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斑马鱼模型在阿尔茨海默病研究中的应用

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【摘要】 阿尔茨海默病(Alzheimer's disease,AD)是一种中枢神经系统退行性疾病,主要表现为认知功能障碍、言语丧失等,其发病与多种因素有关。近年来,斑马鱼因其在大脑结构与功能、神经传导及 AD 致病基因等方面与人具有高度同源性而受到广泛关注。本文就斑马鱼作为动物模型探索 AD 发病机制、进行 AD 药物评估、药物筛选等方面的优势展开综述,以期为 AD 的发病机制及新药开发研究提供新思路。

【关键词】 阿尔茨海默病;斑马鱼;发病机制;药物评估;药物筛选
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Advancements in application of the zebrafish model of Alzheimer's disease

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【**Abstract**】 Alzheimer's disease (AD) is a multifactorial degenerative disorder of the central nervous system that mainly manifests as cognitive dysfunction and loss of speech. In recent years, the zebrafish has attracted extensive attention because of its high homology with humans in terms of brain structure and function, nerve conduction, and pathogenic genes of AD. This article reviews the advantages of the zebrafish as an animal model of AD, covering topics in the pathogenesis of AD and the evaluation and screening of drugs for treatment of AD. The overall goal is to provide new insights into the pathogenesis of AD and development of novel drugs.

【**Keywords**】 Alzheimer's disease; zebrafish; pathogenesis; drug evaluation; drug screening
Conflicts of Interest: The authors declare no conflict of interest.

阿尔茨海默病(Alzheimer's disease,AD)是一种神经性退行性疾病,发病早期较为隐匿,主要症状为记忆减退、认知障碍乃至行动能力丧失等。预计 2040 ~ 2050 年,全球 AD 患病人数达 1 ~ 1.3 亿[1]。斑马鱼(zebrafish)是一种相对新颖的实验动物,具有脊椎动物的神经系统和行为模式[2]。其基因组含 25 对染色体,超过 26 000 个编码基因,与人类参考基因组相比,约 70%的人类基因与斑马鱼同源,其中有 84%与人类疾病基因相关[3-4]。本文就近年来斑马鱼作为模式动物用于 AD 发病和治疗研究的相关进展进行综述,同时总结斑马鱼模型在 AD 药物评估及药物筛选方面的进展。

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1　斑马鱼在神经退行性疾病研究中的优势

中枢神经系统(central nervous system, CNS)退行性疾病主要包括 AD、帕金森病(Parkinson's disease, PD)、肌萎缩侧索硬化症(amyotrophic lateral sclerosis, ALS)、亨廷顿舞蹈病(Huntington's disease, HD)等,症状大多为神经元缺失导致的运动和认知能力下降[5]。在神经退行性疾病研究中,斑马鱼具有与人类相似的功能性血脑屏障(blood brain barrier, BBB),这为斑马鱼模型模拟药物通过 BBB 治疗神经退行性疾病创造了条件[6-7]。

此外,斑马鱼具有高度的透明可视性,研究人员可以对斑马鱼 CNS 进行高分辨率活体成像,直接反应斑马鱼脑内神经细胞的生长情况[8]。斑马鱼脑的结构和功能都与人类具有一定相似性[9]。从结构角度看,斑马鱼受精 24 h 后前脑(forebrain, Fb)、中脑(midbrain, Mb)、后脑(hindbrain, Hb)和脊髓(spinal cord, Sc)大部分的发育与哺乳动物大脑排列相似[10]。从功能上看,斑马鱼的 Fb 具有接受、处理感觉信息并指导行为的功能。在斑马鱼发育的过程中,Fb 还会进一步形成端脑,端脑具有调节行为、记忆、情绪等功能[11]。另外,斑马鱼神经细胞的多样化与哺乳动物具有一定的相似性,除神经元外还包括放射状胶质细胞、小胶质细胞、少突胶质细胞和多纤毛室管膜细胞等。斑马鱼的神经递质系统也表现出与哺乳动物的相似性,包括 5-羟色胺能、去甲肾上腺素能、多巴胺能和组胺能系统[12]。在斑马鱼基因组中目前已发现多个与人神经退行性疾病相关的基因,例如 *psen*1、*psen*2、*appa*、*appb*(AD 致病基因);*snca*、*pink*1、*lrrk*2(PD 致病基因);*sod*1、*tardbp*、*c9orf*72、*fus*(ALS 致病基因);*huntingtin*(HD 致病基因)等,这些都为应用斑马鱼模型进行神经退行性疾病研究奠定了基础[13]。

