DOI:10.3724/cbls.2026070 **CSTR**:32203.14.cbls.2026070
文章编号:1004-0374(2026)04-0677-12

仇子龙,上海交通大学特聘教授、博士生导师,医学院松江研究院副院长,国家杰出青年科学基金获得者,科技部中青年科技创新领军人才,中组部“万人计划”入选者,中国神经科学学会张香桐青年科学家奖获得者。仇子龙教授在神经发育疾病尤其是孤独症的神经机制与基因治疗领域深耕十余年,取得了一系列突破性成果。他建立了世界首个孤独症非人灵长类动物模型(*Nature*, 2016),该成果入选科技部“中国科学十大进展”。在脑内基因编辑技术方面,他在孤独症小鼠模型中实现致病突变的精准修复与行为逆转(*Nature*, 2026),为基因治疗的临床转化奠定了坚实基础。他在*Nature*等国际顶尖期刊发表论文数十篇,累计引用超过7 500次。作为国家重大科技专项“脑科学与类脑研究——孤独症基因编辑治疗”首席科学家,他主持和推动孤独症基因治疗临床研究,正牵头设计针对孤独症核心症状的临床试验方案,已与广州市妇女儿童医疗中心合作,完成了亚洲最大规模的Rett综合征IIT临床研究,填补了国内儿童神经发育障碍罕见病基因治疗临床空白,并推动建立全球规模最大的孤独症基因治疗研究中心。

跨越血脑屏障:AAV 基因疗法在中枢神经系统疾病中的进展与前沿

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摘 要:腺相关病毒(adeno-associated virus,AAV)凭借安全性高、持久表达与多样的血清型组合,已成为治疗中枢神经系统(central nervous system,CNS)疾病的核心载体。本文系统综述AAV基因疗法的生物学基础、装载与表达策略、给药途径与免疫学问题;总结其在阿尔茨海默病、帕金森病等常见神经退行性疾病,及以Rett综合征、黏多糖贮积症、亨廷顿舞蹈症等为代表的罕见神经发育与遗传性脑病中的最新临床与转化进展;重点讨论“面向CNS的AAV衣壳筛选如何确定与最新进展”,以及“靶向全脑 *vs* 特定脑区,面向特定神经细胞类型精准递送”的最新策略,包括跨物种选择、受体靶向的理性设计、增强子/启动子与miRNA调控、聚焦超声辅助递送等;最后,评述AAV递送碱基与引导编辑工具在脑内的应用态势、脱靶与安全性挑战,并提出面向临床的未来方向。本文力求以规范的引文与最新证据为依据,为CNS疾病基因治疗临床研究与产业化提供参考。

关键词:腺相关病毒;神经退行性疾病;靶向递送;基因编辑;临床转化
中图分类号:Q78;R450;R741 **文献标识码**:A

Crossing the blood–brain barrier: Advances and frontiers of AAV gene therapy in central nervous system disorders

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Abstract: Adeno-associated virus (AAV) vectors have emerged as a leading platform for gene therapy targeting central

