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5 × FAD 转基因小鼠在阿尔茨海默病研究中的应用进展

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【摘要】 5 × FAD 转基因小鼠(transgenic mice with five familial Alzheimer's disease)是携带 5 个家族性基因突变的 APP/PS1 转基因小鼠,其中与 β-淀粉样蛋白前体(amyloid precursor protein,APP)相关的突变为 K670N/M671L(Swedish)、I716V(Florida)和 V717I(London),与早老素-1(presenilin 1,PS1)相关的突变为 MI46L 和 L286V。5 × FAD 小鼠在 1.5 月龄时脑内已有大量的 β-淀粉样蛋白(β-amyloid,Aβ),2 月龄时开始出现神经炎性斑(neuritic plaque,NP)。5 × FAD 小鼠的病理表型包括淀粉样斑块聚集、神经元丢失、神经胶质细胞增生和记忆功能障碍等。5 × FAD 小鼠的生物学特性可能涉及脑内 Aβ 斑块的形成变化、Tau 蛋白过度磷酸化、突触功能障碍、神经炎症反应、线粒体功能障碍、血脑屏障损伤、神经元损伤、内质网应激和眼部病变等。作为阿尔茨海默病的经典动物模型,5 × FAD 转基因小鼠在早期即可模拟 AD 患者晚期的神经病理过程及行为学表现,被广泛应用于 AD 发病机制研究和 AD 新药开发。本文对 5 × FAD 转基因小鼠模型的模型构建、生物学背景、生物学特性及 AD 防治药物的研发应用进行总结,以期为 5 × FAD 转基因小鼠在 AD 研究中的应用提供参考与借鉴作用。

【关键词】 阿尔茨海默病;5 × FAD 转基因小鼠;学习记忆;认知功能
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Advances in the application of 5 × FAD transgenic mice in Alzheimer's disease research

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【Abstract】 Transgenic 5 × FAD mice are APP/PS1 transgenic mice carrying five familial Alzheimer's disease (AD) gene mutations. Beta-amyloid precursor protein (amyloid precursor protein, APP) expression is related to the K670N/M671L (Swedish), I716V (Florida), and V717I (London) mutations, and presenilin 1 (PS1) is affected by the M146L and L286V mutations. 5 × FAD mice express high levels of β-amyloid in the brain at 1.5 months old, and neuritic plaques began to appear at 2 months old. The pathological phenotypes of 5 × FAD mice include amyloid plaque

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aggregation, neuronal loss, gliosis, and memory dysfunction, while their biological characteristics include changes in the formation of brain β-amyloid plaques, hyperphosphorylation of Tau protein, synaptic dysfunction, neuroinflammatory response, mitochondrial dysfunction, blood-brain barrier injury, neuronal injury, endoplasmic reticulum stress, and eye lesions. As a classic animal model of AD, 5 × FAD transgenic mice can simulate the neuropathological process and behavioral manifestations of late-stage AD in humans, and these mice are thus widely used in research into the pathogenesis of AD and the development of new drugs. In this review, we summarize the model construction, biological background, and biological characteristics of 5 × FAD transgenic mice, and the development and application of drugs for the prevention and treatment of AD, to provide references for the application of 5 × FAD transgenic transgenic mice in AD research.

【Keywords】 Alzheimer's disease; 5 × FAD transgenic mice; learning and memory; cognitive function

Conflicts of Interest: The authors declare no conflict of interest.

阿尔茨海默病(Alzheimer's disease, AD)作为一种神经退行性疾病,严重威胁着 65 岁及以上人群[1],AD 患者主要存在记忆减退、认知障碍等症状,同时伴有焦虑、抑郁等神经精神症状。根据世界卫生组织(World Health Organization, WHO)的最新数据显示,2050 年全球患有痴呆症的患者预计将达到 1.39 亿[2]。据估计,2023 年 65 岁及以上痴呆患者的治疗及护理费用约为 3450 亿美元,预计 2050 年将略低于 1 万亿美元[3],阿尔茨海默病给社会带来的负担正在日益增加[4-6]。

AD 的发病机制包括脑内淀粉样蛋白沉积[7]和 Tau 蛋白异常磷酸化[8]、神经炎症[9]、氧化应激(oxidative stress, OS)[10]、线粒体功能障碍(mitochondrial dysfunction, MD)[11]、钙稳态失调[12]和神经胶质功能障碍[13]等,但其发病机制尚不明确,同时缺乏系统完整的病理及生理学研究,仍无理想防治药物,因此,急需稳定可靠的动物模型来用于研究治疗 AD。根据现有的 AD 发病机制研究进展及基因型分类,目前常用的 AD 转基因小鼠模型有 10 种(表 1),包括 PDAPP、TG2576、APP23、

表 1　常用的 AD 转基因小鼠模型

Table 1　Commonly used classification of transgenic AD mouse models

AD 小鼠模型 Mouse models of AD	突变基因 Mutant gene	启动子 Promoter	认知记忆损伤 Cognitive and memory dysfunction	病理变化 Pathological change	缺点 Disadvantages	应用 Application
APP 单转基因小鼠 APP single transgenic mice	PDAPP	APP^{V717F}	MWM: ≥ 4 月龄[15-16] MWM: ≥ 4 months of age[15-16]	Aβ 沉积: 6 ~ 9 月龄, Tau 异常磷酸化: 14 月龄[17] Aβ deposition: 6 ~ 9 months of age, Tau abnormal phosphorylation: 14 months of age[17]	识别记忆缺陷的出现晚于空间学习记忆缺陷, Tau 病理出现较晚且无 NFTs Appearance of recognition memory deficit was later than that of spatial learning memory deficit, and the appearance of Tau pathology was later without NFTs	神经退行性变和斑块形成机制的研究 Studies on the mechanisms of neurodegeneration and plaque formation
	TG2576	$APP^{K670N/M671L}$	PrP	MWM: 9 ~ 10 月龄, Y 迷宫: 10 月龄[18] MWM: 9 ~ 10 months of age, Y maze: 10 months of age[18]	Aβ 沉积: 9 ~ 10 月龄[18] Aβ deposition: 9 ~ 10 months of age[18]	识别记忆的缺陷较晚, Aβ 沉积出现时间较晚, 无 Tau 病理和 NFTs Defects of recognition memory were late, the time of Aβ deposition was late, and there was no Tau pathology and NFTs	早期 AD 研究为 Aβ 斑块清除以及星形胶质细胞参与 AD 发生等 Early studies on AD include Aβ plaque clearance and the involvement of astrocytes in the development of AD
	APP23	$APP^{K670N/M671L}$	Thy-1	MWM: 3 月龄, BM: 12 月龄[19] MWM: 3 months of age, BM: 12 months of age[19]	Aβ 沉积: 14 ~ 18 月龄[20], Tau 异常磷酸化: 6 月龄[21] Aβ deposition: 14 ~ 18 months of age[20], Tau abnormal phosphorylation: 6 months of age[21]	非空间性学习记忆缺陷和 Aβ 沉积出现较晚 Non-spatial learning and memory deficits and Aβ deposition appear late	-