2　斑马鱼在 AD 发病机制研究中的应用

AD 作为一种典型的神经退行性疾病,因其发病较为隐匿,早期不易发现,发病后期严重影响患者的生活。AD 的发病机制目前尚不明确。斑马鱼因具有上述利于进行神经退行性疾病研究的特点,已被广泛用于针对 AD 发病机制的研究(表 1)。

表 1　斑马鱼应用于 AD 的发病机制研究

Table 1　Application of zebrafish in the pathogenesis of AD

发病机制 Pathogenesis	斑马鱼模型 Zebrafish model	参考文献 References
Tau 蛋白假说 Tau hypothesis	*TAU-P*301*L* 转基因模型 *TAU-P*301*L* transgenic model	[14]
	*p. A*152*T* 基因突变模型 *p. A*152*T* gene mutation model	[15]
	MAPT 基因模型 *MAPT* gene model	[16]
	葡萄糖代谢异常模型 Abnormal glucose metabolism model	[17]
	朊蛋白相互作用模型 Prion protein interaction model	[18-19]
淀粉样蛋白假说 Amyloid hypothesis	铜绿假单胞菌 FapC 淀粉样蛋白片段相互作用模型 FapC amyloid fragments of *Pseudomonas aeruginosa* interaction model	[20]
	PSEN 基因模型 *PSEN* gene model	[21]
	神经干细胞/祖细胞再生模型 Neural stem cell/progenitor cell regeneration model	[22]
胆碱能假说 Cholinergic hypothesis	重金属毒性模型 Heavy metal toxicity model	[23-26]
	农药残留模型 Pesticide residue model	[27-31]
	环境污染物模型 Environmental pollutant model	[32-34]
	化学诱导 AD 模型 Chemical induced AD model	[35-36]
其他发病机制的研究 Studies on other pathogenesis	叶酸缺乏模型 Folate deficiency model	[37]
	氧化应激模型 Oxidative stress model	[38]
	*SORL*1 基因模型 *SORL*1 gene model	[39]
	线粒体功能障碍模型 Mitochondrial dysfunction model	[40]
	lncRNA 模型 lncRNA model	[41]

2.1　Tau 蛋白假说相关机制研究

神经纤维缠结(neurofibrillary tangles, NFTs)是 AD 的重要病理学特征之一[42]。在 AD 患者脑中,高度磷酸化的 Tau 蛋白失去结合微管的能力,变得不稳定而聚集成 NFTs。Tau 蛋白磷酸化和去磷酸化之间的失衡是 NFTs 形成和 AD 发病的早期症状[43]。有学者利用斑马鱼模型开展了 Tau 蛋白参与 AD 发病机制的研究并取得了一定进展。建立荧

光标记的 Tau 蛋白转基因斑马鱼可以观察到 Tau 蛋白磷酸化、NFTs、神经元死亡等与 AD 相关的病理学特征[44-45]。BARBEREAU 等[14]建立 *TAU-P*301*L* 转基因(引起 Tau 蛋白过磷酸化)斑马鱼模型,研究发现早期 Tau 蛋白引起的神经毒性可以影响脑营养因子(brain-derived neurotrophic factor, BDNF)信号传导,从而影响神经突触的长度以及神经元活性。再生胰岛衍生蛋白 1α(regenerating islet-derived 1α, REG-1α)是一种含有 C 型凝集素结构域的分泌蛋白,REG-1α 可以调节神经突触生长,并在 AD 发病早期高表达。研究发现 REG-1α 在 *TAU-P*301*L* 转基因斑马鱼中过表达,且通过 AKT/GSK3β 途径增加了 Tau 的 S202/T205 残基(Tau 蛋白早期磷酸化位点)磷酸化和 S396 残基(Tau 蛋白晚期磷酸化位点)磷酸化,从而证明了 REG-1α 参与 Tau 蛋白相关疾病的发生发展,特别是 Tau 磷酸化时间序列的修饰,提示了新的 AD 治疗靶点[46]。临床研究发现,Tau 蛋白发生 *p. A*152*T* 突变会造成患者体内 Tau 蛋白的积累,在 *p. A*152*T* 突变的患者身上会表现出神经损伤、语言能力丧失等症状[47]。LOPEZ 等[15]利用转基因斑马鱼模型发现 *p. A*152*T* 突变导致斑马鱼神经损伤,提示 *p. A*152*T* 突变与 AD 发病的关系。