收稿日期:2025-09-30; **修回日期**:2025-12-03
基金项目:国家自然科学基金项目(2025ZD0214700,82430046,32571649,82201632)
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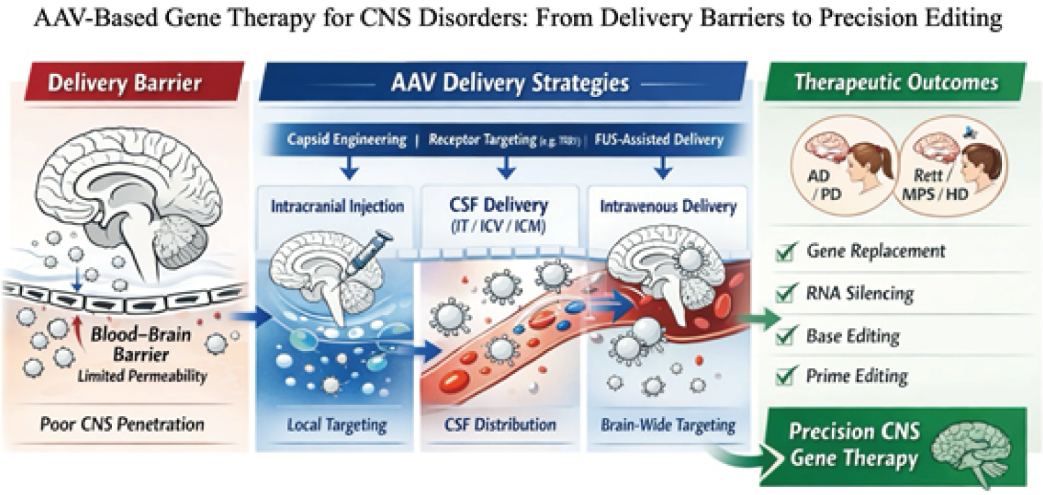
nervous system (CNS) disorders; however, efficient, safe, and scalable delivery across the blood-brain barrier (BBB) remains a central challenge for clinical translation. This review provides a comprehensive and forward-looking synthesis of recent advances in AAV-based CNS gene therapy, focusing on delivery strategies, disease applications, and genome editing technologies, while highlighting key translational factors influencing clinical outcomes. We first outline the biological properties of AAV vectors, including capsid diversity, genome packaging constraints, and regulatory elements that govern transgene expression and durability. We then compare major routes of administration—direct intracranial delivery, cerebrospinal fluid (CSF)-mediated approaches, and systemic intravenous injection—highlighting their advantages and limitations in achieving brain-wide versus region-specific transduction. In addition, we discuss key parameters affecting *in vivo* performance, including dose, distribution, and tissue tropism. Special emphasis is placed on recent advances in capsid engineering, including *in vivo* directed evolution, receptor-guided rational design, and cross-species validation strategies that aim to bridge long-standing translational gaps between rodent models and nonhuman primates or humans. We next summarize therapeutic progress across major CNS disorders, including neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, as well as monogenic neurodevelopmental and lysosomal storage disorders exemplified by Rett syndrome and mucopolysaccharidosis, together with emerging clinical insights from ongoing trials. In parallel, we describe the integration of AAV delivery with genome editing modalities—particularly base and prime editing—which offer precise and potentially safer alternatives to conventional gene replacement strategies, especially for diseases driven by defined genetic variants. Finally, we outline key translational bottlenecks, including host immune responses, dose-dependent toxicity, limited packaging capacity, and the need for improved cell-type specificity and regulatory control. We propose a framework for next-generation CNS gene therapy centered on "human receptor-guided capsid design, multi-layered transcriptional regulation, and precision genome editing". Together with advances in scalable manufacturing, quality control, and regulatory standardization, these developments are expected to improve safety, enhance delivery efficiency, and support more durable therapeutic outcomes. This review provides an integrated perspective to guide future research and facilitate clinical translation of AAV-based therapeutics for CNS disorders.



Key words: AAV; neurodegenerative diseases; targeted delivery; gene therapy; clinical translation

中枢神经系统(central nervous system,CNS)疾病包括神经退行性疾病、神经发育障碍、神经代谢病和罕见遗传病等,在全球范围内造成巨大医疗与社会负担。随着人口老龄化加剧,阿尔茨海默病、帕金森病等高发神经疾病患者数量持续上升,治疗需求愈发迫切。然而,受血脑屏障(blood-brain barrier,BBB)限制,传统小分子和大分子生物制剂难以有效进入脑组织,显著制约疗效。

近年来,基因治疗成为CNS疾病干预的重要方向,其中腺相关病毒(adeno-associated virus,AAV)因其安全性高、表达持久、可通过工程化实现组织特异性而备受关注。2019年以AAV9静脉注射为基础的Zolgensma获批用于脊髓性肌萎缩症(spinal muscular atrophy,SMA),奠定了CNS基因治疗的临床可行性基础[1-3]。此后,AAV基因疗法在CNS疾病中快速推进,全球已有数十项临床试验验证其疗效潜力。与此同时,AAV仍面临装载容量、免疫应答、跨物种差异及剂量相关毒性等挑战。为解决这些难题,研究者持续开发新型AAV衣壳、受体介导的跨BBB策略、物理辅助通道,以及更适配AAV的小型碱基编辑器、增强子与miRNA调控系统,以实现更精准、安全、高效的CNS疾病治疗。

本文将系统综述AAV在CNS疾病中的应用进展,重点聚焦跨BBB策略、代表性疾病的治疗探索、与基因编辑工具的结合、免疫学挑战及未来产业化与监管趋势,以期为研究和临床转化提供参考。

