line. Amyloid deposits are detected by seven months of age, with abundant hippocampal deposition apparent by 19 months [156]. An age dependent decline in long-term potentiation and spatial memory is observed [156,157].

Interestingly, it is now known that the overexpression of FAD mutant forms of APP and PS1 is not necessary to cause pathology. It is possible to produce a model of amyloid deposition using a knock-in of the APP Swedish mutation, and the FAD-linked $PS1^{P264L}$ mutation, both under the control of their respective endogenous murine promoters [158–160]. Amyloid deposits can be detected by six months of age and deposition progresses linearly throughout the life of these animals [159]. The benefit to this model is that, in the absence of ectopic overexpression, APP and PS1 follow their physiological patterns of expression. What is perhaps most interesting in this model is that amyloid deposition still occurs, albeit at a rate slower than other models; until this model was reported, it was widely believed that APP overexpression was required in order to obtain amyloid deposition in rodents. The amyloid deposits in this line also more closely resemble neuritic plaques than in many other lines [161].

One of the most ambitious attempts at creating an AD model was the 5xFAD mouse, which combines five FAD mutations ($APP^{K670N/M671L,I716V,V717I}$, $PS1^{M146V,\ L286V}$) into one line [162]. This model overexpresses APP at high levels and generates abundant $Aβ_{42}$ that is detectable by six weeks of age. Deposition of amyloid has been observed as early as two months of age within cortical layers and intraneuronal Aβ can be detected by approximately 45 days of age [162]. Ultimately these mice develop gliosis, cognitive impairment, based on Y-maze testing, and neuronal loss [162]. This model has been used to demonstrate the importance of BACE1 regulation in AD pathogenesis [163]. The major benefit of this model is the rapid onset of pathology that makes it an ideal biological system for the quick evaluation of novel hypotheses about factors influencing amyloid deposition, and for the rapid assessment of potential therapeutics.

4.2. Mouse models of tau pathology

No known mutations are associated with the tau protein in AD. Because of this, most transgenic lines utilize mutations from other tauopathies, such as frontotemporal dementia (FTD). The JNPL3 transgenic mouse was the first such line to use the FTD mutation tau^{P301L}, under the PrP promoter, to drive tau pathology [164]. NFTs are first detected in these mice at approximately five months, concurrent with observations of motor and cognitive dysfunction [165]. The tangles generated in these mice differ somewhat from AD tangles, with notable differences in tangle morphology, location, and density [165]. Some of these differences can be explained by the fact that the P301L mutation generates 4R length tau protein, where AD tangles are composed of 3R and 4R isoforms [166]. Another difference is that these animals also exhibit neuronal loss throughout the spinal cord [165]. Spinal cord pathology is also observed in Tg2576 mice, which utilizes the same PrP promoter, indicating that the PrP promoter induces significant spinal cord overexpression of the transgene.

The hTau mouse is a knock-out of endogenous rodent *MAPT* coupled with the addition of a transgene expressing full length wild type human *MAPT* [167]. This line expresses the full length human *MAPT* gene and alternative splicing occurs, resulting in the expression

of all six isoforms of the human tau protein. These mice show signs of tau hyperphosphorylation at approximately six months and NFT formation by 15 months [167]. NFTs in these mice are distributed throughout the hippocampus and neocortex, roughly following the progression of tangle pathology seen in AD [168]. A decline in memory and cognitive function can be observed by approximately 12 months of age, before the onset of neurodegeneration [169].

4.3. Mouse models of both amyloid and tau pathology

In a further effort to create a more accurate model of AD, LaFerla et al. generated a trigenic model which displays both amyloid and tau pathology. The 3xTgAD line was generated by introducing $MAPT^{P301L}$ and $APP^{K670N/M671L}$ transgenes by the simultaneous embryonic microinjection into mouse embryos carrying a PS1 mutant knock-in [34]. This resulted in the cointegration of the *MAPT* and *APP* transgenes. Since the cointegrated genes do not assort independently, breeding logistics are more manageable than they would be for a line carrying three independent mutations. Extracellular amyloid can be first observed at approximately six months in the frontal cortex and spreads through the hippocampus by 12 months [34,170,171]. The detection of tau accumulation within pyramidal neurons occurs at approximately 12 months and PHFs are first observed around 15 months of age [34,172]. These mice have been shown to have disruptions in long-term potentiation, but suffer no neuronal loss and have disrupted sleep patterns [170,173]. It is the combination of amyloid and tau pathologies which makes this a popular model to study AD. This model has developed a rich history in the AD field. Immunotherapy studies with this line showed that clearance of Aβ can reduce tau pathology and prevent cognitive decline in these animals at an early age [174]. This gave more credit to the role Aβ has in promoting tau pathology. This model also incited a significant public debate (http://www.alzforum.org/res/for/journal/detail.asp?liveID=193) about the true disease relevance of intraneuronal Aβ [175,176].

5. The future of mouse models in AD research

Many of the existing models of AD exhibit the basic pathological hallmarks of the disease, providing a good platform for future genetic modification. Investigators looking to study complex interactions involved with AD and other forms of pathology have combined the existing murine AD models with models of other diseases. For example, crossing AD models with existing models of diabetes has allowed some of the complex interactions between the two pathologies to be observed [177]. Crossing existing models to create hybrids allows researchers to avoid committing the large amount of resources required to generate an entirely new transgenic mouse, making the entire process much more feasible. However, this approach is not without its own potential problems. Some lines may not cross well, resulting in unexpected phenotypes which, although occasionally interesting, are often of little value. Further, creating this new line still requires several generations of backcrossing, genotyping, and characterization, which remains costly and time consuming. Also, introducing a fourth genetic modification is nearly always logistically impossible. Fortunately, recent technological advances in viral transgenesis have given researchers another option. Combining viral transgenesis with existing mouse models could allow for more in depth and complex studies, and has the potential for the creation of AD mouse models that more closely parallel the human condition.

