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·评述与综述·

张毅,郑州大学第一附属医院生物细胞治疗中心主任、教授,主要从事肿瘤细胞与免疫治疗的基础研究及临床转化,带领团队先后主持国家级等项目60余项及69项临床试验,多项创新技术成果实现重大临床转化,获得中国侨界贡献奖及多项省部级以上科技奖项。担任中国免疫学会肿瘤免疫和生物治疗分会主任委员,连续入选全球前2%顶尖科学家, 发表SCI论文300篇,获授权专利17项。

体内CAR-T细胞疗法的研究进展与挑战

黄逸凡,张 毅*
(郑州大学第一附属医院生物细胞治疗中心,郑州 450052)

摘 要:嵌合抗原受体T细胞(CAR-T)疗法是肿瘤免疫治疗的革命性技术,其存在体外操作复杂、成本高、治疗周期长等局限性。体内CAR-T细胞疗法作为一种新型的肿瘤免疫疗法,正在成为癌症治疗领域的研究热点。T细胞可以通过递送载体直接在体内诱导产生具有抗肿瘤活性的CAR-T细胞。随着基因递送系统、基因编辑技术和CAR结构的不断优化,体内CAR-T疗法在安全性、有效性和临床转化方面都取得了重大进展。本文回顾了利用载体技术进行体内CAR-T治疗的主要优势以及相关研究进展,并讨论了体内CAR-T疗法面临的挑战,以期为临床应用提供参考。

关键词:体内CAR-T细胞疗法;免疫治疗;基因递送
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Research progress and challenges in *in vivo* CAR-T cell therapy

HUANG Yi-Fan, ZHANG Yi*
(Biotherapy Center and Cancer Center, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China)

Abstract: Chimeric antigen receptor T-cell (CAR-T) immunotherapy has emerged as a revolutionary technology in cancer treatment, demonstrating exceptional efficacy particularly in hematologic malignancies. However, traditional *ex vivo* CAR-T therapies still face significant limitations, including complex preparation processes, high production costs, lengthy preparation cycles, and the requirement for patients to undergo lymphodepletion pretreatment, which restrict their widespread clinical accessibility. As a highly promising innovative strategy, *in vivo* CAR-T cell therapy delivers gene drugs directly within the body to reprogram the patient′s own T cells in situ, offering the potential to fundamentally resolve these challenges. This paper comprehensively reviews the latest research advances in *in vivo* CAR-T therapy, focusing on key technological breakthroughs in the two core delivery platforms: viral and non-viral. Regarding viral vectors, lentivirus (LV) and adeno-associated virus (AAV) are achieving specific targeting of T cells through surface modification techniques to

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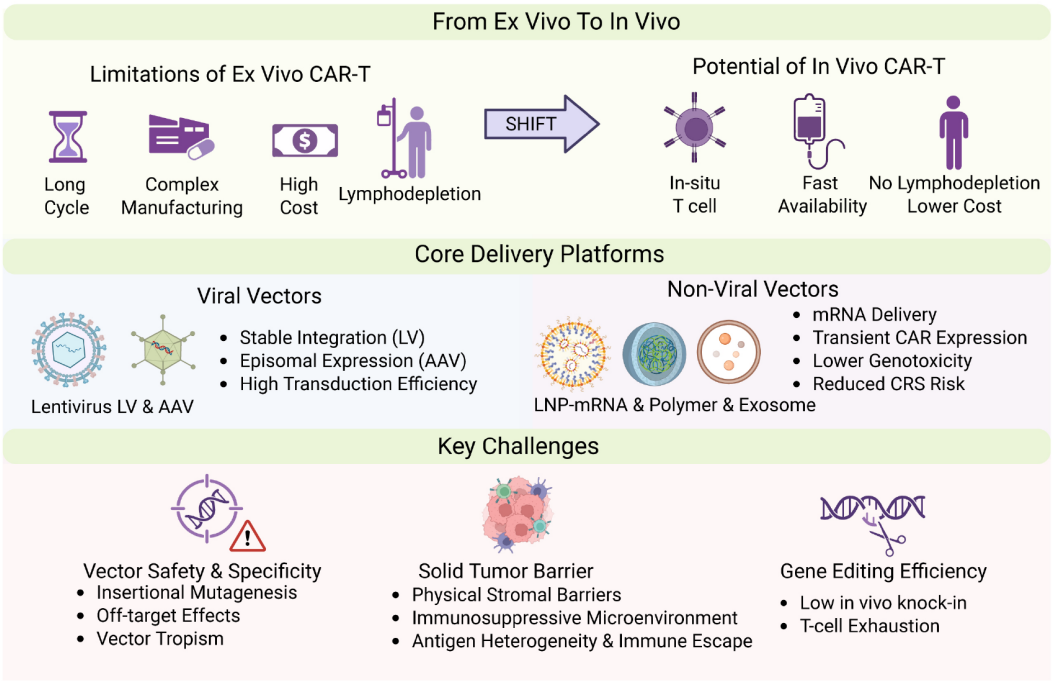
***通信作者**:E-mail: yizhang@zzu.edu.cn