1 AAV 基因疗法的生物学基础

1.1 AAV 的结构与特征

AAV属于细小病毒科依赖病毒属,为无包膜、直径约20~25 nm 的单链DNA病毒,不具备自身复制能力,需依赖腺病毒、疱疹病毒等辅助。其基因组约4.7 kb,由反向末端重复序列(inverted terminal repeats,ITRs)和两个主要开放阅读框(Rep与Cap,分别负责复制与衣壳装配)组成,通过在 ITRs 之间插入外源序列可构建重组AAV(recombinant AAV,rAAV)载体。相较慢病毒、腺病毒、单纯疱疹病毒与脂质纳米颗粒(表1),AAV主要以非整合环状形式存在于细胞核内,插入突变风险低、免疫原性较弱,尤其适用于非分裂细胞(如神经元)的长期表达,其多血清型组织嗜性为CNS疾病基因治疗奠定了重要基础[4-6]。

表1 CNS疾病基因治疗领域AAV 与其他递送工具的比较

Table1 Comparison of AAV and other delivery platforms for CNS disorders gene therapy

分类	优势	限制
慢病毒	装载容量高(8~10 kb)	存在基因组整合风险
腺病毒	高水平瞬时表达	免疫原性强
单纯疱疹病毒	嗜神经,容量大	安全性与稳定性不足
脂质纳米颗粒	mRNA递送强, 高瞬时表达	跨BBB效率有限

1.2 AAV的表达与载荷

AAV的表达受启动子、调控元件及载荷容量限制。天然单链 AAV(single-stranded AAV,ssAAV)的载荷上限约为4.7 kb,超过后滴度与表达稳定性会明显下降。这推动了双 AAV系统的发展,其通过转录/转译拼接或mRNA转剪接技术(RNA editing for variable exon recognition and targeting,REVeRT)重构大基因或多组分编辑系统,解决载荷上限问题[7-9]。为提升表达效率,研究者开发了自互补AAV(self-complementary AAV,scAAV),它通过改写 ITR 序列,使病毒基因组以双链形式包装,可直接进入转录阶段,大幅提升表达速度[10]。ssAAV与scAAV在表达动力学、载荷能力和适用场景上互补(表2)。随着更高比例"正确基因组"的包装工艺、降低细菌序列交叉包装等质控优化[11],AAV的递送效率与可预测性持续提高,进一步拓展了其在中枢复杂疾病中的应用潜力。

表2 ssAAV与scAAV的比较及发展趋势

Table 2 Comparison and development trends of ssAAV and scAAV

分类	ssAAV	scAAV
基因结构	ssDNA,需互补链合成	dsDNA,可直接转录
表达速度	慢	快
载荷容量	4.5~4.7 kb	2.2~2.5 kb
适用基因	中大基因长期表达	小基因快速起效
适用场景	各类细胞,适合长期表达	非分裂细胞优势显著
使用限制	表达延迟,分裂缓慢组织表达低	载荷减半

1.3 跨BBB递送策略与CNS到达

BBB由内皮细胞、基底膜、星形胶质细胞构成,其高选择性极大限制病毒与大分子进入CNS。AAV进入CNS主要依赖直接给药与静脉给药两类策略。其中,AAV直接给药(如脑实质立体定向注射、脑脊液途径、椎管内IT、枕大池注射ICM、脑室内注射ICV、鞘内注射)可绕开BBB,在局部或脑脊液系统获得稳定且高效的转导,已在 SMA等疾病的批准或后期临床试验中验证其可行性[1],但局限在于侵入性强。AAV静脉给药更具临床吸引力,但需解决跨BBB效率不足的问题。随着衣壳工程的突破,多种跨BBB衣壳(AAV-PHP.eB、AAV.CAP-B10 系列等)显著提高了皮层、海马等区域的转导效率[12,13]。伴随跨BBB受体机制的逐步明确[14,15],静脉给药具有全脑均一分布,无需颅内操作的优势,进一步推动了静脉给药向全脑分布与临床应用发展。不同给药路径在全脑覆盖、脊髓与背根神经节暴露、外周器官暴露与安全性之间存在权衡,需要载体与剂量、体积与流速的系统优化[16-18]。

1.4 免疫学与安全考量

AAV 神经嗜性与长期表达优势使其成为 CNS疾病基因治疗最核心的载体,但其外源衣壳及先天免疫因素会显著影响疗效与再给药可行性。AAV衣壳抗原性[19]、患者中和抗体[20]以及脑内递送后逆向运输可触发体液与细胞免疫,引发转氨酶升高、炎症反应及 $CD8^+$ T 细胞介导的表达下降等剂量相关毒性,这类免疫相关不良事件已在SMA及帕金森病等多项临床研究中被系统报道[21-23]。较高剂量静脉给药中还可见补体激活、肝毒性等安全隐患,背根神经节(dorsal root ganglion,DRG)神经毒性在非