续表 1

AD 小鼠模型 Mouse models of AD	突变基因 Mutant gene	启动子 Promoter	认知记忆损伤 Cognitive and memory dysfunction	病理变化 Pathological change	缺点 Disadvantages	应用 Application
APP 双转基因小鼠 APP double transgenic mouse	TgCRND8	$APP^{K670N/M671L}$ APP^{V717F}	PrP	MWM:6 月龄[22],Y 迷宫和 T 迷宫:6、9 月龄[23] MWM: 6 months of age[22], Y maze and T maze: 6 and 9 months of age[23]	Aβ 沉积:3 月龄[22] Aβ deposition: 3 months of age[22]	无 Tau 病理和 NFTs No Tau pathology and NFTs	AD 短期研究 Short-term studies in AD
	J20	$APP^{K670N/M671L}$ APP^{V717F}	PDGF	–	无 Aβ 沉积,存在 Aβ 突触毒性[24] There was no Aβ deposition and Aβ synaptic toxicity was present[24]	不表现识别记忆功能障碍,无 Aβ 沉积,无 Tau 病理和 NFTs There was no recognition memory dysfunction, no Aβ deposition, no Tau pathology, and no NFTs	AD 相关的血管样变与神经炎性变的研究 Study on vasoid and neuroinflammatory changes associated with AD
	APP/PS1	$APP^{K670N/M671L}$ $PS1^{L166P}$	PrP	RAWM:3 月龄[25], MWM:6 月龄[26-27] RAWM: 3 months of age[25], MWM: 6 months of age[26-27]	Aβ 沉积: 2.5 月龄[28] Aβ deposition: 2.5 months of age[28]	空间学习记忆障碍和识别记忆障碍的进展相对缓慢,不表现交替任务障碍 Spatial learning and recognition memory impairments progress relatively slowly and do not exhibit alternating task impairments	人/鼠基因同系物研究,神经退行性变化的神经生物学研究 Human/mouse gene homolog studies, neurobiological studies of neurodegenerative changes
APP/PS 转基因小鼠 APP/PS transgenic mice	TG2576 + PS1 (M146L)	$APP^{K670N/M671L}$ $PS1^{M146L}$	PrP	Y 迷宫: 12 ~ 14 周龄[29] Y maze: 12 ~ 14 weeks of age[29]	Aβ 沉积: 23 周龄[30] Aβ deposition: 23 weeks of age[30]	认知记忆损伤进展相对缓慢,Aβ 沉积出现较晚 Progression of cognitive memory impairment was relatively slow, and Aβ deposition appeared late	–
	APP/PS1 KI	–	Endogenous	RAWM:9 月龄[31] RAWM: 9 months of age[31]	Aβ 沉积:6 月龄[32] Aβ deposition: 6 months of age[32]	认知障碍出现较晚,没有 Tau 病理和 NFTs Cognitive impairment appeared late, no Tau pathology, NFTs	–
	5 × FAD	$APP^{K670N/M671L}$ APP^{I716V} APP^{V717I} $PS1^{M146L}$ $PS1^{L286V}$	Thy-1	BM:3 月龄[33] Y 迷宫:4 ~ 5 月龄[34] BM: 3 months of age[33], Y maze: 4 ~ 5 months of age[34]	Aβ 沉积:2 月龄[34] Aβ deposition: 2 months of age[34]	Tau 病理和 NFTs 的存在仍有争议 Tau pathology and the existence of NFTs remain controversial	神经元内 $Aβ_{42}$ 诱导的神经变性和淀粉样斑块形成的相关研究 Studies related to $Aβ_{42}$-induced neurodegeneration and amyloid plaque formation in neurons
APP/PS/Tau 转基因小鼠 APP/PS/Tau transgenic mice	3 × Tg-AD	$APP^{K670N/M671L}$ $PS1^{L146V}$ Tau^{P301L}	Thy-1	MWM: 6 月龄, NOR:9 ~ 11 月龄, BM:12 月龄[35] MWM: 6 months of age, NOR: 9 ~ 11 months of age, BM: 12 months of age[35]	Aβ 沉积: 12 月龄[35], Tau 异常磷酸化:12 月龄[36] Aβ deposition: 12 months of age[35], Abnormal phosphorylation of Tau: 12 months of age[36]	与 AD 病理特征最为接近,但外源性基因表达稳定性较差、造模较困难且造价高 It is most similar to the pathological characteristics of AD, but the exogenous gene expression stability is poor, the modeling is difficult and the cost is high	与突触功能障碍和 AD 相关的斑块和神经缠结病理的研究 Studies of plaque and neurotangle pathology associated with synaptic dysfunction and AD

TgCRND8、J20、APP/PS1、TG2576 + PS1(M146L)、APP/PS1 KI、5 × FAD 和 3 × Tg-AD 小鼠[14]。

AD 疾病复杂,尽管还没有理想的模型,然而与上述模型相比,5 × FAD 模型具有明显优势,重现了 AD 淀粉样蛋白病理的主要特征且认知记忆损害及病理变化出现时间相对较早,成为早发性 AD 的理想模型。同时该模型对 $A\beta_{42}$ 的产生有着极高水平的累积效应,可用于研究神经元内 $A\beta_{42}$ 诱导的神经变性和淀粉样斑块形成等相关领域。5 × FAD 小鼠模型涵盖了与 AD 相关的几乎所有神经病理特征:淀粉样斑块、胶质细胞增生、神经传递缺陷、突触丧失和神经退行性病变,唯一缺失的是 Tau 异常磷酸化,至今仍存在争议。同 AD 患者一样,5 × FAD 小鼠的 AD 样病理可分为 3 个阶段:2 月龄左右的无症状期,其特征是存在淀粉样斑块和出现胶质样增生;6 月龄左右的症状期,其特征是出现大量的淀粉样蛋白病理、神经炎症、显著的认知缺陷和神经传递障碍;9 月龄左右的症状晚期,其特征是出现突触和神经元损失。因此本文将围绕 5 × FAD 小鼠展开论述,为 5 × FAD 转基因小鼠在 AD 研究中的应用提供参考与借鉴作用。