enhance transduction efficiency and reduce off-target risks. In the non-viral vector domain, lipid nanoparticles (LNPs) combined with mRNA technology have garnered significant attention for enabling transient CAR expression, reducing cytokine release syndrome (CRS) risks, and avoiding genomic insertion mutations. Recent clinical trials have demonstrated their potential for treating solid tumors. Additionally, the article highlights the advantages of emerging carriers like polymeric nanoparticles and engineered exosomes in terms of low immunogenicity and controllable delivery. Although *in vivo* CAR-T therapies have progressed from proof-of-concept to multiple human clinical trials, including platforms like INT2104 and VivoVec, widespread adoption remains hindered by significant challenges. Key obstacles include potential genotoxicity and insertion mutation risks associated with viral vectors, immune escape driven by the complex immunosuppressive microenvironment, dense physical barriers, and antigenic heterogeneity in solid tumors. The article concludes that future research must focus on further optimizing the targeting precision and safety of delivery systems, while integrating gene editing technologies to overcome T-cell exhaustion. This approach will advance *in vivo* CAR-T therapy into an efficient, universal, and readily available precision treatment solution for tumors.



Key words: *in vivo* CAR-T cell therapy; immunotherapy; gene delivery

癌症治疗是全球性的难题,手术、放化疗等常规癌症治疗手段疗效有限[1]。相比之下,免疫疗法利用激活的免疫细胞精准靶向肿瘤,具有独特优势[2]。为克服传统T细胞疗法依赖MHC识别、抗原呈递缺失等局限,嵌合抗原受体T细胞(chimeric antigen receptor T cell,CAR-T)疗法应运而生[3]。

传统CAR-T细胞疗法需要从患者外周血中分离T细胞,利用病毒或非病毒载体导入CAR基因,再经体外扩增后回输到患者体内[4,5]。该技术虽在血液肿瘤(如B细胞白血病、淋巴瘤、多发性骨髓瘤)及部分实体瘤中疗效显著[6-8],且已有靶向CD19、BCMA等抗原的产品获批临床[9,10],但其应用仍面临多重挑战。体外制备工艺复杂、成本高昂且周期漫长,患者可及性受限[11-13];生产过程中易受工艺与环境因素影响[14],需遵循严格的GMP规范与监管要求[15];漫长制备期间患者需接受桥接治疗与淋巴细胞清除,可能引入额外风险并影响疗效[16-18];此外,抗原逃逸、回输细胞在体内的持久性不足、异体免疫排斥及移植物抗宿主病等挑战共同限制了该疗法的长期效果与临床应用[19-21]。

为突破体外CAR-T细胞疗法的局限性,研究人员开发了直接在体内诱导生成CAR-T细胞的新技术(图1)。该技术通过工程化病毒载体、脂质纳米颗粒等靶向递送系统,将CAR基因特异性导入患者体内的T细胞中[22],有效规避了传统体外制备工艺复杂、周期长、成本高以及可能引起的T细胞耗竭与异质性等问题[23],治疗周期更短,患者可及性更高。此外,该方法还能通过精准调控CAR基因递送,实现对CAR-T细胞功能的精确控制[24,25]。动物实验已证明,体内CAR-T疗法具有良好的抗肿瘤活性与安