人灵长类动物(non-human primates,NHP)及大动物研究中受关注,神经丝轻链蛋白(neurofilament light chain,NfL)等神经损伤标志物及免疫谱系分析逐渐成为安全监测和伴随诊断的重要手段[1-3,16,24,25]。总体而言,通过衣壳工程、剂量控制与递送路径优化,AAV在CNS疾病中的应用正不断突破瓶颈,并加速向临床转化。

2 面向脑疾病的AAV基因治疗进展

在多数神经退行性与遗传性脑病中,现有治疗仍以对症为主,难以逆转病程。小分子药物多仅短期改善症状,难以阻止神经元丢失;抗体类药物受限于BBB穿透率低及长期高剂量风险;酶替代疗法难进入CNS,无法改善发育性损伤。因此,AAV基因疗法因其在CNS中的长期表达与靶向递送优势,被视为最具病程修饰潜力的策略,近年来多个项目已取得临床进展。

2.1 常见神经退行性疾病

2.1.1 阿尔茨海默病(AD)

AD是最常见的,以进行性认知功能障碍为特征的中枢神经系统退行性病变,全球约有 5 000 万人罹患该病。AD的病因包括早发家族型,由APP(amyloid precursor protein)、Presenilin 1/2等基因突变直接驱动的β-淀粉样蛋白(Aβ)生成异常,和散发型,受载脂蛋白E (apolipoprotein E,APOE)基因型和代谢因素影响所致的Aβ代谢与清除障碍,核心病理包括Aβ沉积、Tau 蛋白异常磷酸化与突触退化[26-28]。其中,神经生长因子(nerve growth factor,NGF)、脑源性神经营养因子(brain-derived neurotrophic factor,BDNF)可激活 Trk 受体相关信号通路,增强神经元抗凋亡能力,延缓突触和神经元丢失[29,30]。基于此通路的治疗研究中,AAV2-NGF在前脑底核区立体定向输注的多中心试验证实安全可行但疗效有限,提示需优化靶区覆盖[31,32];AAV2-BDNF临床正在进行(NCT05040217),动物与NHP实验证实对内嗅皮层-海马回路具有保护作用[33]。

APOE作为散发型AD的主要风险因子,其不同亚型(APOE2、APOE3、APOE4)分别参与AD的发生及预防。APOE2对AD有预防作用,APOE4会增强AD发病风险,患者同时携带两个APOE4的患病风险会提高9~15倍,显著加重患者Aβ和Tau的病理表型[34-36]。AAV-APOE2能调控小胶质细胞功能,促进迁移和吞噬,增强 Aβ 的细胞清除[37];APOE2通过改善CSF流动和脑膜淋巴功能,提高 Aβ 清除效率,并影响Tau相关表型[38-40]。临床上LX1001(AAV-APOE2)在APOE4纯合AD患者中显示 CSF中APOE2剂量依赖性上升,并伴随磷酸化Tau标志物下降,安全性良好[41,42]。

2.1.2 帕金森病(PD)

PD以多巴胺能神经元丧失与α-突触核蛋白聚集为特征,伴随神经炎症、线粒体功能障碍与轴突运输受损。AAV可用于长期表达神经营养因子或重建多巴胺合成。在神经营养因子治疗方案中,AAV2-GDNF在部分研究中改善运动症状,但大规模试验未达终点,提示递送效率与病程分层是关键[43-45]。在多巴胺合成重建方面,AAV递送酪氨酸羟化酶(TH)、芳香氨基酸脱羧酶(AADC)和鸟氨酸脱羧酶(GCH1)等以恢复多巴胺合成,代表性方案AAV2-GAD II 期试验显示良好安全性并改善运动症状,是回路调控型基因治疗的重要证据[24,25],AADC 补充疗法已进展至临床III期[46]。此外,以病因修饰为目标的AAV9-GBA1(PR001/LY3884961)采用枕大池/CSF给药,正在开展GBA-PD人群早期临床(NCT04127578),多中心长期随访进行中。AAV在PD治疗领域已取得较早临床突破,优势在于可对基底核、纹状体等特定回路实现定点、长期的分子修饰,从而直接作用于疾病回路而非短效系统药物难以精确覆盖的靶点,但疗效稳定性与患者异质性仍是难点,未来或需与细胞及免疫疗法结合。