1　5 × FAD 转基因小鼠

1.1　5 × FAD 转基因小鼠的生物学背景

目前,研究者通过对已存在的 AD 转基因小鼠相杂交、基因敲入等制备了双转或多转基因小鼠,其病理学变化接近人类患者,并可对 AD 多种相关因素的相互作用进行研究。

为了更快地促进 $A\beta_{42}$ 产生、建立 AD 淀粉样蛋白模型,国内外相关的研究团队和学者一直在探索建立多转基因 AD 动物模型,通过在 APP/PS1 转基因模型小鼠中引入人类基因 5 个家族性 AD 的突变位点繁育而成的转基因小鼠为最近较新的 AD 模型小鼠[34],品系全称为 B6. Cg-Tg(APPSwFlLon, PSEN1 * M146L * L286V)6799Vas/Mmjax,也有文献称其为 5 × FAD、Tg-5 × FAD、5XFAD 或 Tg6799,该小鼠具有 AD 相关的 5 个突变位点,其中与 β-淀粉样蛋白前体(amyloid precursor protein, APP)相关的突变为 K670N/M671L(Swedish)、I716V(Florida)和 V7171(London),与早老素 1(presenilin 1, PS1)相关的突变为 MI46L 和 L286V。这两个转基因的表达受到 Thy1 启动子神经特异性元件的调控,在脑神经元中特异性过表达[37]。在 B6/SJL 杂交背景下 5 × FAD 小鼠可作为半合子保持 10 代以上,并稳定地表达 APP 和 PS1 基因[34]。

1.2　5 × FAD 转基因小鼠的生物学特性

1.2.1　5 × FAD 转基因小鼠的行为学特征

Morris 水迷宫实验(Morris water maze, MWM)主要用于测试实验动物对空间位置感和方向感(空间定位)的学习记忆能力[38]。与同月龄野生型(wild type, WT)小鼠相比,5 月龄的 5 × FAD 小鼠游泳速度显著降低[39],6 月龄的 5 × FAD 小鼠检测期逃避潜伏期显著延长[40]、目标象限停留时间显著减少[41],11 月龄雄性 5 × FAD 小鼠的目标象限停留时间百分比显著下降[42],12 月龄 5 × FAD 小鼠学期的逃避潜伏期和检测期的目标象限停留时间显著延长[43],说明 5 × FAD 小鼠的空间学习记忆能力下降。

GIRARD 等[44]使用一种新开发的自动化设备——嗅觉 H 迷宫(olfactory H-maze, OHM),比较 2、4 和 6 月龄 5 × FAD 小鼠与其野生型同窝的表现,发现 4 和 6 月龄 5 × FAD 小鼠在延迟的交替任务(alternation task, ALT)、延迟的非交替任务(non-alternation task, N-ALT)、延迟的反转任务(reversal task, REV)均表现出前额叶皮层依赖学习能力的下降。

Y 迷宫(Y-maze, YM)可用于评估小鼠的短期记忆,是由啮齿动物天生的好奇心驱动以探索先前未曾探视过的区域,通过小鼠探索迷宫的三个臂来评估其自发交替能力以及空间学习记忆能力。与 WT 对照组相比,2 月龄雌性 5 × FAD 小鼠自发交替百分比显著下降[45],11 月龄雄性 5 × FAD 小鼠的新臂进入次数显著下降[42],说明 5 × FAD 小鼠的短期记忆能力降低。

旷场实验(open field test, OFT)又称敞箱实验,是评价实验动物在新异环境中自主行为、探究行为与紧张度的一种方法。与 WT 对照组相比,6 月龄和 12 月龄 5 × FAD 小鼠的运动距离显著增加,总休息时间也相应减少[46]。

新物体识别实验(novel object recognition, NOR)用于检测啮齿类动物的学习记忆能力。与 WT 对照组相比,3 月龄雄性 5 × FAD 小鼠的逃避潜伏期从第 2 天开始显著升高[33]。5 月龄 5 × FAD 小鼠的雌性比雄性新物体识别时间更短[47]。在检测期,12 月龄的 5 × FAD 小鼠的新物体识别时间和新物体偏爱指数均显著降低,6、12 月龄的 5 × FAD 小鼠的辨别指数显著减少[48]。

巴恩斯迷宫实验(Barnes maze test, BM)被广泛

用于测试在较小压力下的空间学习和记忆能力。与 WT 对照组相比,3 月龄的雄性 5 × FAD 小鼠检测期目标象限停留时间显著减少[33],5 ~ 6 月龄 5 × FAD 小鼠在训练期和检测期潜伏期均显著增加[49]。

T 迷宫实验(T maze test, TM)可用来评估自发交替行为,是检测啮齿类动物空间工作记忆的一种经典行为学方法。与 WT 对照组小鼠相比,5 ~ 6 月龄 5 × FAD 小鼠的错误次数显著上升[41]。

采用高架十字迷宫(elevated plus maze, EPM)实验分析小鼠焦虑样行为,与杂合 5 × FAD 小鼠相比,2 月龄和 5 月龄的纯合 5 × FAD 小鼠开臂进入百分比显著增加,表明正常焦虑表型的丧失[39]。

在转棒实验(rotarod test, RRT)中,OBLAK 等[46]发现与 WT 对照组相比,6 月龄和 12 月龄 5 × FAD 小鼠转棒上停留时间更长,小鼠跌倒潜伏期延长。

以上研究结果表明,5 × FAD 转基因小鼠行为学异常改变最早可出现在 2 月龄,且雌性较同龄雄性更易发生,主要包括空间学习记忆能力、自主行为和紧张度、物体识别记忆能力、焦虑情绪和运动协调能力(表 2)。

