动物模型在神经退行性疾病中的应用

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摘要 神经退行性疾病的主要临床症状表现为记忆丧失、认知障碍、运动能力丧失和感觉缺失等。随着人口老龄化的加剧, 神经退行性疾病的发病率也逐渐上升。目前, 人们对这类疾病的认知尚浅, 因此, 对应的治疗和干预方法也很紧缺。动物模型在神经退行性疾病中的广泛应用为我们提供了良好的实验材料, 为研究发病机制及治疗方式提供了重要平台。该文总结了在阿尔兹海默症、帕金森症、亨廷顿病以及肌萎缩侧索硬化症这四种常见神经退行性疾病的相关研究中成功构建的动物模型, 涉及动物包括秀丽隐杆线虫、黑腹果蝇、斑马鱼、啮齿类动物、小型猪和非人灵长类动物。

关键词 神经退行性疾病; 阿尔兹海默症; 帕金森症; 亨廷顿病; 肌萎缩侧索硬化症; 动物模型

Application of Animal Models in Neurodegenerative Diseases

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Abstract The main clinical symptoms of neurodegenerative diseases contain memory loss, cognitive impairment, loss of motor ability, and loss of sense. As the ageing of the population intensifies worldwide, its incidence rate increases. Due to its complex pathogenesis and manifestations, our knowledge of these diseases is still shallow, and the corresponding methods of treatment are scarce. The extensive use of animal models in the study of neurodegenerative diseases provides us with good experimental materials. This review summarizes the successful constructed animal models in Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis. Animal species involved include *Caenorhabditis elegans*, *Drosophila melanogaster*, zebrafish, rodents, minipigs and non-human primates.

Keywords neurodegenerative diseases; Alzheimer's disease; Parkinson's disease; Huntington's disease; amyotrophic lateral sclerosis; animal models

1 神经退行性疾病概述

神经退行性疾病的共同特征表现为脑和脊髓中神经元和神经胶质细胞的损伤和死亡, 临床症状表现为记忆丧失、认知障碍、运动能力丧失和感觉缺失等, 主要包括阿尔兹海默症(Alzheimer's disease, AD)、帕金森症(Parkinson's disease, PD)、亨廷顿病(Huntington's disease, HD)、肌萎缩侧索硬化症(amyotrophic lateral sclerosis, ALS)等。神经退行性疾病通常在中年或老年时期发病, 发病率随着人口老龄化的加剧而逐年上升。尽管对神经退行性疾病的研究日益增多, 但我们对这些疾病复杂的发病机制依然知之甚少, 对应的治疗方案和药物更是凤毛

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麟角。

对于神经退行性疾病的研究, 一般从三个层次开展。分子水平, 可以研究疾病相关基因和蛋白功能, 探索分子间的相互作用。细胞水平, 可以研究疾病对细胞功能和一些细胞活动的影响。个体水平, 可以从整体的高度比较全面地了解疾病对于机体的影响。通过构建动物模型, 我们可以更加清楚地理解神经退行性疾病在个体水平上的表现, 同时也为寻找对应的治疗方式提供重要的研究平台。目前被广泛使用在此类研究中的动物主要有秀丽隐杆线虫、黑腹果蝇、斑马鱼、啮齿类动物、小型猪和非人灵长类动物等。

AD属于中枢神经系统退行性疾病, 除了散发型AD(sAD)外, 已知*APP*、*PSEN1*和*PSEN2*等基因的突变会引发家族型AD(fAD)[1]。AD的主要病理特点是细胞内由过度磷酸化的Tau蛋白异常聚集引起的神经原纤维缠结的形成和Aβ(β-amyloid peptides)多肽为核心的老年斑在前脑基底、大脑皮层和海马体中的沉积[2]。

PD是继AD之后第二常见的神经退行性疾病, 也可分为散发型和家族型(5%~10%)。目前已经发现的PD致病基因包括*α-Synuclein*、*Parkin*、*PINK1*、*LRRK2*、*DJ-1*和*ATP13A2*等[3]。此外, 暴露于MPTP和6-OHDA等的神经毒素也被证明是PD的风险因素。PD的主要病理学特征是位于黑质致密部的多巴胺能神经元(DA神经元)的进行性变性和丢失以及在受累脑区形成包含α-Synuclein蛋白和泛素的嗜酸性路易小体(Lewy bodies)[4]。