Lentiviruses have been used extensively in the nervous systems of rodents and non-human primates with high efficiency and expression [178,179]. In AD research, lentiviruses have been used to introduce stably expressing genes related to disease. Lentiviral transductions of wild type tau and the P301L mutant have been utilized in rats to show altered kinase activity [180]. Overexpressing NF-E2 related factor-2 in APP/PS1 mice displayed improved spatial learning as determined by Morris Water Maze test [181]. One of the more common uses of lentiviruses is for RNA interference. For example, lentiviral transduction of BACE1 siRNA reduced levels of BACE1 and AD related pathology [182,183]. Mice lentivirally transduced with APP_{695} siRNA had reduced overall levels of APP_{695} mRNA, protein, and extracellular Aβ [184].

AAVs have been used in a similar way. In a study by Jaworski et al., non-transgenic mice were intracerebrally transduced with AAV vectors carrying APP.SLA mutants, which contain the Swedish, London and Australian mutations [185]. By six months plaque deposition was observed in the cortex and the hippocampus, yet no degeneration was observed [185]. In the same study, AAVs delivering wild-type and mutant P301L transgenes induce neurodegeneration of pyramidal neurons of the CA1 and CA2 by 12 weeks and induce post-mitotic neurons to activate cell-cycle machinery. Additionally, AAV serotypes 9 and 10 have been shown to effectively deliver tau mutants and to rapidly induce neurodegeneration of dopaminergic neurons of the striatum [186]. Gene knock-down has also been achieved within the CNS *via* delivery of siRNAs. Delivery of mTOR siRNA *via* AAV5 reduced mTOR levels by 80% within the CNS [187]. A significant reduction of CDK5 was observed in 3xTgAD mice transduced with AAV CDK5-miRNA, which resulted in a five-fold decrease in tau phosphorylation [188]. A mouse model of amyloid pathology (TASPM; $APP^{K670N/M671L}$ x $PS\text{-}1^{M146V}$) transduced with pathogenic tau mutations AAV6 TauP301S or AAV6 3PO displayed increased tau phosphorylation, tau aggregation, amyloid plaque deposition, neuronal loss and cognitive impairment [189].

Viral transgenesis not only has the potential for the creation of better mouse models, but should also allow investigators to explore the role of newly discovered genetic linkages in a more efficient and cost effective manner. For instance, several recent GWAS studies have indicated that gene *BIN1*, encoding the protein bridging integrator 1, may be a risk factor in AD [190,191]. It has recently been shown that BIN1 may be directly connected to the development of tau pathology, and that it strongly colocalizes and coimmunoprecipitates with tau *in vitro* and *in vivo* [192]. BIN1 is a protein with many complex cellular actions, including cellular trafficking, synaptic vesicular endocytosis and cytoskeletal organization [193]. Alternative splicing and misregulation of BIN1 has been observed in other diseases, such as myotonic dystrophy [194]. There are 17 known splice forms of BIN1 and six splice forms of tau [195,196], creating a very large array of potential interactions, any combination of which might be important for the development of AD. Undertaking a study of this nature purely from the standpoint of generating and crossing different mouse models would require a huge investment in both time and physical resources. Viral transgenesis, however, could be an ideal solution to this problem. For example, the hTau mouse expresses all 6 splice forms of human tau [167]. Transduction of these mice with viruses expressing each individual BIN1 isoforms would allow investigators to observe not only the effects of BIN1 isoforms on tangle pathology, but also determine if there are preferential interactions between BIN1 and these isoforms. The process of generating the different viral vectors, followed by transducing hTau mice, would be faster, more efficient, and probably more likely to succeed at answering the question.

6. Conclusions

Despite the impressive progress investigators have made in generating more complex and relevant animal models of AD, no model fully reflects the biochemical hallmarks combined with the cognitive and behavioral symptoms present in AD. Many of these models are excellent at emulating specific facets of disease. There are models which recapitulate amyloid pathology, others that generate tau pathology and even some which combine these models. Though all of these models show some pathology that parallels the actual disease state, their spatial and temporal distribution can be vastly different.

Combining new technologies with existing models of AD can theoretically allow for better models, and overcome some of these limitations.

Both AAVs and lentiviruses have been used in combination with existing AD mouse models with some success. There have been studies which have utilized viral technologies to overexpress proteins implicated in AD, such as the oxidative stress protein NF-E2, and a variety of tau mutants. Others have knocked-down AD-related proteins in existing mouse models using short hairpin constructs. These studies, however, represent only a fraction of the potential that somatic viral transgenesis has for advancing AD research. These viral technologies can be used to introduce many genetic changes at once for the screening of many individual factors (and their interactions) simultaneously. Large scale screens such as this could allow for more in depth studies at a more rapid pace than researchers have previously been able to accomplish.

In truth, it may be unrealistic to believe that a perfect animal model of AD will ever exist, as the disease is so complex that the species barrier may be too great to overcome. However, utilizing somatic viral transgenesis in tandem with existing mouse lines could allow for the creation of better models than what are currently available. We can study more complex genetic problems, at a faster pace, and at a lower cost. The use of viral gene delivery may our current best option for studying the plethora of complex interactions involved in AD pathogenesis, screen potential therapeutics, and to bring investigators closer to an ideal mouse model.

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