表 2 5 × FAD 转基因小鼠的行为学特征

Table 2 Behavioral characteristics of 5 × FAD transgenic mice

认知类型 Cognitive type	方法 Methods	月龄 Months	性别 Sex	对照小鼠 Control mice	检测时期 Period of detection	指标 Index	变化 Change
空间学习记忆 Spatial learning and memory	MWM	12[43]	雌、雄 Female and male	WT	学习期 Learning period	逃避潜伏期 Escape latency	↑
					检测期 Detection period	目标象限停留时间 Time in the target quadrant	↑
		11[42]	雄 Male	WT	检测期 Detection period	目标象限停留时间 Time in the target quadrant	↓
		6[40]	雌、雄 Female and male	WT	学习期 Learning period	逃避潜伏期 Escape latency	–
					检测期 Detection period	逃避潜伏期 Escape latency	↑
		6[41]	雌、雄 Female and male	WT	检测期 Detection period	目标象限停留时间 Time in the target quadrant	↓
	YM	2[45]	雌 Female	WT	检测期 Detection period	自发交替百分比 Percentage of spontaneous alternation	↓
		11[42]	雄 Male	WT	检测期 Detection period	自发交替百分比 Percentage of spontaneous alternation	↓
	OHM	2[44]	雌、雄 Female and male	WT	检测期 Detection period	延迟的交替任务 Alternation task	–
						延迟的非交替任务 Non-alternation task	↑
						延迟的反转任务 Reversal task	↓
		4、6[44]	雌、雄 Female and male	WT	检测期 Detection period	延迟的交替任务 Alternation task	↓
						延迟的非交替任务 Non-alternation task	↓
						延迟的反转任务 Reversal task	↓
	BM	3[33]	雄 Male	WT	检测期 Detection period	目标象限停留时间 Time in the target quadrant	↓
		5 ~ 6[49]	雌、雄 Female and male	WT	检测期 Detection period	逃避潜伏期 Escape latency	↑
	TM	5 ~ 6[41]	雌、雄 Female and male	WT	检测期 Detection period	错误次数 Number of errors	↑
自主行为和紧张度 Autonomic behavior and tension	OFT	6、12[46]	雌、雄 Female and male	WT	检测期 Detection period	运动距离 Moving distance	↑

续表 2

认知类型 Cognitive type	方法 Methods	月龄 Months	性别 Sex	对照小鼠 Control mice	检测时期 Period of detection	指标 Index	变化 Change
新异物体识别能力 New object recognition ability	NOR	5[47]	雌、雄 Female and male	WT	适应期 Adaptation period	新物体识别时间 New object recognition time	-
			雌 Female	5 × FAD 雄 5 × FAD Male	检测期 Detection period	新物体识别时间 New object recognition time	↓
		3[33]	雄 Male	WT	学习期 Learning period	潜伏期时间 Latency period	↓
		12[48]	雌、雄 Female and male	WT	检测期 Detection period	新物体识别时间 New object recognition time	↓
						偏爱指数 Index of preference	↓
焦虑情绪 Anxiety	EPM	2、5[39]	雌、雄 Female and male	杂合子 Heterozygote	检测期 Detection period	开臂进入次数百分比 Percentage of open arm entry times	↑
运动协调能力 Motor coordination ability	RRT	4、6、12[46]	雌、雄 Female and male	WT	检测期 Detection period	转棒上停留时间 The time stayed on the rod	↑

注：↑：升高或增加；↓：降低或减少；-：无差异。（下表同）
Note. ↑. Increase or extension. ↓. Decrease or decrease. -. Indicates no difference. (The same in the following tables)

1.2.2　5 × FAD 转基因小鼠的病理特征

淀粉样蛋白斑块是 β-淀粉样蛋白（Aβ）的沉积，是 AD 的典型病理特征。小 Aβ 肽的生成并无天然毒性，而由大量极度聚集的 Aβ 原纤维组成的淀粉样斑块则象征着异常的病理改变，Aβ 寡聚物可诱导突触功能障碍[50]，进一步导致 AD 中神经元损伤和记忆丧失[51]。Aβ 蛋白主要有两种亚型：可溶性 $Aβ_{40}$ 和不可溶性 $Aβ_{42}$[52]，在正常人中，$Aβ_{40}$ 比例较 $Aβ_{42}$ 高[34]。APP、PS1 和早老素 2（presenilin2，PS2）基因的常染色体显性突变增加了 $Aβ_{42}$ 的产生[53]。APP 转基因上的 FAD（flavine adenosine dinucleotide）突变容易出现在 β-或 γ-分泌酶结合位点附近，导致 Aβ 总量增加（K670N/M671L 瑞典型突变[54]）或 $Aβ_{42}$ 增加（I716V 佛罗里达型突变和 V717I 伦敦型突变[55]），PSEN1 转基因（γ-分泌酶的催化亚基）上的 FAD 突变增加了 $Aβ_{42}/Aβ_{40}$ 比值。大多数 AD 转基因小鼠需要 6 ~ 12 月或更长时间才能形成淀粉样斑块[56]，但 5 × FAD 转基因小鼠神经元细胞内的 $Aβ_{42}$ 蛋白自 1.5 月龄起就会出现[34]。5 × FAD 小鼠中雌性比雄性 APP 基因表达更多，可能是由于 Thy1 启动子中的雌激素相关部位用于驱动 APP 转基因表达，并产生更高水平的 Aβ[57]。随着小鼠月龄增加，$Aβ_{42}$ 蛋白表达几乎呈线性增加，而 $Aβ_{40}$ 的增加通常较 $Aβ_{42}$ 滞后 2 ~ 3 个月[34]。淀粉样蛋白病理变化始于大脑皮层的深层和亚皮层，随着小鼠月龄增加，皮层和海马上斑块扩散面积增大。2 月龄时皮层中形成致密的淀粉样斑块，5 ~ 6 月龄 5 × FAD 小鼠皮层和海马中 Aβ 斑块更加清晰[49]，6 月龄 5 × FAD 小鼠海马 DG 区 Aβ 斑块显著增加[58]。