HD是由*HTT*单基因突变引起的, 从而导致亨廷顿蛋白(HTT)氨基端区域的聚谷氨酰胺(Poly-glutamines, Poly-Q)片段的异常扩增(>37个重复)。

ALS是一种累及上、下运动神经元的神经退行性疾病, 分为散发型(sALS)和家族型(fALS)两类。目前已鉴定的fALS的致病基因和位点近40个, 主要包括*C9orf72*(约占45% fALS和10% sALS)、*SOD1*(约占15% fALS和1%~3% sALS)、*TDP-43*和*FUS*等。

本文总结了在AD、PD、HD以及ALS这四种神经退行性疾病相关研究中成功构建的动物模型, 并罗列了一些已有的基因改造模型(表1), 涉及动物包括秀丽隐杆线虫、黑腹果蝇、斑马鱼、啮齿类动物、小型猪和非人灵长类动物。

2 神经退行性疾病相关动物模型简介

2.1 秀丽隐杆线虫

秀丽隐杆线虫(*Caenorhabditis elegans*, 简称线虫)饲养简单、繁殖快、成本低, 可以液氮冷冻保存。线虫的遗传信息研究透彻, 已知约35%的线虫基因在人类中有同源物, 约42%的人类疾病基因在线虫中有同源物[5]。线虫的神经系统非常简单, 仅有302个神经元。线虫的发育及分化谱系明确, 基因编辑操作简单, 是研究神经退行性疾病良好的动物模型。

在AD的研究中, 多个实验组揭示, 在肌肉细胞中表达人类$A\beta_{42}$多肽会导致线虫残疾, 并且发现一些分子伴侣蛋白参与抵抗毒性$A\beta_{42}$多肽[6-8]。纯合缺失*APP*同源基因*apl-1*会导致线虫幼虫死亡[5]。在谷氨酸能神经元中, 表达$A\beta_{42}$多肽会导致细胞骨架蛋白功能改变[9]。表达人类Tau蛋白及其突变体(P301L和V337M)的线虫运动协调性差, 会出现神经元丢失或神经系统紊乱现象[10]。

PD的线虫模型主要包括使用MPTP、6-OHDA、鱼藤酮和百草枯等神经毒素的诱导模型和转基因模型。过表达人类α-Synuclein和突变体(A53T和A30P)的线虫出现非渐进性的DA神经元损伤[11]。在线虫中敲除或敲减*Parkin*的同源基因*pdr-1*会增加线虫对线粒体复合体I抑制剂的敏感性, 表达突变体(lg103)的线虫会产生类似PD患者的蛋白聚集物[12-13]。此外, *DJ-1*(线虫同源为*djr-1.1*和*djr-1.2*)[14]、*PINK1*(线虫同源为*pink1*)以及*LRRK2*(线虫同源为*lrk-1*)[15]等基因敲除或致病突变体转基因线虫也已获得。

在HD的研究中, 研究者已获得多种不同长度Poly-Q连接的HTT片段的转基因(Htt-Q150等)或基因敲入(HdhQ111等)的线虫[16]。尽管线虫中没有*HTT*直系同源基因, 但这些转基因线虫均表现出年龄依赖性的感觉缺陷和神经元功能障碍[17]。

最早的ALS线虫模型是在线虫中表达人类SOD1及其ALS相关突变体(A4V、G37R和G93A), 该研究阐述了氧化应激在衰老过程中对ALS的影响[18]。此外, 表达人类TDP-43及其ALS相关突变体(G290A、A315T和M337V)的线虫表现出运动功能不协调和异常神经突触的产生[19], 表达人类FUS蛋白的ALS致病突变体(R514G、R521G、R522G和P525L)[20]的线虫会导致该突变体蛋白的错误定位和聚集, 进而伴随运动功能损伤。