此外,*MAPT* 是编码 Tau 蛋白的基因,目前在斑马鱼基因组中已经发现 *mapta* 和 *maptb* 两个与人类编码 Tau 蛋白的 *MAPT* 同源的基因[16]。与性别相关的 *USP*9 基因可以调节 *MAPT* 基因的表达,引起 Tau 蛋白磷酸化[48-49]。KÖGLSBERGER 等[50]通过敲低斑马鱼体内 *usp*9 基因的表达,发现 *mapta* 和 *maptb* 的表达降低,提示 *USP*9 基因调控 Tau 蛋白磷酸化的机制。另外,在 *TAU-P*301*L* 转基因斑马鱼体内敲低 FK506 结合蛋白 52(Fkbp52)蛋白,重新激活了斑马鱼神经元及轴突活性[51]。SEPULVEDA-DIAZ 等[52]在斑马鱼中抑制 *hs*3*st*2 基因的表达后,斑马鱼大脑和脊髓内的 Tau 蛋白磷酸化水平降低,且与运动相关的神经元长度明显恢复,提示 *HS*3*ST*2 可以作为 AD 治疗的一个新靶点。上述研究表明成功建立 Tau 蛋白过表达的斑马鱼模型可为探索 AD 的发病机制开辟新途径。

2.2 淀粉样蛋白假说相关机制研究

淀粉样蛋白(amyloid-beta peptides, Aβ)假说最早提出于 20 世纪 80 年代[53]。该假说认为 Aβ 的沉积是 AD 发病的始动因素,可引起脑内葡萄糖代谢异常,也会导致脑内 Tau 蛋白磷酸化和 Aβ 的过度积累[17]。以斑马鱼为模型,不同实验室分别研究了重复性低氧(repeated hypoxia, RH)暴露和慢性反复睡眠剥夺(repeated sleep deprivation, RSD)对脑损伤的影响。研究表明 RH 暴露[54]和 RSD[55]均引发斑马鱼脑内葡萄糖水平下调,而补充乙酰葡萄糖胺(N-acetylglucosamine, GlcN)则抑制了斑马鱼脑内炎症、Tau 蛋白磷酸化及 Aβ 沉积的发生。SEMPOU 等[18]研究发现加入人源 Aβ 寡聚体可以激活斑马鱼胚胎细胞的 PrP/SFK/E-cadherin 通路介导神经损伤。在后续的实验研究中,通过敲除斑马鱼体内调控 Aβ 生成的 *appa* 基因和调控朊蛋白形成的 *PRP*1 基因,进一步证实了 Aβ 与朊蛋白之间的相互作用关系[19]。随着对微生物脑肠轴(microbiotagut-brain axis, MGBA)研究的不断深入,已发现 Aβ 可以与铜绿假单胞菌分泌的 FapC 结合并定植于肠道,在斑马鱼中 FapC 与 Aβ 的结合加剧了 Aβ 引起的斑马鱼认知能力下降[20]。最近,ALI 等[56]发现 Aβ 单体可以靶向解聚由机会性肠道病原体铜绿假单胞菌和大肠杆菌形成的 FapC 和 CsgA 微生物淀粉样蛋白,提示 Aβ 单体在脑肠轴中潜在的抗生物膜作用,有助于未来选择性治疗 AD 和抗感染药物的开发。