AD 另一特征性病理改变——神经纤维缠结（neurofibrillary tangle，NFTs），形成的主要原因是 Tau 蛋白的过度磷酸化[59]。NFTs 是神经元细胞质和突起中直的、纤维状的、高度不溶的斑块，会导致神经元之间通信和信号处理的异常丢失，最终导致神经元凋亡[59]。在 5 × FAD 小鼠的首次实验报道中缺乏 Tau 相关内容[34]，后期有研究通过 ELISA 法检测到 6 月龄 5 × FAD 小鼠皮层和血清中 p-Tau 蛋白水平显著升高[40]，甚至发现 5 × FAD 小鼠大脑中的 Tau 过度磷酸化比学习记忆障碍出现的更早[60]，但 MAAROUF 等[61]的实验中 5 × FAD 小鼠没有出现 NFTs。因此有关 5 × FAD 小鼠 Tau 蛋白表型的病理研究至今仍存在争议，导致结果异质性的原因可能是动物的年龄、性别、遗传背景，以及方法学和分析等的差异，对此仍需进一步的研究证明。

脑萎缩（brain atrophy，BA）也是 AD 非常重要的病理特征之一，AD 患者神经元丢失导致相关大脑区域的严重萎缩，导致认知和记忆功能的进行性丧失，最终导致痴呆。采用苏木精-伊红（hematoxylin-eosin，HE）染色法观察小鼠大脑皮层和海马 DG 区组织结构，6 月龄 5 × FAD 转基因小鼠细胞核和神经元萎缩[40]。6 月龄 5 × FAD 小鼠躯体感觉皮层的锥体细胞中的突触数量较 WT 小鼠显著减少[62-63]。采用透射电子显微镜（transmission electron microscope，TEM）观察 4 月龄小鼠脑组织超

微结构,发现 5 × FAD 小鼠大脑皮层中的突触数量减少,在大脑皮层和海马中的突触神经递质囊泡的数量也减少[64],9 月龄 5 × FAD 转基因小鼠皮层第 5 层的神经元数量明显减少[34]。基于设计的体视学方法对 12 月龄 5 × FAD 小鼠皮层进行计数,发现皮层第 5 层神经元数量明显减少[65]。通过免疫荧光法(immunofluorescence, IF)检测 5 × FAD 小鼠皮层和海马 DG 区的电离钙结合适配器分子 1(ionized calcium-binding adapter molecule 1, Iba-1)高表达的小胶质细胞和胶质纤维酸性蛋白(glial fibrillary acidic protein, GFAP)高表达的星形胶质细胞均呈现激活状态,细胞数量显著减少[66]。

综合以上研究结果,5 × FAD 转基因小鼠早在 2 月龄时就会出现 Aβ 沉积,5 ~ 6 月龄时显著增多; 4 月龄时皮层中突触数量减少,6 月龄时显著减少; 6 月龄时出现神经元的萎缩,9 月龄时可观察到密集的神经元丢失。这些研究表明,5 × FAD 转基因小鼠具有 AD 的典型病理特征(表 3)。

1.2.3 5 × FAD 转基因小鼠的生理生化表型

突触标记物如突触素(synaptophysin, SYP)、突触融合蛋白(syntaxin, STX)和突触后致密蛋白 95(postsynaptic density-95, PSD-95)的表达会随着小鼠月龄增加而降低。采用 ELISA 方法检测小鼠全脑匀浆中突触素水平,5 × FAD 转基因小鼠全脑匀浆中突触素水平在 4 月龄开始下降,9 月龄时约为对照组的 75%,12 月龄时突触标记物的水平显著降低[34]。4 ~ 12 月龄的 5 × FAD 小鼠的皮层和海马中突触素和 PSD-95 显著减少[33,48,68]。但也有研究称这两种标记物水平没有变化,可能是因为大多数研究只适用于 B6/SJL 混合背景的雄性小鼠,对照组小鼠通常从不同的供应商处购买。

细胞周期素依赖蛋白激酶 5(cyclin-dependent kinase 5, CDK5)在成人大脑中发挥着包括学习和记忆形成等高级认知功能[69]。CDK5 与 p25 蛋白(CDK5R1)结合后, CDK5 活性在神经系统疾病(AD、PD 和 HD)中失去控制,最终导致神经毒

表 3 5 × FAD 转基因小鼠的病理特征

Table 3 Pathological characteristics of 5 × FAD transgenic mice

指标 Index	方法 Methods	对照 Control	月龄 Months	部位 Part	变化 Change
Aβ 斑块 Aβ aggregation	IHC	WT	2[34]	皮层 Cerebral cortex	↑
	IHC	WT	5 ~ 6[34]	海马和皮层 Hippocampus and cerebral cortex	↑
	IHC	WT	6[58]	海马 DG 区 Hippocampal DG areas	↑
	MSD	WT	4、6、12[46]	海马 Hippocampus	↑
				全脑 Whole-brain	–
p-Tau	ELISA	WT	6[40]	皮层和血清 Cerebral cortex and serum	↑
GFAP、Iba1	IF	WT	4[66]	皮层和海马 DG 区 Cerebral cortex and hippocampal DG areas	↑
神经元萎缩 Neuronatrophy	HE	WT	6[40]	皮层和海马 DG 区 Cerebral cortex and hippocampal DG areas	↑
树突长度 Dendritic length	BS	WT	4[67]	海马 Hippocampus	↓
树突棘数量 Number of dendritic spines	BS	WT	6、12[48]	皮层 Cerebral cortex	↓
	BS	6 月龄 5 × FAD 6 months old 5 × FAD	12[48]	皮层 Cerebral cortex	↓
突触数量 Number of synapses	BS	WT	6[62-63]	躯体感觉皮层的锥体细胞 Pyramidal cells in the somatosensory cortex	↓
突触神经递质囊泡 Synaptic neurotransmitter vesicles	TEM	WT	4[64]	海马和皮层 Hippocampus and cerebral cortex	↓
神经元数量 Number of neurons	BS	WT	12[65]	皮层第五层 Layer 5 of cerebral cortex	↓
	Nissl	WT	9[34]		↓

性[70]。将小鼠的皮层匀浆进行 Western Blot 分析后发现,3 月龄 5 × FAD 小鼠皮层中的 p25 水平呈上升趋势,同时神经元内 Aβ 聚集物出现;9、12 月龄时 5 × FAD 小鼠皮层中的 p25 水平增加到同源野生型的 150%,同时出现显著的神经变性和大锥体神经元丢失。因此,p25 水平上升神经元丢失和神经元内 Aβ 聚集关系密切。