表1 四种神经退行性疾病的基因改造动物模型

Table 1 Genetic modification of animal models in four neurodegenerative diseases

动物模型 Animal model	阿尔兹海默症 Alzheimer's disease	帕金森症 Parkinson's disease	亨廷顿病 Huntington's disease	肌萎缩侧索硬化症 Amyotrophic lateral sclerosis
Caenorhabditis elegans	unc-54 ::h Aβ eat-4 ::h Aβ aex-3 ::h Tau rgef-1 ::h PHP Tau mec-7 ::h Tau rab-3 ::h Tau[28]	dat-1 ::h α-syn (WT, A30P, A53T) unc-51 ::h α-syn dat-1 ::LRRK2 G2019S Tg: *pdr-1*(lg103); *djr-1.2* mutant; *djr-1.1* mutant Tg/KD/KO; *pink-1* mutant Tg/KO; lrk-1 mutant Tg/KO[11]	osm-10 ::HtnQ95, HtnQ150 pmec-3 ::Htt57Q88, Htt57Q128[28,95]	hsp16-2/myo-3 ::h SOD1 Tg: hSOD1 (WT, G85R, G93A, C6S/C57S/C111S/C146S) Tg: hTDP-43 (WT, G290A, A315T, M337V) Tg: alfa-1 mutation Tg: hFUS (WT, R514G, R521G, R522G, P525L, FUS^{513}, FUS^{501}, S57Δ)[44]
Drosophila melanogaster	Tg: hTau, Tg: hAPP Tg: hBACE1 *Appl* KO[28,96]	Tg: h α-Syn(WT, A30P, A53T) Tg: h LRRK2(WT, I1122V, Y1699C, G2019S, I2020T, G2385R) *Parkin* KO, *Pink1* KO, *Dj-1* KO[11,28,96-97]	gmr ::N-HttQ128 Httex1pQ93, Htt128Q, HttQ120, HttQ75 elav ::Httex1pQ93, Htt128Q, N-HttQ128, Htt128Q FL apVNC ::Htt128QFL[28,95-97]	Tg: hSOD1 (WT, A4V, D83G, D83S); *Sod1* KO Tg: hTDP-43 (WT, F147L/F149L, G287S, A315T, G348C, A382T, ΔNLS); *Tdp43* KO Tg: C9orf72 (36-103, 160, 30, 58 repeats) Tg: hFUS (WT, R524S, P525L, R518K, R521C, R521H[44,97]
Zebrafish	*Appa*/*Appb* KD Tg: hTau, hTau-P301L	KD *dj-1*, *lrrk2, parkin, pink1*[11]	KD *htt* Tg: Htt-Q102-GFP[95]	Tg: SOD1 (A4V, G93A, G93R, G37R) *C13H9orf72* KD[44]
Mouse	Tg: hAPP (V717I, V717F, D23N, K670N/M671L) PS1, 5×FAD, 3×Tg; *App* KO, *Psen2* KO, *Psen1* KO (lethal)[28,71,98]	Thy1 ::h α-Syn (WT, A30P, A53T) PrP ::h α-Syn (WT, A30P, A53T) Tg: Y39C, G2019, R1441G, Q311X; *atg7* KO, *Parkin* KO, *Dj-1* KO, *Lrrk2* KO, *Pink1* KO, α-*Syn* KO, *Vmat2*-deficient[11,71,98]	Lines: R6/1, R6/2, N171-Q82, HD94, HD150QG, HD190QG, HD48, HD89, YAC128, YAC48, YAC72, BACHD; CAG140, HdhQ92, HdhQ111, HdhQ150, HdhQ200[28,71,95,98]	Tg: SOD1 (WT, A4V, G37R, H46R, D83G, L84V, G85R, G86R, D90A, G93A, L126Z, G127X); hTDP-43 (WT, A315T, Q331K, Q331K, M337V, G348C); hFUS (WT, R521G); C9orf72(repeats 80, 66, 100-1 000, 500, 450) *Sod1* KO, *Fus* KO[44]
Rat	Tg: PSAPP[71]	Tg: AAV α-Syn (WT, A53T)[11,99]	Tg: HD51 BACHD[95,98]	hSOD1 (H4,G93A); hTDP-43 (WT, M337V); hFUS (WT, R521C); *FUS* KO[44,95]
Non-human primates			Htt84Q (macaque monkey)[94]	
Minipig			N208-105Q N548-124Q[91]	hSOD1 G93A[92]