2.2 罕见遗传及神经发育性脑疾病

2.2.1 黏多糖贮积症IIIA(MPS IIIA, Sanfilippo A)

MPS IIIA是一种常染色体隐性遗传病,核心病因在于溶酶体内肝素硫酸(heparan sulfate,HS)降解途径的阻断,其中SGSH(N-sulfoglucosamine sulfohydrolase)和SUMF1 (sulfatase modifying factor 1)是决定HS是否能有效分解的关键酶分子。SGSH的缺陷会导致HS无法进入后续的分解流程,从而在神经元和胶质细胞内逐渐积累,导致白质退变与认知受损,进而造成显著的学习与行为倒退。患儿通常在3~6岁进入快速认知衰退期,语言、适应行为与运动功能均迅速下降,AAV递送正常酶基因可部分恢复代谢功能[47]。

早期 AAVrh.10-SGSH/SUMF1颅内多点注射 I/II 期试验(NCT01474343)在24~48个月随访中显示良好

耐受性,并提示认知退化显著放缓,患儿2年内VABS (Vineland adaptive behavior scales)下降速度减少约40%~60%,部分早期治疗儿童的DQ(developmental quotient)年下降<10,个别病例在30~48个月仍维持语言与社交功能稳定,明显优于自然病程[48,49]。后续的 scAAV9(ABO-102/UX111)静脉或CSF给药亦获得一致信号,治疗后CSF HS降低30%~70%,且伴随认知改善(12~36个月)[48-50]。总体来看,AAV基因疗法可显著延缓MPS IIIA的中枢退化进程。

2.2.2 Rett综合征

Rett综合征由MECP2(methyl-CpG binding protein 2)基因突变引起,该基因剂量敏感性高,过表达存在风险,因此剂量敏感性促使“表达自调控”成为核心设计目标。TSHA-102(scAAV9-miniMECP2)引入基于microRNA的自适应调控元件(microRNA-responsive autoregulatory element, miRARE)实现自适应调控,早期临床披露低剂量安全可耐受,并见跨域量表改善初步信号[51,52]。

2.2.3 亨廷顿舞蹈症(HD)与脊髓小脑共济失调(SCA)

HD和SCA疾病均为CAG重复扩增导致PolyQ蛋白病,伴随蛋白聚集毒性与线粒体功能障碍。RNA干预方面,AAV递送miRNA/shRNA可持久降低突变毒性蛋白的表达,改善小鼠表型[53-55];基因编辑方面,AAV递送编辑工具可靶向致病等位基因,部分恢复疾病模型的运动与神经表型[56]。

2.2.4 肌萎缩侧索硬化(amyotrophic lateral sclerosis, ALS)

ALS部分病例与SOD1、C9orf72突变相关。SOD1靶向AAV9-shRNA 在小鼠中可延缓发病并延长寿命[57,58];AAVrh10或AAV9在成人模型经脊髓或IV给药也显示改善运动与呼吸功能,部分已进入早期临床[59,60]。C9orf72的G4C2六核苷酸扩增可致毒性RNA聚集与多肽DPRs(dipeptide repeat proteins),AAV-CRISPR在近年的预临床研究中疗效显著,但尚需解决递送与免疫风险难题[61]。

3 AAV递送的基因编辑策略在遗传性脑病中的进展

3.1 从体内证据出发:小鼠到NHP

碱基编辑作为当前基因工程的前沿技术,其通过脱氨酶介导的胞嘧啶和腺嘌呤脱氨,作为直接修改DNA和RNA中单个碱基的精准方法,为体内 CNS 疾病治疗提供较高安全性与可控性[62-65]。在小鼠中,AAV递送的编辑器可在大脑、肝脏、心脏等组织长期表达并获得稳定编辑,显著改善遗传性听力缺陷、代谢病、三核苷酸重复扩增病等模型表型[66-74]。这些研究在小鼠层面为确定有效剂量、安全窗口、脱靶风险及免疫反应提供了系统性证据[56,75-77]。

在NHP中,AAV介导的碱基编辑可在大脑皮层、丘脑、肝脏与眼组织实现数月至一年以上的稳定表达,并获得具有生物学效应的编辑效率[78]。但也暴露出数个临床转化瓶颈,包括大剂量引发的肝毒性与免疫反应、不同脑区和个体间的效率差异,以及编辑器体积超出AAV载量的限制等[79-81]。构建跨层级证据体系,对于后续 I 期试验的剂量设计、安全评估及递送策略优化至关重要。