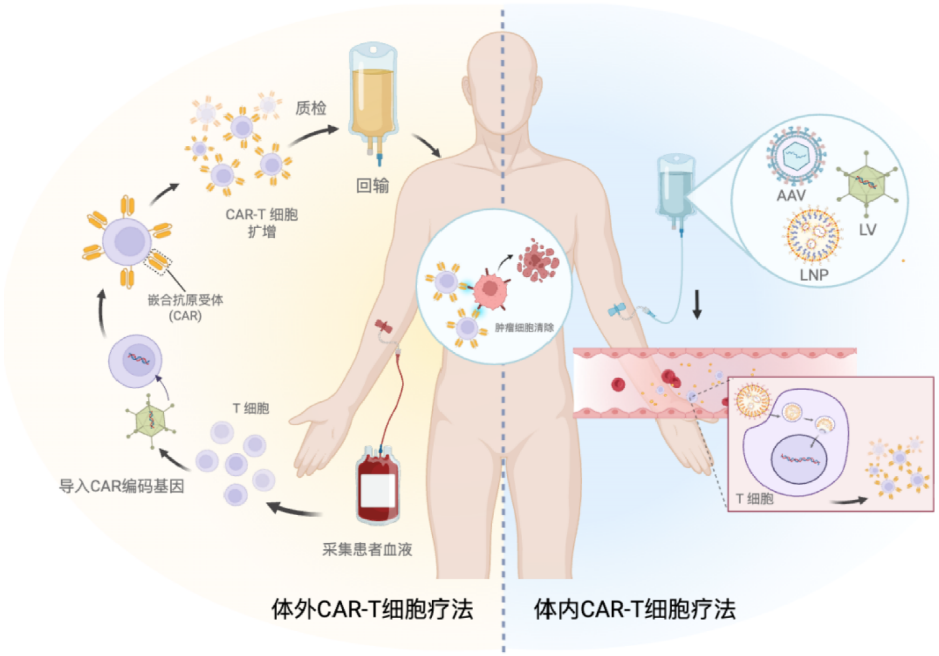


图1 体外CAR-T细胞与体内CAR-T细胞的制备过程

左:传统体外CAR-T细胞疗法。该疗法需经过复杂的体外制备流程,包括采集患者外周血分离T细胞、利用病毒载体在体外导入嵌合抗原受体(CAR)编码基因、体外扩增以及严格的质量检测,最后将制备好的CAR-T细胞回输至患者体内。右:新兴体内CAR-T细胞疗法。该疗法采用简化的原位重编程策略,将装载CAR基因的靶向递送载体(如腺相关病毒AAV、慢病毒LV或脂质纳米颗粒LNPs)直接注射入患者体内。载体进入血液循环后,在体内直接靶向并修饰内源性T细胞,使其转化为具有靶向清除肿瘤细胞能力的CAR-T细胞。

Figure 1 Preparation process of *ex vivo* CAR-T cells and *in vivo* CAR-T cells

Left: Traditional *ex vivo* CAR-T cell therapy. This requires a complex in vitro manufacturing process, including collecting the patient's peripheral blood to isolate T cells, utilizing viral vectors to introduce the chimeric antigen receptor (CAR) encoding gene in vitro, expanding the cells in vitro, and conducting strict quality control testing, before finally re-infusing the prepared CAR-T cells back into the patient. Right: Emerging *in vivo* CAR-T cell therapy. This adopts a simplified in situ reprogramming strategy by directly injecting targeted delivery vectors loaded with the CAR gene (such as adeno-associated virus (AAV), lentivirus (LV), or lipid nanoparticles (LNP)) into the patient. Once the vectors enter the blood circulation, they directly target and modify endogenous T cells *in vivo*, transforming them into CAR-T cells capable of specifically eradicating tumor cells.