2.2 黑腹果蝇

黑腹果蝇(*Drosophila melanogaster*, 简称果蝇)生命周期短、繁殖迅速、体型小、易于在实验室培养和维持。果蝇的遗传信息和背景清楚, 各种基因操作方法成熟, 如UAS/GAL4系统等。约有60%的果蝇基因在人类中有保守基因, 约75%已知的人类疾病基因在果蝇中有同源物[21]。此外, 果蝇具有复杂的神经系统, 含有神经元、神经胶质细胞和血脑屏障等, 对神经科学的研究有较明显的优势。

在AD相关研究中, 纯合缺失*APP*同源基因*appl*的果蝇出现明显的行为缺陷[22]。表达毒性Aβ蛋白的果蝇出现神经退化及视力损伤[23]。果蝇感觉神经元中表达人类Tau蛋白或GSK-3β突变体均会导致轴突退化[24-25]。表达Tau蛋白及其突变体(R406W、V337M和P301M)的果蝇出现神经元缺失, 但未见神经原纤维缠结形成[26-27]。表达人类BACE1和APP的果蝇常用作药物测试, 具有一定的现实价值[28]。

在PD相关研究中, 表达人类α-Synuclein及其突变体(A53T和A30P)的果蝇模型病理重现性好, 出现年龄依赖性的DA神经元缺失和路易小体类似物的形成[29-31]。 *Parkin*同源基因缺失和组织特异性RNA干扰果蝇系则表现出线粒体功能异常和飞行肌肉受损[32-33]。表达人类Parkin致病突变体(Q311X、T240R和R275W)的果蝇则会出现年龄依赖性的神经变性和功能障碍[34-35]。*PINK1*同源基因缺失和RNA干扰果蝇系[36]、*DJ-1*同源(*DJ-1A*和*DJ-1B*)基因敲除和RNA干扰果蝇系[37-38]以及表达人类LRRK2及其突变体(G2019S、G2385R、I2012T和Y1699C)的果蝇系均已获得[39-40]。其中, 表达人类α-Synuclein及其突变体的果蝇是所有α-Synuclein转基因动物模型中唯一出现渐进性DA神经元变性的模型[11]。此外, 鱼藤酮等药物诱导也同样适用于果蝇的PD造模。

在HD相关研究中, 表达毒性Poly-Q连接的HTT一号外显子片段(Q75和Q120)会诱导果蝇发生迟发性视网膜病变[41]。因其神经变性程度可以直观、简单地通过复眼的损伤情况来判断, 果蝇被认为是比较优质的HD模型[28]。

在ALS相关研究中, 纯合缺失*SOD1*同源基因会减短果蝇寿命, 增加对氧化应激的敏感性[42]; 纯合缺失*TDP-43*同源基因会导致果蝇幼虫死亡。此外, 表达TDP-43突变体和其RNA干扰果蝇系[43]以及表达不同长度G_4C_2重复的C9orf72果蝇系也均已获得[44]。

2.3 斑马鱼

斑马鱼(zebrafish)是一种常用的脊椎动物模型, 体型小、繁殖能力强, 可用来进行大规模的药物筛选。斑马鱼胚胎透明、发育迅速, 非常适合研究受精卵发育和器官发生。斑马鱼基因组与人类大约有70%的同源性, 大约82%的人类疾病基因在斑马鱼中有同源基因[45]。用于斑马鱼基因改造、遗传分析的工具丰富, 如反义吗啉代寡核苷酸(MO)技术等, 此外细胞标记、组织移植技术也比较成熟[46]。

在AD相关研究中, 使用MO技术敲减*Appa*和*Appb*(人类*APP*同源)会导致斑马鱼原肠体长度缩短以及原肠胚聚集, 这些缺陷可以通过补充正常形式的*APP* mRNA得到缓解[47]。通过GATA-2启动子在斑马鱼的泛神经元中表达突变形式Tau-GFP蛋白, 或者在神经元中表达突变Tau^{P301L}蛋白的斑马鱼都揭示了突变形式Tau蛋白的毒性[48-49]。