血液中脂质的组成包括甘油三酯(triglyceride, TAG)、总胆固醇(total cholesterol, TC)、低密度脂蛋白(low density lipoprotein, LDL)和高密度脂蛋白(high density lipoprotein, HDL)。其中 LDL 和 TAG 水平升高会抑制神经元连接,促进大脑中淀粉样斑块的沉积[71],与 AD 风险呈正相关[72],而 HDL 水平与 AD 风险呈负相关[73]。与雄性相比,雌性 5 × FAD 和 WT 小鼠的 LDL 水平更高、HDL 水平更低,雌性总胆固醇的显著差异仅存在于 4 ~ 12 个月的 5 × FAD 小鼠之间。4 月龄时,雄性 5 × FAD 和 WT 小鼠之间的总胆固醇没有显著性差异,6 月龄时 5 × FAD 小鼠的总胆固醇有降低趋势,12 月龄时 5 × FAD 小鼠的总胆固醇显著降低[46]。

中枢神经系统(central nervous system, CNS)中的炎症称为神经炎症,是机体为防止大脑损伤而产生的保护性应激反应,在多种脑部疾病中发挥重要作用,抑制神经炎症可以保护神经元、减轻学习记忆障碍,但目前尚无有效的抗神经炎症治疗药物。与 WT 小鼠相比,6 月龄的 5 × FAD 小鼠大脑皮层中的白细胞介素-1β(interleukin-1β, IL-1β)和肿瘤坏死因子-α(tumor necrosis factor-α, TNF-α)表达均显著升高,自 3 月龄起小鼠海马中 TNF-α、IL-1β 等神经炎症因子呈时间依赖性增加[74],4 月龄 5 × FAD 小鼠额叶皮层的星形胶质细胞激活和 TNF-α 水平显著降低[75],6 月龄 5 × FAD 小鼠海马相关危险因素(IL-1β、TNF-α、APOE、GFAP)表达显著增加[58],6.5 月龄 5 × FAD 小鼠海马中炎症介质 TNF-α 和白细胞介素-6(interleukin-6, IL-6)、白血病病毒整合基因-1(friend leukaemia integration-1, Fli-1)mRNA 水平显著增加[76]。

微小 RNA-128(miR-128)是一种在神经系统中高表达的 miRNA,在神经系统的发育及正常生理功能的维持中发挥重要作用,其异常表达与阿尔茨海默病密切相关。采用实时定量 PCR(real-time quantitative PCR, RT-qPCR)检测 2 月龄(初始 AD 病理)、8 月龄(重度 AD 病理)和 12 月龄(慢性重度 AD 病理)的小鼠海马中 miR-128 表达水平变化,与对照组 WT 小鼠相比,8 月龄 5 × FAD 小鼠的 miR-128 水平显著下调,但在 2 或 12 月龄时没有变化[77]。5 × FAD 小鼠模型尿液中外泌体(exosomes, *Exos*)miRNA 在 Aβ 斑块沉积前存在表达差异,是潜在的诊断早期 AD 的非侵袭性生物标志物,对于早期干预 AD 具有重要意义[78]。

综合以上研究结果,在 5 × FAD 转基因小鼠脑内,与神经信号传导、神经系统发育、神经系统功能表达和神经炎症等密切相关的生理生化表型较 WT 小鼠发生显著改变(表 4)。

2　5 × FAD 小鼠的其他生物学改变

目前研究表明,5 × FAD 转基因小鼠的其他生物学特性可能涉及突触功能障碍(synaptic dysfunction)、神经炎症(neuroinflammatory, NI)、线粒体功能障碍及血脑屏障(blood brain barrier, BBB)损伤、神经元损伤(neuron damage)、内质网应激(endoplasmic reticulum stress, ERS)和眼部病变等,下面进行详述。

2.1　5 × FAD 中的突触功能障碍

突触结构是学习和记忆的生物学基础,突触病理是 AD 和衰老的早期标志[82-84],在 AD 患者的尸检样本中,突触损失比神经元死亡更常见[85]。Aβ 驱动的神经突触萎缩和突触丢失在 AD 早期开始,并直接引发记忆障碍。早期突触功能障碍会引起突触室损伤并最终导致突触丢失,与认知功能下降密切相关[86-89]。

突触丢失和突触强度的改变被认为是 AD 认知障碍的基础,通过测量小鼠在虚拟现实任务中的突触功能发现,5 × FAD 小鼠海马的 CA1 区中间神经元与锥体细胞的突触连接存在缺陷[90]。5 × FAD 小鼠海马突触中的线粒体和糖酵解功能选择性受损,而非突触的线粒体功能得以维持,证明了海马突触线粒体功能的破坏是 5 × FAD 小鼠模型早期病理的主要事件[64]。KIMURA 等[91]的研究表明,5 × FAD 小鼠从 6 月龄开始出现突触受损。5 × FAD 小鼠突触线粒体呈现分裂与融合之间的不平衡,即线粒体动力学紊乱,同时伴有线粒体自噬 Parkin 通路的激活以及自噬标记物微管相关蛋白 1 轻链 3B-Ⅱ(microtubule-associated protein 1 light chain 3B-Ⅱ, LC3B-Ⅱ)的募集,证明 5 × FAD 小鼠出现显著的突触线粒体功能障碍[92]。

表 4 5 × FAD 转基因小鼠的生理生化表型

Table 4 Physiological and biochemical phenotypes of 5 × FAD transgenic mice

生化指标 Biochemical phenotype	方法 Methods	月龄 Months	部位 Part	结果 Results
$A\beta_{42}$	ELISA	4 ~ 9[34]	全脑 Whole-brain	↑
$A\beta_{40}$	ELISA	4 ~ 9[34]	全脑 Whole-brain	↑
SYP	ELISA	4 ~ 9[34]	全脑 Whole-brain	↓
突触融合蛋白 Syntaxin	Western Blot	4-12[33,48,68]	海马、皮层 Hippocampus, cerebral cortex	↓
PSD-95	Western Blot	9、12[34]	皮层 Cerebral cortex	↓
		4-12[33,48,68]	海马、皮层 Hippocampus, cerebral cortex	↓
P25	Western Blot	3、9、12[46]	皮层 Cerebral cortex	↑
胆固醇 Cholesterol	血糖分析 Analysis of glucose	4[46]	非空腹血浆 Non-fasting plasma	–
		12[46]	丘脑 Thalamus	↓
BACE1	Western Blot	6、9[57,79-81]	半脑匀浆 Half brain homogenate	↑
		6[40]	皮层 Cerebral cortex	↑
TNF-α	ELISA	4[75]	额叶皮层 Frontal cortex	↑
APOE、GFAP	qPCR	6[58]	海马 DG 区 Hippocampal DG areas	↑
IL-1β	qRT-PCR	6[40]	皮层 Cerebral cortex	↑
	Western Blot	3[74]	海马 Hippocampus	↑
Fli-1 mRNA	PCR	6.5[76]	海马、皮层 Hippocampus, cerebral cortex	↑
IL-6	IHC		海马 Hippocampus	
miR-128	qRT-PCR	8[77]	海马 Hippocampus	↓