3.2 朊蛋白病(PRNP)的编辑策略

表观遗传编辑(CRISPR-based epigenetic activation/repression modulation,CHARM)在小鼠脑内通过AAV递送可显著下调朊病毒蛋白表达,呈现疾病修饰潜力[59];进一步的体内DNA碱基编辑策略实现了朊病毒蛋白的永久敲低,并延长人源化模型的生存时间,将该策略推向更贴近临床转化的层级[66,82]。

3.3 Prime editing 在脑内的递送策略

先导编辑技术(prime editing,PE) 作为一种写入式基因修复的编辑技术,能够在无需外源供体模板的情况下,实现小片段碱基序列的替换,在编辑多样性上优于传统的DNA碱基编辑方案[83]。因CNS神经元高度不可再生、对DNA断裂高度敏感,PE系统可显著降低神经毒性及染色体重排风险[84]。在递送策略上,PE系统需使用双AAV重组体系进行递送,最新研究已验证AAV-PE 可实现小鼠与NHP脑内基因矫正,并在神经元中维持数月至一年以上的表达,展示其应用的可行性[68]。

3.4 Rett综合征致病突变的碱基编辑进展

Rett 综合征由MECP2基因X连锁功能缺失突变导致,极适合点突变纠正策略。AAV编辑在小鼠Rett 模型中已被证明可部分恢复 MECP2表达,改善运动功能、焦虑行为和寿命,并显著降低神经炎症与突触异常[85]。改造的Nme2-ABE在体外可精准更正两种常见MECP2致病突变,并在体内检测到编辑活性[69]。相较传统基因替代策略,碱基编辑可避免

MECP2过表达毒性,并允许等位基因选择性矫正[86]。但仍面临AAV递送在脑内分布不均、旁观者编辑、RNA 脱靶以及人类/小鼠调控差异带来的转化挑战,仍需NHP与脑类器官进一步验证。

3.5 风险边界:脱靶与结构变异

虽然碱基编辑避免双链断裂,但仍需关注序列依赖性脱靶与潜在结构变异风险。通过全基因组测序与单细胞多组学解析碱基编辑器的结构变异与转录扰动,将为临床前安全性评估建立必要基线[87]。

4 新增专题一:靶向CNS疾病的AAV衣壳筛选——方法学与里程碑

4.1 体内定向进化与CRE依赖选择(CREATE)

CNS定向衣壳筛选一直是跨 BBB 递送的核心挑战。2016年提出的CRE-dependent AAV targeted evolution (CREATE)方法,利用在目标细胞中表达Cre重组酶的小鼠模型,将含loxP-STOP-loxP条形码的AAV衣壳文库进行体内进化,实现了“活体中以功能为导向”的筛选模式,成为AAV工程的重要里程碑[88]。

4.2 受体谱系与物种特异性:从PHP.B到LY6A/LY6C1

AAV-PHP.B/PHP.eB能在小鼠中极大提升全脑转导效率,显著降低所需剂量和外周毒性,是跨BBB工程化的标志性成果[12],但效果依赖小鼠特异的 LY6A/LY6C1与PHP.B的结合,导致在大鼠和NHP中难以复现[15]。基于受体理解的理性筛选已生成在缺乏LY6A的小鼠系仍有效的PHP.eB,并通过NHP体内条形码筛选获得适用于CSF到达路径的新衣壳[14,89],减少了跨物种失配风险。

4.3 面向人的理性设计:转铁蛋白受体TfR1靶向的突破

2024年,靶向人TfR1的BI-hTFR1在“人TFRC KI”小鼠中实现40~50倍的 CNS增强,并显著提升GBA1酶活,提示“人受体靶向+体内验证”的可转化路径,是推进成人CNS疾病基因治疗的关键进展[90]。

4.4 跨物种与NHP优先的筛选

AAV-PHP虽在小鼠中高效,但在NHP中受受体表达、免疫与剂量窗口影响而性能较差[89]。针对CSF路径或BBB跨越能力,直接在NHP中进行筛选与富集可减少跨物种失配风险,但该策略筛选成本较高;未来可结合结构生物学与机器学习实现更精确的衣壳优化,增强衣壳的跨物种表现[91]。基于NHP的体内选择已获得“按需到达脊髓”的CSF递送衣壳,为脊髓疾病提供了可转化工具[14]。