在斑马鱼基因组中存在 PSEN 基因的 2 个同源基因,分别为 *psen*1 和 *psen*2[21]。NERY 等[57]利用 morpholino 反义核苷酸靶向斑马鱼 *psen*1 的第 8 外显子剪接位点进行干扰,诱导了斑马鱼幼虫认知障碍、突触减少、Aβ42 沉积等早发性 AD 的表现。研究发现在散发型 AD 患者脑中 PSEN2 的截断异构体 PS2V 水平上调,且通常由缺氧和高胆固醇摄入所诱导。PS2V 可以增加 γ-分泌酶活性,从而影响 Aβ 的生成。由于啮齿类动物缺乏这种 PSEN 亚型,因此缺少合适的遗传可操作动物模型,研究发现斑马鱼体内 *ps*1IV 与人类的 PS2V 同源,研究者利用 Morpholino 反义核苷酸斑马鱼 *ps*1IV 的表达后发现与 AD 炎症相关的 *il*1*b*、*ccr*5 基因和细胞增殖相关的基因表达发生了变化,提示 PS2V 在散发型 AD 中发挥重要作用[58]。另外,*psen* 突变对斑马鱼的影响与斑马鱼的年龄有关,成年斑马鱼体内 *psen*1 基因突变影响大脑对低氧的反应[59],而 7 日龄斑马鱼体内 *psen*1 会改变机体铁离子稳态、氧化磷酸化等[60]。

促进 AD 患者脑内神经细胞再生也是治疗 AD 的一种有效手段。通过在斑马鱼脑室内注射 Aβ42

建立 AD 模型,发现斑马鱼出现了神经元凋亡、小胶质细胞激活、突触变性和学习缺陷等典型 AD 病理学特征。然而 Aβ 并非只具有神经毒性,成年斑马鱼脑内 Aβ 可以增加神经干细胞(neural stem/progenitor cells, NSPCs)的可塑性,并促进 NSPCs 的增殖[22]。BHATTARAI 等[61]认为成年斑马鱼脑内神经元和小胶质细胞/巨噬细胞被 Aβ 激活后产生的白细胞介素-4(interleukin-4, IL-4),通过与 NSPCs 中 IL-4 受体结合将活化信号逐级传递,最终激活信号传导转录激活因子 6(signal transducer and activator of transcription 6, STAT6)使其发生磷酸化来促进 NSPCs 的增殖和神经发生。SIDDIQUI 等[62]研究发现斑马鱼脑内色氨酸代谢物犬尿喹啉酸(kynurenic acid, KYNA)通过其芳烃受体 2(aryl hydrocarbon receptor 2, Ahr2)负向调节成年斑马鱼大脑 NSPCs 的可塑性。KYNA 代谢和芳烃受体(aryl hydrocarbon receptor, AHR)信号通路相关基因在晚发型 AD 中存在差异表达,这表明 KYNA/Ahr2 信号通路与神经发生存在密切联系,为探索 AD 发病机制提供新思路。

2.3　胆碱能假说相关机制研究

胆碱能假说是关于 AD 发病机制最早提出的假说,其表现为 AD 脑中乙酰胆碱酯酶(acetylcholinesterase, AChE)、乙酰胆碱转移酶(choline acetyltransferase, ChAT)异常以及乙酰胆碱(acetylcholine, ACh)水平下降。基底前脑胆碱能神经元是最早受 AD 影响的神经元之一,有研究表明 AChE 活性降低与 Aβ 聚集有关[63]。金属暴露是造成乙酰胆碱活性降低的主要原因。使用铝或者 $AlCl_3$ 引起斑马鱼脑组织中 ACh 水平下降,AD 相关致病基因和蛋白表达增加,同时斑马鱼的学习记忆能力受损[23]。急性和慢性暴露于 $ZnCl_2$ 均会导致斑马鱼脑内 Tau 蛋白过度磷酸化、Aβ 沉积,当暴露剂量 > 1 ppm 时,对 ACh 活性有明显抑制作用[24]。斑马鱼胚胎暴露于重金属铜[25]或汞[26]时,斑马鱼体内 ACh 活性受到明显抑制,而且干扰斑马鱼的运动和回避行为。

斑马鱼作为一种海洋鱼类,水中农药残留和环境中污染物毒性暴露通常引起斑马鱼脑内神经递质水平的改变及行为障碍。将斑马鱼胚胎暴露于水产系统广谱杀菌剂噻呋酰胺(thifluzamide)后观察到斑马鱼体内 AchE 活性降低,神经递质如 5-羟色胺(5-hydroxytryptamine, 5-HT)、去甲肾上腺素(norepinephrine, NE)含量增加、神经系统相关基因的转录变化、胚胎大脑内褪黑素(melatonin, MT)水平的显著变化,严重影响了斑马鱼胚胎昼夜节律和生长发育[27]。同样,将斑马鱼持续暴露于新型烟碱类杀虫剂吡虫啉(imidacloprid)后引起斑马鱼行为障碍,通过转录组学分析发现昼夜节律紊乱、精氨酸和脯氨酸代谢失衡及神经递质紊乱是引起行为障碍的主要原因[28]。研究人员将斑马鱼胚胎暴露于敌百虫(trichlorfon)[29]或甲基异苯磷(isofenphos-methyl, IFP)[30],发现两种杀虫剂均能抑制 AChE 的活性。影响神经系统发育的环境污染物磷酸二苯酯(diphenyl phosphate, RDP)也表现出与 AChE 较强的结合亲和力,斑马鱼暴露在 RDP 后发现体内与 CNS 发育相关的关键基因和蛋白的表达均明显下降[31]。