2.2 5 × FAD 中的神经炎症反应

炎症通常伴随免疫系统的激活,且严重的炎症易发生在细胞外 Aβ 沉积物周围[93]。在小胶质细胞、星形胶质细胞、神经元和认知层面,淀粉样蛋白能促进神经炎症细胞因子的合成,形成继发性炎症[94],引起神经元损伤,以及学习记忆功能障碍[95-96]。小胶质细胞控制着大脑的代谢平衡,包括促炎或抗炎细胞因子的分泌以及异常蛋白的吞噬[97],在神经元可塑性和突触重塑中也起着至关重要的作用[98]。星形胶质细胞是大脑中的第二种免疫细胞,其激活程度与 $A\beta_{42}$ 及淀粉样斑块的水平呈正比[34]。活化的小胶质细胞和星形胶质细胞主要分布于 Aβ 沉积部位,Aβ 斑块会激活小胶质细胞并分泌促炎细胞因子和趋化因子。小胶质细胞的促炎激活是 AD 的标志之一[99]。

髓样细胞 2(triggering receptor expressed on myeloid cells 2,TREM2)上的触发受体在 AD 中具有抗炎及神经保护作用,主要表达在中枢神经系统的小胶质细胞上[100-101]。TREM2 在 5 × FAD 小鼠海马中表达上调,而腺相关病毒(adeno-associated virus,AAV)介导的 TREM2 敲除显著增加了 5 × FAD 小鼠海马中的促炎细胞因子水平,加重了认知缺陷[58]。3 月龄时雌性 5 × FAD 小鼠炎症介质和胶质细胞标志物的表达变化更为明显,且这种变化呈年龄依赖性增加,而雄性 5 × FAD 小鼠在 5 月龄前没有明显趋势[102]。因此,在 5 × FAD 小鼠模型中,3 月龄前给予抗炎剂将是最有效的。值得注意的是,有研究发现 5 × FAD 小鼠的核心体温升高,可能是应激或炎症诱导的高热反应[46]。

2.3 5 × FAD 中的线粒体功能障碍

线粒体是细胞内普遍存在的细胞器,主要通过氧化磷酸化为生命活动提供能量。此外,线粒体也参与钙信号传导、细胞代谢调节及细胞凋亡调控[103]。Aβ 寡聚体会诱导 Ca^{2+} 内流到神经元细胞,促进线粒体 Ca^{2+} 超载[104]。在散发性 AD 小鼠模型中,合成膜蛋白中糖基化终产物受体(receptor for advanced glycation endproducts,RAGE)片段的治疗降低了皮层和海马中线粒体呼吸酶的活性[105]。5 × FAD 小鼠大脑中氧化还原的线粒体复合物 Ⅰ(NADH-泛醌氧化还原酶)中 Fe-S 簇比例增加,释放铁和过氧化氢,过氧化氢的亚铁离子通过芬顿反应形成有毒的氢氧自由基,引发线粒体功能障碍[106]。5 × FAD 小鼠在 9 月龄出现认知功能障碍伴随海马内突触的结构降解和线粒体的异常,其中线粒体功能损伤表现为海马中 ATP 水平和呼吸链复合物 Ⅰ 活性的降低[107]。通过超微结构分析发现在 5 × FAD 小鼠大脑皮层和海马的细胞质、突触中无嵴线粒体显著增加[64],由于嵴是线粒体能量产生的机制,因此支持了 5 × FAD 小鼠线粒体代谢下降的观点,并进一步表明线粒体功能的改变可能驱动

早期 AD 的生物学缺陷。

2.4　5 × FAD 中的血脑屏障损伤

近年来,脑血管对 AD 病理生理的影响引起了人们的关注。脑血管功能障碍,如脑血流量减少和血脑屏障损伤,被认为是 AD 发病机制的早期和关键因素。血脑屏障将神经元从循环血液成分中分离出来,通过限制血源性溶质的被动扩散,以及主动向大脑输送营养物质,严格调节分子进出大脑的运输[108-109]。血脑屏障完整性的丧失使血液中的神经毒性成分进入大脑,导致免疫反应和神经炎症,进而引发神经退行性变的多种途径[110-111]。据报道,AD 在神经退行性变、痴呆和脑萎缩之前就会发生血脑屏障功能障碍[112]。电子顺磁共振波谱(electron paramagnetic resonance, EPR)可用于检测 5 × FAD 小鼠模型血脑屏障损伤及神经元组织氧化还原状态的改变[106]。阻断血脑屏障后的凝血酶原(prothrombin, PRO)及前动力蛋白受体 2(prokineticin receptor 2, PKR2)的升高可能导致 5 × FAD 小鼠的海马神经退行性病变,并使其物体识别能力下降[74]。在 5 × FAD 模型中,血脑屏障功能障碍的证据包括血脑屏障中受体介导的 Aβ 清除受损[113]、内皮细胞和星形胶质细胞之间的接触受损[114]、血脑屏障完整性改变[115]以及血脑屏障渗漏[116]。

2.5　5 × FAD 中的神经元损伤

氧化应激可引起神经退行性疾病中的神经元损伤,对 AD 的早期发病和疾病进展有显著促进作用[117-118]。在 AD 发展过程中,病理性 Aβ 和 Tau 积聚并错误定位到突触,会导致突触功能障碍和神经元损伤。神经元分泌 Aβ 以响应突触活动,Aβ 反过来会下调突触传递[119],这种负反馈调节可以作为一种生理稳态机制来限制神经元活动的水平[120]。研究表明,4 月龄前 5 × FAD 小鼠海马的神经元活性无显著变化,12 月龄起观察到皮层第 5 层锥体神经元损伤严重[34]。5 × FAD 小鼠模型再现了人类 AD 特征的神经元损失,且海马和皮层区的神经元损失尤为明显,然而许多其他 AD 动物模型没有表现出任何神经元损伤。