4.5 靶向小胶质细胞与困难细胞型

在CNS内部,不同细胞类型的转导效率差异显著。AAV对小胶质细胞天然难以转导,其原因包括吞噬清除强、受体缺乏、染色质阻遏和神经元竞争性吸附[92-94]。因此,目前能实现小胶质细胞表达的AAV多依赖 Cre系统,临床转化价值有限。未来的解决方案包括基于小胶质细胞受体的衣壳定向进化、特异受体新靶点设计,以及基于合成启动子和进化型增强子策略开发非 Cre 依赖的基因调控系统;并利用AAV-LNP 混合或完全非病毒递送系统,结合以吞噬信号为基础的主动摄取策略,有望突破小胶质细胞难以转导的长期瓶颈[81,93]。当前成功案例(如AAV-MG)多停留在小鼠水平,其跨物种可行性与临床转化潜力仍有待进一步评估[95]。

总体而言,CNS衣壳工程应遵循“人受体可用性验证,跨物种及人源化模型验证,NHP功能验证,药代、免疫、安全并行评估”的管线化思路,这不仅有助于避免因鼠类特异性受体而带来的转化偏差,也为未来从基础筛选到临床试验的无缝衔接提供了可操作的路线图[14,15,90,95]。

5 新增专题二:靶向“全脑 vs 特定脑区 vs 特定细胞类型”的最新策略

5.1 全脑靶向

静脉给药:AAV9在婴幼儿的SMN1中证明了跨脑递送可行性[1],但成人BBB更成熟与致密,以及个体差异导致的衣壳不稳定、人体免疫和外周毒性,导致了全脑转导效率的下降。低免疫原且跨物种保守的受体靶向衣壳(如 TfR1 定向 AAV)为成人全脑递送提供新方案[90]。CSF给药:可覆盖脑表面与脊髓,但分布取决于受体、脑脊液比例、注射体积/流速及解剖层级,动物到人的比例转换仍是核心瓶颈[3,17,18]。FUS辅助给药:FUS-微泡可实现可逆BBB开窗,增强局灶或半脑级入脑能力,并在PD与AD患者中有早期临床探索[96-99]。

5.2 特定脑区靶向

对于PD、MPS 等疾病,需对深部核团进行高浓度局部递送。立体定向注射及对流增强输注等策略

已在多项AAV临床研究中得到应用(如AAV2-GAD、AAV-AADC及MPS IIIA多点注射),并呈现良好分布可行性[24,46,48]。相比于开颅手术路径,特定脑区靶向覆盖范围受限,灌注压力与速率若控制不当会造成组织损伤或回流。聚焦超声(focused ultrasound, FUS)为"非侵入式区域靶向"提供潜在替代方案,并在NHP中已能将AAV9定向导入目标脑区,为未来实现"开颅手术替代"提供了可行路径[96]。

5.3 特定细胞类型靶向

细胞类型特异启动子(如神经元特异性启动子hSyn、新皮质和海马体中兴奋性神经元特异性启动子CaMKIIα、星形胶质细胞特异性启动子GFAP)可实现对细胞类型的粗粒度限制,但存在神经元或少突胶质细胞漏表达,核心原因包括AAV自身神经元偏好、剂量相关背景表达、星形胶质细胞异质性等(表3)。

表3 星形胶质细胞启动子的表达分析

Table 3 Expression analysis of astrocyte-specific promoters

启动子名称	优点	缺点	泄漏程度
GFAP	表达强,覆盖广	序列长;受炎症状态影响大	神经元表达泄漏
GfaABC1D	截短型GFAP,特异性好	表达活性弱于GFAP	泄漏低
ALDH1L1	表达稳定,特异性好	序列长,表达弱	泄漏低
GLAST	部分脑区特异性高	序列长,表达弱	泄漏低

针对这些难题,可以采用诸如工程化细胞类型特异AAV衣壳,设计启动子/miRNA开关等多层级调控系统等策略,改善AAV在星形胶质细胞中的精准表达。工程化AAV衣壳的最新趋势是将受体靶向衣壳(如BI-hTFR1)与细胞类型特异启动子/增强子进行正交组合,有望在成人中实现"全脑入脑,及细胞类型精确表达"的两级选择,从而降低剂量并提升治疗指数,尚需在NHP及人源化模型中进行严格验证[90,100,101]。miRNA靶位去靶向的策略是在转基因3′UTR植入特定miRNA靶位(可以利用宿主组织的内源性miRNA实现"负调控",从而在非目标组织(如肝脏、造血系统)中沉默转基因表达),有效提升安全窗口。例如,Rett综合征中的miRARE技术展示了对剂量敏感基因的动态反馈抑制[51]。此外,近期增强子AAV资源库的开发进一步拓展了精细调控工具箱,使研究者能在神经元亚群水平(如皮层兴奋/抑制亚型)实现更高分辨率的调控[51,100-102]。