PD的相关致病基因在斑马鱼中均有直系同源, 包括*parkin*、*pink1*、*dj-1*和*lrrk2*。对上述基因的转基因或基因敲减斑马鱼模型的研究揭示了这些基因在DA神经元的发育和存活过程中具有保守功能[50-55]。在过表达α-Synuclein的活体斑马鱼模型中, 使用荧光标记物追踪的实验首次证明了轴突肿胀会诱导神经细胞死亡, 推进了PD的病理研究。另外, 使用6-OHDA、MPTP等神经毒素也可构建PD的斑马鱼模型。

在HD相关研究中, 敲减内源*htt*基因的斑马鱼模型的研究揭示了htt蛋白在细胞内参与铁离子的利用[56]。表达毒性Poly-Q连接的htt氨基端片段(Q102-GFP)的斑马鱼可用于候选药物的活性鉴定, 使用该模型发现, PGL-135、刚果红、293G02和306H03这四种化合物可以抑制毒性Htt聚集物的形成, 这为寻找HD的潜在药物提供了帮助[57]。

在ALS相关研究中, 通过对表达TDP-43突变体(A315T和G348C)和FUS突变体(S57Δ和R521H)斑马鱼的研究, 研究者揭示了亚甲蓝对细胞可能的保护作用[58]。mTDP-43转基因斑马鱼是首个利用体内表型来筛选ALS候选化学药物的模型。此外, 表达人类SOD1及其突变体(A4V、G93A、G93R和G37R)的斑马鱼以及*C13H9orf72*(*C9orf72*同源)基因敲减的斑马鱼均已获得[44]。

2.4 啮齿类

啮齿类动物是实验室常用哺乳动物模型, 主

要有小鼠和大鼠。小鼠大多来源于小家鼠(*Mus musculus*), 性情温顺、体型小、易于饲养、繁殖快。小鼠是一种研究最详尽、用途最广泛的模式动物。99%的小鼠基因在人类基因组中有同源序列, 与人类高度同源[59]。实验室使用的大鼠(Rats)主要来自于褐家鼠(*Rattus norvegicus*), 形似小鼠但比小鼠大。大鼠基因组相比于小鼠更接近人类, 已被广泛应用于高级神经活动的研究中。

在AD相关研究中, 研究者可以通过直接向小鼠和大鼠脑内注射毒性Aβ多肽或使用东莨菪碱等化合物损伤胆碱能神经元来模拟AD病症[60-61]。通过手术或药物处理的损伤模型操作简单, 造模时间短, 但个体差异较大。表达人类APP突变体(K670N/M671L、V717I、V717F和D23N系), PSEN1(PS1系)和PSEN2(PS2系)突变体的小鼠均表现出渐进的、早发型的痴呆症状, 但都缺乏神经元纤维缠结的形成。在此基础上, 研究者通过杂交得到了双转基因PSAPP系小鼠和三转基因3×Tg小鼠系(包含人类APP、PS1突变和Tau突变)[62-65]。5×FAD小鼠系是结合三个人类APP突变体和两个PS1突变建立起来的, 该小鼠模型更早地出现淀粉样病变、认知障碍及神经元损失。这些多转基因小鼠较单转基因小鼠的病理表现与AD更相似, 具有更高的应用前景。此外, *App*、*Psen2*和*Bace1*基因敲除小鼠也已获得, 而*Psen1*基因敲除会导致小鼠死亡[66-68]。

在PD相关研究中, 最经典、最广泛使用的模型是由6-OHDA诱导的大鼠和MPTP诱导的小鼠模型, 常用于潜在药物的筛选。利血平、鱼藤酮和百草枯等也有相似作用[69]。此外, 还有表达人类α-Synuclein及其致病突变体的小鼠(Thy1/PrP-WT、A30P和A53T)和大鼠(AAV-WT和A53T)。在这些模型中, 使用mPrP启动子的A53T转基因小鼠是脊椎动物PD模型中病理特征表现最全面的[70]。敲除*Parkin*、*PINK1*和*DJ-1*同源基因的小鼠以及表达人类LRRK2及其致病突变(R1441G和G2019S)的小鼠可以帮助理解由这些基因缺失或突变引起的黑质纹状体DA的早期异常[11,71]。