2.6　5 × FAD 中的内质网应激

内质网应激主要发生在神经元,持续的应激可促进神经元的功能退化。大脑长时间暴露于过表达的转基因膜蛋白或淀粉样蛋白病理可能会诱导 5 × FAD 小鼠的未折叠蛋白反应(unfolded protein response, UPR)。通过 Western Blot 发现与 WT 小鼠相比,5 × FAD 小鼠的 UPR 无变化,内质网应激标志物显著升高[57]。理论上过表达的跨膜蛋白 APP 和 PS1 在内质网的积累会引发慢性 ERS 和 UPR 的激活[57],但 5 × FAD 小鼠中没有发现 ERS,可能是因为在 1.0 ~ 1.5 月龄 APP 和 PS1 转基因的初始表达阶段,ERS 水平被短暂恢复正常并持续到稳态机制重新平衡,以处理增加的蛋白质折叠负荷。尽管越来越多的证据表明,高水平的 Aβ 不会在 5 × FAD 转基因小鼠中诱导 UPR,但来自人类 AD 患者的组织学数据却支持这一假设,即在人类 AD 和其他神经退行性疾病中,UPR 在某些神经元中被激活[121]。因此,未来的实验需要在 5 × FAD 大脑中转基因表达开始之前、期间和之后分别分析内质网应激蛋白水平。

2.7　5 × FAD 中的眼部病变

虽然与 AD 相关的病理改变大多发生在大脑,但其实眼睛也是受影响的器官之一。越来越多的患者会出现视力障碍、视野丧失、色觉异常、对比敏感性降低、视觉注意丧失、视觉记忆减退、运动视觉减弱以及眼球运动和固定障碍等视功能障碍症状。

3 月龄时 5 × FAD 小鼠视网膜血流动力学和氧指标、厚度和组织 $A\beta_{42}$ 蛋白水平的同时改变与先前报道的人类 AD 的发现相一致,即在 5 × FAD 小鼠的视网膜和脑组织中均观察到 $A\beta_{42}$ 水平的增加[122]。5 × FAD 小鼠视网膜中存在神经元内淀粉样蛋白-β 寡聚物(oligomers of amyloid-β, AβO)[123],AβO 被认为是 AD 突触毒性和淀粉样蛋白发生的关键,参与了体内外 Aβ 聚集的突触毒性和传播,其积累与视网膜 Aβ 斑块沉积呈负相关[124]。利用视网膜厚度作为评价指标,5 × FAD 小鼠视网膜厚度明显低于同龄非转基因小鼠,Aβ 疫苗处理后的 5 × FAD 小鼠,不仅 Aβ 斑块减少,视网膜厚度也增加[125]。总之,在发育和衰老过程中,5 × FAD 小鼠视网膜和视神经上的淀粉样前体蛋白、磷酸化淀粉样前体蛋白、β 蛋白裂解酶、γ 淀粉裂解酶、总 Tau 蛋白和磷酸化 Tau 蛋白的表达变化与大脑组织中的变化具有一致性。

3　5 × FAD 转基因小鼠在 AD 防治药物研发中的应用

由于 5 × FAD 转基因小鼠具有 AD 典型的认知行为学与病理特征,并参与 AD 的发病机制,其已被用于各类 AD 防治药物的研究中(表 5)。

表 5 5 × FAD 转基因小鼠在 AD 防治药物研发中的应用

Table 5 Application of 5 × FAD transgenic mice in the development of anti-AD drugs

药物种类 Drug types	治疗药物 Therapeutic	给药剂量 Administration dosage	给药方式 Administration mode	给药时长 Duration of administration	月龄 Month ages	方法 Methods	检测结果 Results
中药 Traditional Chinese medicine	棉花素 Gossypetin	10 mg/kg	灌胃 Gavage	91 d	1.6[45]	MWM	目标穿越次数 Number of target crossing
	蝉翼素 Pteryxin	16 mg/kg	皮下注射 Subcutaneous injections	7 d	12[43]	MWM	目标象限停留时间 Time in the target quadrant
	丁香酚 Eugenol	30 mg/kg	灌胃 Gavage	30 d	11[42]	MWM	目标象限停留时间 Time in the target quadrant
	姜黄素 Theracurmin	100, 300 and 1000 mg/kg	灌胃 Gavage	84 d	3[33]	BM	目标象限停留时间 Time in the target quadrant
	PF	5 mg/kg	皮下注射 Subcutaneous injections	28 d	6[41]	MWM	逃避潜伏期 Escape latency
	T4	5, 25 μg/kg	皮下注射 Subcutaneous injections	60 d	5[126]	MWM	逃避潜伏期 Escape latency
	SLCP	100 mg/kg	灌胃 Gavage	60 d	6、12[48]	MWM	目标象限停留时间 Time in the target quadrant
	OABL	20 mg/kg	皮下注射 Subcutaneous injections	21 d	6[40]	MWM	目标象限停留时间 Time in the target quadrant
化学药 Chemical drugs	MOS	0.12%, w/v	灌胃 Gavage	56 d	0.5[127]	MWM	逃避潜伏期 Escape latency
	3NCP	10, 20 and 40 mg/kg	皮下注射 Subcutaneous injections	28 d	5 ~ 6[49]	BM	错误次数 Total errors

4 总结

5 × FAD 转基因小鼠模型优势在于重现了 AD 相关淀粉样蛋白病理的主要特征,具有许多其他 AD 动物模型所没有的神经元损伤,可在小鼠模型早期模拟 AD 患者晚期的神经病理过程及行为学表现,被认为是关于 AD 表型再现最详尽的模型之一。但其在解释 5 × FAD 表型及其与人类 AD 的关系时,也存在一定劣势:首先,没有 AD 病例是由多个 FAD 突变引起的,并且在 5 × FAD 小鼠中突变组合可能会对 APP 处理产生意想不到的影响;其次,5 × FAD 中 $A\beta_{42}/A\beta_{40}$ 的比值似乎高于人类 AD,因此增加了 5 × FAD 中 $A\beta_{42}$ 毒性水平高于典型 AD 大脑的可能性;最后,小鼠是否存在神经原纤维缠结(NFTs)仍有争议。总的来说,该模型概括了人类患者临床 AD 的众多特征,在临床前评估新药物对 AD 的疾病治疗潜力方面仍然具有很好的前景。

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