全性[26],这为该技术的临床转化及在肿瘤治疗中的应用奠定了基础。

1 体内T细胞工程化的递送平台

高效、安全、特异性地进行体内基因递送是实现体内T细胞工程化的首要任务,这催生了多种递送平台的快速发展。目前已有多个基于病毒载体或纳米颗粒的靶向递送平台被开发用于原位生成CAR-T细胞。

1.1 病毒载体

病毒载体被广泛应用于CAR-T细胞制备,体内T细胞工程主要采用慢病毒(lentivirus,LV)和腺相关病毒(adeno-associated virus,AAV)两类载体。

1.1.1 慢病毒

慢病毒是基于HIV的基因递送系统,作为主流基因治疗载体,能将外源基因随机整合至宿主基因组,实现长期稳定表达[27]。但因慢病毒天然缺乏细胞靶向性,无法在体内特异性靶向T细胞。为实现T细胞特异性靶向,需对慢病毒进行包膜假型化改造[28]。研究人员开发了受体靶向型慢病毒,通过替换包膜蛋白实现T细胞特异性转导。该技术无需预激活T细胞,能直接靶向递送基因,显著提高转导效率并降低脱靶效应[25]。

通过在载体表面表达CD3特异性scFv,工程化慢病毒可选择性靶向静息T细胞,在体内仍保持高转导效率[29]。CD3靶向的慢病毒能在人源化小鼠体内高效生成$CD19^-$ CAR-T细胞,并有效清除B细

胞[30]。在肿瘤模型中,CD4靶向慢病毒较CD8靶向载体展现出更强的抑瘤效果,可能与避免$CD8^+$ T细胞耗竭有关[31]。此外,慢病毒载体还存在插入突变风险和载体容量有限等问题。

1.1.2 腺相关病毒

AAV为4.7 kb无包膜单链DNA病毒,经改造后可介导DNA靶向递送[32]。作为一种非整合型病毒,其基因组以附加体形式稳定存在,可实现CAR基因的瞬时高表达。相比腺病毒,AAV兼具低毒性与低免疫原性[33-35]。大量临床证据支持其安全性[36],高转导效率与稳定表达特性使其成为体内CAR-T治疗的理想载体[37]。但T细胞靶向性差,需依赖抗体修饰。已有研究者利用DNA改组技术融合8种野生型AAV血清型功能域,开发出嵌合AAV-DJ载体,显著提升了T细胞转导效率。在人源化白血病小鼠模型中,注射携带CD4-CAR的AAV成功在体内生成功能性CAR-T细胞,并有效抑制T细胞白血病进展[26]。该研究不仅证明了AAV平台用于体内CAR-T治疗的可行性,更揭示了其在靶向特定T细胞亚群方面的精准调控潜力。

1.2 非病毒载体

1.2.1 脂质纳米颗粒(lipid nanoparticles, LNPs)

LNPs是由脂质构成的10~1 000 nm级球形囊泡,具有单层或多层磷脂双分子层结构[38]。LNPs能通过封装有效保护mRNA免受降解,维持其稳定性[39-41],并通过其可电离脂质成分介导高效的内体逃逸,这是其介导功能性蛋白表达的前提。基于此机制,LNPs成为体内递送CAR-mRNA的理想平台。LNPs通过靶向修饰(如抗CD5或抗CD3抗体),可实现体内T细胞的精准识别与转染[42]。经靶向配体修饰后,LNPs可特异性识别T细胞,在体内实现CAR-mRNA的瞬时表达,从而避免长期CAR表达引发的细胞因子释放综合征(cytokine release syndrome, CRS)等患病风险[43-45]。

LNPs的应用已超越肿瘤领域。装载成纤维细胞活化蛋白特异性CAR mRNA的CD5靶向LNPs,成功在小鼠体内生成CAR-T细胞,显著改善心力衰竭小鼠的心功能[46]。此外,有研究团队设计了一种无抗体修饰的LNP,可递送CAR环状RNA并在体内生成功能性CAR-T细胞,用于靶向炎性衰老疾病[47]。

为突破传统LNPs靶向性不足、T细胞转染效率低等瓶颈,新型载体技术不断涌现。新型LNPs(如CD8靶向的L829-tLNP)通过系统性优化mRNA与脂质成分,在非人灵长类模型中成功实现高效、安全的体内CAR-T细胞生成,有望突破传统LNPs靶向性差、表达效率低及安全性不足三大瓶颈[48]。