塑料降解具有一定的环境毒性,将斑马鱼胚胎分别暴露于增塑剂邻苯二甲酸二乙基己基酸(Di(2-ethylhexyl) phthalate, DEHP)的代谢物邻苯二甲酸单乙基己基酸(mono-2-ethylhexyl phthalic acid, MEHP)[32]和塑料降解产生纳米级塑料颗粒(nano-sized plastic particles, NP)[33]中,最终发现两种物质均阻碍斑马鱼胚胎的生长和神经发育相关基因的表达,还会引起斑马鱼胚胎的氧化应激反应和脑细胞凋亡,并伴有 AChE 活性降低。DING 等[34]将斑马鱼暴露于光老化聚苯乙烯(photoaged polystyrene, P-PS)中,发现 P-PS 显著增加了 AChE、ChAT 的活性,影响斑马鱼胚胎神经传递和氧化应激相关基因的表达。此外,冈田酸(okadaic acid, OKA)和东莨菪碱(scopolamine, SCOP)常被用作斑马鱼 AD 模型的诱导物。OKA 可以诱导斑马鱼发生 ACh 功能障碍、诱发谷氨酸兴奋毒性、氧化应激等 AD 相关的症状[35];在斑马鱼腹腔注射 SCOP 后会产生胆碱功能障碍造成学习记忆能力丧失[36]。

2.4　与 AD 相关的新机制探索

斑马鱼模型应用于 AD 的新机制研究为 AD 的治疗提供了新方向。叶酸缺乏与许多神经系统疾病有关,研究者通过异位过表达重组 EGFP-γ-谷氨酰水解酶(γ-glutamyl hydrolase, γ-GH),建立了持续叶酸缺乏的老年斑马鱼转基因模型。随后在斑马鱼脑冷冻切片中发现 Aβ 和 Tau 蛋白磷酸化水平显著上升,提示叶酸缺乏与 AD 发病之间的关系[37]。在低水平活性氧(reactive oxygen species, ROS)应激下,人类 p53 亚型 Δ133p53 的表达上调,通过增强

抗氧化基因的表达来促进细胞存活和减缓细胞衰老,而 AD 患者脑组织中 Δ133p53 表达减少,但 Δ133p53 在脑衰老中的确切功能尚不清楚。ZHAO 等[38]利用斑马鱼模型证实斑马鱼脑内人 Δ133p53 的同源物 Δ113p53 通过其抗氧化功能延缓神经细胞衰老且与学习记忆能力相关,提示 Δ113p53/Δ133p53 的过度消耗可能会导致长期的 ROS 应激,并最终诱发 AD 等与年龄相关的疾病。

研究发现,*SORL*1 基因与早发家族性 AD(early-onset familial AD, EOFAD)和 LOAD 都有遗传关联,SORL1 蛋白已被证明在 Aβ 运输中起作用。BARTHELSON 等[39]对斑马鱼 *sorl*1 基因进行了靶向诱变获得斑马鱼 EOFAD 模型,且 *sorl*1 基因突变会影响线粒体和核糖体功能,提示 *SORL*1 基因与 AD 之间的潜在联系。线粒体功能障碍以及与线粒体粘度异常与多种神经系统疾病的发病机制有关。XIE 等[40]利用 MG2I 精准靶向斑马鱼神经元线粒体,MG2I 与激活氟化物的蛋白 dL5** 结合并暴露在远红光下时,会产生单线态氧。神经元线粒体内表达 dL5** 的转基因斑马鱼表现出严重的光依赖性神经行为缺陷,提示线粒体功能与神经系统疾病之间的直接关系。