在HD相关研究中, 研究者可以在小鼠中使用线粒体毒素如丙二酸和3-硝基丙酸(3-NPA)使动物产生纹状体损伤来模拟HD病症, 这种药物损伤模型起效快、重点突出, 但表现不全面。此外, 已有超过20种啮齿类HD转基因模型, 包括表达人类突变HTT的氨基端区域, 如R6/2和N171-82Q等小鼠系[72-73]; 表达全长人类HTT及其突变体的基因敲入模型, 如YAC128和BACHD等小鼠系[74-75]; 表达毒性Poly-Q连接的HTT一号外显子的基因敲入模型, 如HdhQ111、CAG140和HdhQ150等小鼠系[76-80]。其中, R6/2小鼠系因其发病快、表型明显等特点而最为广泛使用。

在ALS相关研究中, 表达SOD1^{G93A}突变体的小鼠是最早的ALS疾病小鼠模型。该小鼠可以模拟ALS的大部分临床及病理变化[81]。然而, *Sod1*基因敲除小鼠未出现运动神经元损伤。此后多种表达SOD1突变体(G37R、G85R、D90A和G86R)小鼠系相继被建立[82-84]。表达SOD1突变体(H46R和G93A)的大鼠出现年龄依赖性的运动神经元变性, 逐渐会导致瘫痪和死亡[85-86]。多种使用不同启动子表达不同长度G_4C_2重复的C9orf72的小鼠表型不尽相同[44]。表达TDP-43突变体的小鼠(A315T)和大鼠(M337V)模型也已获得[87-88]。*FUS*同源基因敲除小鼠出生后不久即死亡[89]。

2.5 小型猪

我国小型猪资源丰富、品种多样、遗传稳定。它们与人类的解剖学、遗传学和生理学相似性高, 比啮齿类动物更接近人类。小型猪体型小、易于饲养维持、方便实验操作和微生物控制, 近年来逐渐成为生物、医学研究中重要的动物模型。

在HD相关研究中, 表达人类毒性Poly-Q连接的HTT片段的N208-105Q西藏小型猪仅有一只四个月龄时仍无表型的幼崽存活。随后的研究中获得了有繁殖能力的N548-124Q小型猪, 但其病理变化与HD患者仍有一定差异[90-91]。

在ALS相关研究中, 表达人类SOD1^{G93A}突变体的猪模型表出现可遗传的运动缺陷以及剂量和年龄依赖性的神经元变性, 该模型相比于已有的ALS小鼠模型更接近于人类病理表型[92]。

2.6 非人灵长类动物

非人灵长类动物是所有模式动物中与人类同源性最高的, 具有高级的脑功能和神经活动, 主要包括猴、猿等。但因其繁殖率低、幼体生长缓慢、实验周期长、培养和维持费用高而不能广泛应用。

在PD相关研究中, MPTP诱导的PD猴模型具有最接近人类的PD病理特征, 包括在病人样本中观察到的黑质纹状体多巴胺消耗和纹状体多巴胺、去甲肾上腺素和血清素的改变, 是药物预测的首选PD模

型[93]。

在HD相关研究中, 使用丙二酸和3-NPA诱导的非人灵长类动物HD模型可以模拟得到纹状体损伤表型。恒河猴(猕猴)表达人类毒性Poly-Q连接的HTT片段的转基因模型能体现HD的主要标志, 并且病理表现较R6/2系小鼠更接近于人类HD, 具有重要的实验价值[94]。

3 总结与展望

动物模型在神经退行性疾病中的广泛应用为疾病的研究做出了重要贡献, 但是仍与人类的病理学和临床表现差异较大。几乎所有已有的模式动物都只能模拟疾病的部分变化, 在研究过程中我们可能需要组合利用多种动物模型, 防止因为模式动物自身的影响而被误导。据报道, 许多在啮齿类动物模型中表现良好的新药在临床试验时失败率高达90%, 提示我们在实验后期使用进化程度更高的小型猪或非人灵长类模型具有重要意义。此外, 随着基因编辑技术的发展和成熟, 大量基因改造动物模型成功构建, 但大多数自然发生的神经退行性疾病并不具有遗传特性。综合上述方面可知, 动物模型在神经退行性疾病的研究和治疗方案的探索中为我们提供了重要的实验材料, 但并不完美, 寻找和建立更合适的动物模型或者综合使用多种动物模型对我们以后的实验将大有裨益。

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