1.2.2 聚合物纳米颗粒和外泌体

除了LNPs外,聚合物纳米颗粒和外泌体也可用作构建体内CAR-T的载体递送平台。聚合物纳米颗粒以聚(β-氨基酯)(PBAE)等生物可降解聚合物为代表,能通过静电作用封装核酸实现可控递送。其优势在于结构更易修饰,可精准调控粒径形态,提升载物能力与递送性能[49]。聚合物纳米颗粒虽具有高负载、可定制和低毒性等优势,但仍面临T细胞转染效率低、生产工艺复杂等挑战[50]。临床前研究显示,装载前列腺肿瘤特异性CAR mRNA的PBAE纳米颗粒可有效控制肿瘤生长并显著延长小鼠生存期[51,52]。

外泌体则是一种内源性的天然纳米载体,凭借其高稳定性和低免疫原性成为CAR mRNA的理想递送系统[53,54]。工程化外泌体通过表面表达抗CD3/CD28 scFv靶向T细胞,并利用MS2-LAMP-2B系统封装mRNA,已在体外成功生成功能性CAR-T细胞,展现体内应用潜力[55]。此外,表达抗CD3/EGFR的树突状细胞来源外泌体(tDC-Exo)可激活内源性T细胞并促进其与癌细胞结合,模拟CAR-T疗法从而增强抗肿瘤效果[56]。但是,外泌体仍面临分离纯化工艺复杂等问题。

2 体内CAR-T疗法的挑战与应对策略

体内CAR-T细胞疗法凭借其无需体外扩增、治疗周期短等优势,已成为肿瘤免疫治疗的研究热点,但其临床应用仍面临多重挑战。

基因递送系统的安全性是体内CAR-T技术发展的主要挑战。病毒载体存在插入突变风险,可能引发细胞异常增殖。目前通过优化CAR结构及载体组分可降低该风险[57,58]。在非病毒载体中,LNP-mRNA系统面临递送效率低、靶向性差等局限[59,60]。有研究通过开发pH敏感肽(如KAALA)促进溶酶体逃逸[61],或采用KALA肽修饰纳米颗粒提升基因表达效率[62],显著改善了非病毒载体的递送能力。

T细胞耗竭是制约体内CAR-T细胞临床疗效的另一关键因素[63]。通过引入CD28/4-1BB共刺激域,可增强T细胞活性与持久性[64];联合免疫检查点

抑制剂,可逆转T细胞衰竭[65,66];利用CRISPR/Cas9等技术编辑耗竭相关基因或抑制CD38表达,可改善细胞代谢状态并延长存活时间[67,68]。

相比血液肿瘤,体内CAR-T疗法在实体瘤治疗中面临更大的挑战[69]。在实体瘤中,体内CAR-T疗法面临三大核心挑战:免疫抑制微环境、物理屏障及实体瘤抗原异质性。在免疫抑制微环境中,Treg、MDSC与TAM通过分泌TGF-β、IL-10等因子形成抑制网络[70];癌症相关成纤维细胞及其产生的致密细胞外基质(如肝癌中富含的透明质酸与胶原)则构成物理屏障,阻碍CAR-T浸润[71,72];同时,肿瘤细胞通过下调GPC3、AFP等靶抗原实现免疫逃逸[73]。针对这些障碍,研究者已开发多种应对策略:如通过药物或基因编辑阻断免疫抑制细胞功能[74-76],增强CAR-T细胞在肿瘤中的活性和持久性;设计多靶点/双特异性CAR,克服抗原异质性[77,78];优化基因递送系统,实现对CAR-T细胞的精准调控:如在肝癌治疗中,肝靶向载体可提升CAR基因递送效率并降低全身毒性[79,80],而肝移植后患者因处于免疫抑制窗口期,体内CAR-T疗法可能尤为适用。

3 体内CAR-T疗法临床转化前景

体内CAR-T疗法正从概念验证迅速走向临床探索,目前多项体内CAR-T疗法临床试验正在全球开展,标志着肿瘤治疗正迈向体内精准免疫治疗新时代(表1)。在临床转化方面,体内CAR-T疗法通过创新递送系统、精准基因编辑等策略,在提升靶向性和安全性方面取得重要进展。