另外,通过斑马鱼基因测序发现了更多与 AD 相关的基因位点。ERBABA 等[64]在斑马鱼发育的各个阶段(胚胎、成年、老年)分别对斑马鱼大脑分离的神经元进行 RNA 测序并与 AD 患者大脑样本测序结果进行比对,发现 AD 患者大脑中 GJC2 和 ALCAM 两个基因的表达与斑马鱼衰老进程中的表达结果一致,揭示大脑衰老过程中基因的表达变化,并为神经退行性疾病的治疗提供了新的靶点。缺氧引起的氧化应激对脑功能产生了严重影响,通过对缺氧和常氧条件下的斑马鱼 Fb、Mb 和 Hb 区域分别进行 RNA 测序,测序结果提示 Fb 和 Hb 区域的 lncRNA (adrb3b, cav1, stat3, bac2, apoeb, psen1, s100b)参与了 AD 的发病过程[41]。

3 斑马鱼在 AD 药物评估与筛选中的应用

研究人员针对 AD 的发病机制构建出斑马鱼 AD 模型,使斑马鱼表现出 AD 样病理学特征,为 AD 药物评估和筛选提供了有效模型。

3.1 斑马鱼在 AD 药物评估中的应用

多奈哌齐(donepezil, DPZ)是一种有效的乙酰胆碱酯酶抑制剂,临床上用于治疗神经退行性疾病的认知增强剂,常作为阳性药物进行 AD 药物效果评估。MUTHURAMAN 等[65]将斑马鱼在香烟烟雾下暴露 5 d,期间利用选择性内皮素受体拮抗剂安利生坦(Ambrisentan)和 DPZ 进行治疗,结果发现 Ambrisentan 与 DPZ 治疗组都表现出神经炎症减轻、神经递质功能的恢复。RISHITHA 等[66]发现槲皮素固体脂质纳米颗粒对戊四唑(pentylenetetrazole, PTZ)诱导的神经认知障碍具有减弱作用,效果与 DPZ 治疗组一致。SANG 等[67]通过化学合成含有芹菜素-多奈哌齐衍生物(apigenin-donepezil derivatives)、柚皮素-多奈哌齐衍生物(naringenin-donepezil derivatives)、染料木素-多奈哌齐衍生物(genistein-donepezil derivatives)和查尔酮-多奈哌齐衍生物(chalcone-donepezil derivatives)的物质 TM-4,结果提示 TM-4 在 $AlCl_3$ 诱导的斑马鱼 AD 模型中表现出显著提升的运动障碍恢复率和应答效率。TM-4 对 SCOP 诱发的记忆障碍有改善作用。通过转录组测序进一步确认 TM-4 对多靶点的调控。BOOPATHI 等[68]使用 DPZ、加兰他敏和白藜芦醇对铬离子(Cr^{4+})暴露诱导的斑马鱼进行治疗,结果显示 DPZ 和 Res 有效地保护斑马鱼免受 Cr^{4+} 诱导的焦虑和记忆障碍。

3.2 斑马鱼在 AD 药物筛选中的应用

BBB 是靶向治疗 AD 药物向大脑传递过程中的主要屏障,斑马鱼胚胎形成早期的 BBB 具有不完整性,因此在斑马鱼体内应用纳米颗粒药物,可以加快药物传递效率。JAVED 等[69]用酪蛋白包埋的金纳米颗粒(casein coated-gold nanoparticles, βCas AuNPs)对斑马鱼 AD 模型中 Aβ 的毒性进行了阻断,并证实 βCas AuNPs 恢复了斑马鱼的活动和认知能力。ZHOU 等[70]使用新型纳米载体碳点与柠檬酸和邻苯二胺相结合形成的纳米药物 Y-CDs 越过 5 日龄斑马鱼模型 BBB,抑制斑马鱼体内 Aβ 的产生。ANDRIKOPOULOS 等[71]用酪蛋白包埋的氧化铁纳米颗粒对 Aβ 诱导的斑马鱼胚胎进行治疗,有效抑制了 Aβ 的毒性。另外,SALEEM 等[72]对斑马鱼 AD 模型使用包埋白菊花素的壳聚糖纳米颗粒 Chr-Chi NPs 进行治疗,发现该药物有助于保持记忆、认知能力和增加突触连接,减少 Aβ 的聚集、神经元死亡和活 ROS 的产生。利用转铁蛋白修饰的介孔二氧化硅纳米颗粒 TF-MSNs 与已知的 AChE 活化剂 HI-6 偶联形成的纳米药物可以快速透过斑马