6 挑战与未来方向

6.1 递送策略及免疫学约束

CNS递送中的全身给药与CSF给药(IT/ICM/ICV)在剂量上限、外周暴露与免疫反应上各有侧重,因此建立可控的免疫学策略已成为 AAV 临床应用的核心。当前研究重点在于降低衣壳免疫原性,包括通过定点突变关键表位减少中和抗体结合、利用 PEG 化或糖基化进行表面遮蔽[15,103],以及采用交叉包被或嵌合衣壳的方式筛选能逃逸既有免疫识别的新型 AAV 衣壳[19,104-107]。此外,结合短程免疫调节方案(如类固醇、利妥昔单抗)以及通过血浆置换或IgG裂解酶去除中和抗体,也可提升首剂给药效率并改善重复给药的可行性[16-19,108,109]。整体而言,去免疫化衣壳设计正成为提升CNS基因治疗安全范围和治疗成功率的关键方向,并有望在未来显著扩展AAV在脑疾病中的适应证与治疗深度。

6.2 安全性与监测

脑内 AAV 治疗的安全评估需综合考量局部注射创伤、免疫原性、剂量相关毒性、过量表达导致的神经毒性以及长期基因组学后果。其中,与注射操作以及局部外源蛋白表达相关的药物递送风险,如注射相关损伤与脑内压力改变和免疫与炎症反应需要重点监测[18,110]。高剂量相关的补体激活与肝毒性、背根神经节毒性和神经炎症与非目标组织脱靶表达需纳入伴随诊断与生物标志物监测,具体包括血清NfL、影像学(MRI、PET)及免疫谱监测等。在开发阶段充分开展大动物GLP研究,以提前识别安全风险并指导剂量窗口[3,18,111],对于在脑内长期表达的药物,需要监测脱靶转导和表达插入性突变与长期肿瘤风险[18,103,112]。只有在完善的风险识别、工程学优化与严密的临床监测体系三管齐下的前提下,AAV在脑疾病中的安全转化才能得到最大化保障。

6.3 衣壳工程的"以人受体为中心"范式

未来AAV衣壳迭代有望以"人BBB受体"为锚点(如TfR1),辅以跨物种人源化验证和NHP药代药效评估来确保转化可行性,避免"鼠特异性受体"带来的失配。同时,结合FUS-BBB可逆开窗等手段,减少对超高剂量的依赖,从而提升安全性与成本可控性[14,15,90,95-99]。

6.4 精准编辑的CNS转化路径

碱基编辑与引导编辑工具在神经系统中的应用正在进入实用阶段,随着高灵敏度脱靶检测和编辑效率评估工具链的逐步成熟,CNS中“编辑器-AA递送-NHP验证”的技术路径正成为新趋势。针对重复扩展类疾病(HD、SCA)与显性致病错义突变(Rett、Prion)的治疗最有可能率先进入临床转化[66-69,74-76,82,87]。

6.5 产业化与规范化

CNS疾病基因治疗的可及性不仅依赖科学突破,也取决于生产与监管体系的成熟度。生产一致性(AAV阳性颗粒比、基因组完整性、批次间差异)、质量方法学与可比性研究、全球监管对再给药与长期随访要求以及支付模式的建立,将决定CNS疾病基因疗法的未来产业化进程[11,16,18]。

7 结语

AAV基因疗法在CNS领域正从最初的“能否到达”迈向“精准到达、精准表达、精准编辑”的新阶段。以人受体为锚的衣壳工程、结合FUS物理开窗与剂量优化,叠加转录与转录后层级调控逻辑与紧凑编辑器的组合应用策略,将在成人全脑疗法与局灶核团疗法之间建立新的平衡。对AD、PD等神经退行性疾病的治疗修饰、对Rett综合征、MPS、SCA等罕见病的早期干预和根治,将在未来5~10年迎来里程碑式的临床验证,推动CNS疾病基因疗法进入真正的“精准神经基因治疗”时代。

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