慢病毒载体凭借其介导的长期、稳定基因表达,成为临床转化的先行者。Interius BioTherapeutics开发的INT2104为全球首个进入人体临床试验的体内CAR-T疗法(ClinicalTrials.gov:NCT06539338)。Umoja Biopharma开发的VivoVec平台利用表面抗CD3 scFv的慢病毒载体,也可在无需淋巴清除的条件下实现体内CAR-T细胞生成。临床前研究显示,该平台能在人源化小鼠中有效诱导抗CD19 CAR-T细胞,清除B细胞且未出现显著毒性[81]。针对CD19和CD22的候选药物UB-VV111与UB-VV400/410,已在早期临床试验中展示了良好的应用前景(ClinicalTrials.gov:NCT06539338、NCT06743503)。针对经典靶点CD19及CD19/CD20双靶点的探索仍在继续,JY231(ClinicalTrials.gov:NCT06890065)、LVIVO-TaVec100(ClinicalTrials.gov:NCT07002112)等疗法进一步丰富了病毒载体平台在B细胞恶性肿瘤领域的临床证据。

针对多发性骨髓瘤的BCMA靶点也成为目前的研发热点。有研究报道了慢病毒载体介导的ESO-T01在多发性骨髓瘤患者中的临床数据:4例患者中2例达到严格完全缓解,2例部分缓解,不良反应可控,且在多个组织中检测到CAR-T细胞,证实其体内扩增能力[82]。KLN-1010、ESO-T01等疗法旨在通过慢病毒载体为复发难治性多发性骨髓瘤患者提供新的治疗选择,目前多项临床试验正在进行中(ClinicalTrials.gov:NCT06688435、NCT06791681)。

针对实体瘤治疗挑战,新型递送策略不断涌现。以LNPs为代表的非病毒载体凭借其独特优势,正在开辟体内CAR-T疗法新途径。Myelioid Therapeutics开发的MT-302(靶向TROP2)和MT-303(靶向GPC3)在实体瘤治疗中展现出巨大潜力(ClinicalTrials.gov:NCT05969041、NCT06478693)。近期临床数据显示,MT-302在晚期上皮肿瘤患者中展现出明确的抗肿瘤能力和安全性,首次在人体中证实体内生成的CAR-T细胞能够有效穿透实体瘤并重塑免疫抑制微环境[83]。

表1 基于不同递送载体的体内CAR-T疗法临床研究进展

Table 1 Clinical research progress of *in vivo* CAR-T therapies based on different delivery vectors

载体类型	平台	研发公司	靶点	适应证	研究阶段	临床试验编号
LV	INT2104	Interius BioTherapeutics	CD20	B细胞恶性肿瘤	Phase 1	NCT06539338
LV	UB-VV111	Umoja Biopharma	CD19	B细胞恶性肿瘤	Phase 1	NCT06528301
LV	UB-VV400/410	驯鹿生物	CD22	B细胞恶性肿瘤	Phase 1	NCT06743503
LV	KLN-1010	石药集团中奇制药	BCMA	复发/难治性多发性骨髓瘤	Phase 1	NCT06688435
LNP	MT-302	Myeloid Therapeutics	TROP	实体瘤(上皮肿瘤)	Phase 1	NCT05969041
LNP	MT-303	Myeloid Therapeutics	GPC3	实体瘤(肝癌)	Phase 1	NCT06478693
LV	JY-231	济因生物	CD19	B细胞淋巴瘤/白血病	Phase 1	NCT06890065
tLNP	CPTX2309	Capstan Therapeutics	CD19	B细胞恶性肿瘤/自身免疫性疾病	Phase 1	NCT06917742
LV	ESO-T01	Capstan Therapeutics	BCM	复发/难治性多发性骨髓瘤	Phase 1	NCT06791681

Capstan Therapeutics开发了基于抗体偶联LNP的mRNA体内CAR-T候选药物CPTX2309,用于治疗B细胞介导的自身免疫性疾病,目前I期临床试验正在进行中(ClinicalTrials.gov:NCT06917742)[48]。

4 总结

体内CAR-T疗法作为新一代肿瘤免疫治疗方案,在简化制备流程和降低治疗成本方面展现出显著优势。然而,受限于递送平台效率不足、疗效与安全性尚未充分验证等因素,该技术目前仍处于发展阶段。随着递送精准度和安全性等关键环节的深度优化,体内CAR-T疗法有望成为肿瘤免疫治疗领域的重要发展方向之一。

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