鱼 BBB,通过释放 HI-6 恢复大脑 AChE 活性,防止中毒引起脑损伤[73]。有研究人员使用斑马鱼进行毒性实验,证明脂质体包埋姜黄素形成的纳米药物无毒性,并能显著降低 SH-SY5Y 神经元细胞的氧化应激,发挥神经保护作用[74]。

斑马鱼模型的出现也让传统药物在 AD 治疗方面焕发出新的活力。西非传统药物塞内加尔吉耶尔木(Guiera senegalensis,GS)主要用于治疗癫痫和抑郁症等疾病。DAMO 等[75]研究发现 GS 可以改善 SCOP 诱导的斑马鱼记忆损伤,在脑氧化应激方面存在潜在益处,提示 GS 作为治疗 AD 等神经退行性疾病的潜力。杜仲(Eucommia ulmoides,EUO)作为一种传统中药被广泛用于治疗各种神经退行性疾病。SUN 等[76]从 EUO 雄花中提取相关物质 EUMF,并利用斑马鱼 AD 模型进行实验研究,结果发现 EUMF 能显著缓解斑马鱼运动障碍,抑制 $AlCl_3$ 诱导的斑马鱼头部 Aβ 沉积、减少细胞凋亡,机制研究表明 EUMF 可能通过抑制自噬基因的异常表达来改善 AD 样症状。LI 等[77]利用管花肉苁蓉(Cistanche tubulosa,CT)提取物中的 21 种成分证实管花肉苁蓉对 $AlCl_3$ 诱导的斑马鱼 AD 模型具有神经保护作用。麝香草(Thymus vulgaris L.)具有抗菌、抗氧化、抗炎、抗肿瘤等药用价值。CAPATINA 等[78]将 SCOP 诱导的斑马鱼记忆损伤模型连续 13 d 浸泡在梯度浓度的麝香精油(Thymus vulgaris essential oil,TEO)中,每天 1 次。结果发现 TEO 提高了 AChE 活性,并改善了 SCOP 诱发的斑马鱼健忘、焦虑等症状。

表 2　斑马鱼 AD 模型筛选 AD 治疗药物及其作用机制

Table 2　Screening of AD therapeutic drugs and their mechanism in zebrafish AD model

斑马鱼 AD 模型 Zebrafish AD model	AD 治疗药物 Medications for treating AD	作用机制 Action mechanism	参考文献 References
Tg(huc:eGFP)转基因斑马鱼 Tg(huc:eGFP) zebrafish line	桧木醇 Hinokitiol(Hino)	改善斑马鱼运动和神经发育缺陷 Improve motor and neurodevelopmental defects of zebrafish	[85]
–	嗜酸乳酸球菌 LAB4 和植物乳杆菌 LAB12 *Pediococcus acidilactici* LAB4 and lactobacillus plantarum LAB12	下调斑马鱼脑内 *appa* 基因的表达,改善空间学习和记忆能力 Down-regulating the expression of *appa* gene in zebrafish brain, improved spatial learning and memory ability	[86]
–	可替宁和 6-羟基-l-尼古丁 Cotinine and 6-hydroxy-l-nicotine	减轻斑马鱼焦虑样行为和记忆障碍,并降低斑马鱼大脑氧化应激和 AChE 活性 Alleviates anxiety-like behavior and memory disorders, reduces brain oxidative stress and AChE activity	[87]
东莨菪碱诱导的 AD 模型 Scopolamine induced AD model	马缨丹叶提取物 Lantana camara leaf extract	增强斑马鱼的学习记忆能力、减少脑内炎症反应 Enhance the learning and memory ability of zebrafish, reduce inflammation in brain	[88]
	芫荽精油 *Coriandrum sativum var. microcarpum* essential oil	减轻斑马鱼焦虑样行为和记忆障碍 Alleviates anxiety-like behavior and memory disorders	[89]
	野漆树苷 Rhoifolin	恢复胆碱能活性和改善大脑氧化应激 Restore cholinergic activity and improve brain oxidative stress	[90]
脂多糖和铝诱导的 AD 模型 AD model induced by lipopolysaccharideand aluminum	羊栖菜功能性精油 Hizikia fusiforme functional oil	降低脑内炎症,改善斑马鱼减轻 LPS/$AlCl_3$ 诱导的斑马鱼认知缺陷 Reduce brain inflammation, improve zebrafish and alleviate LPS/ $ALCL_3$-induced cognitive deficits	[91]
–	嗜黏蛋白阿克曼菌 *Akkermansia muciniphila*	改善和预防糖尿病及 AD 症状,改善斑马鱼的记忆、攻击和焦虑行为 Improving and preventing diabetes with AD improves memory, aggression, and anxious behavior in zebrafish	[92]
–	骨分化小分子 DIPQUO Osteoblast differentiation DIPQUO	抑制丝氨酸/苏氨酸激酶(GSK3-β),抑制 Tau 蛋白的激活 Inhibit serine/threonine kinase (GSK3-β), inhibit Tau protein activation	[93]
冈田酸诱导的 AD 模型 AD model induced by okada acid	4-苄基-2-甲基-1,2,4-噻二唑烷-3,5-二酮 4-benzyl-2-methyl-1,2,4-thiadiazolidine-3,5-dione	抑制丝氨酸/苏氨酸激酶(GSK3-β) Inhibit serine/threonine kinase (GSK3-β)	[94]

由于化学合成药物可以通过多靶点定向配体(multi-target-directed ligands, MTDLs)治疗策略治疗AD,因此利用斑马鱼模型对化学合成药物筛选受到广泛关注。KALSOOM 等[79]建立硒二唑类化合物库,证实了硒二唑类化合物可以减轻 Aβ 对胚胎期斑马鱼的毒性。HU 等[80]设计了 3-(4-氨基苯基)-香豆素系列衍生物,通过斑马鱼体内实验证实化合物 4m 对 AChE 的抑制作用最强,并可以改善 $AlCl_3$ 诱导的斑马鱼行为能力损伤。YANG 等[81]合成 3-芳基香豆素衍生物,发现其有潜力作为治疗 AD 的候选药物。化学合成药物多为 AChE 和丁酰胆碱(butyrylcholinesterase, BChE)的抑制剂。新型喹啉-邻氨基甲酸酯(uinoline-O-carbamate derivatives)衍生化合物 3f 可提高 ACh 水平并改善 $AlCl_3$ 诱导的斑马鱼 AD 模型的运动障碍和反应能力[82]。WANG 等[83]将 1,2,3,4-四氢异喹啉和苄基胡椒啶基团结合成肉桂酸(cinnamic acid hybrids)衍生化合物 4e, 4e 可显著提高斑马鱼 AD 模型的运动障碍恢复率和反应效率,该研究合理设计并合成了新的化合物 3d,结果表明化合物 3d 显著改善斑马鱼 AD 模型的运动障碍恢复率和应答效率,对 $Aβ_{1-40}$ 介导的斑马鱼血管损伤具有保护作用[83]。更重要的是,化合物 3d 在高达 2000 mg/kg 的剂量下没有明显的急性毒性,并改善了 SCOP 引起的记忆损伤[84]。表 2 总结了近年来基于斑马鱼 AD 模型筛选的 AD 治疗药物及其作用机制。

4 结论与展望

斑马鱼等小型鱼类是目前广泛应用于神经生物学研究的动物模型,在揭示神经退行性疾病如 AD 等的发病机制方面表现出诸多优势。目前利用斑马鱼模型进行 AD 药物筛选与评估主要集中于传统药物功效、纳米药物研发、化学药物合成等方面。随着我国人口老龄化的加剧,AD 患病率呈逐年上升的趋势,AD 药物研发面临着巨大挑战。斑马鱼模型在昼夜节律、学习记忆能力等方面表现出较好的应用价值,但是目前应用仍然存在一定的局限性,如斑马鱼虽然具有一定的社交行为但与人类相比行为过于简单,且在药物开发方面很难确定斑马鱼对药物的吸收以及利用率。此外,由于 AD 的发病时间、发病机制等过于复杂,在利用斑马鱼进行 AD 研究的过程中很难按照临床标准对斑马鱼中的 AD 表型进行区分,目前还难以针对临床上的不同 AD 分型进行深入研究。随着技术的不断进步,斑马鱼模型有望更多地应用于阐明临床上不同分型 AD 的发病机制及相关药物评价和筛选,在 AD 研究中发挥重要作用。

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