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靶向非传统T细胞亚群的嵌合抗原受体细胞疗法的研究进展及展望

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摘要 嵌合抗原受体T细胞疗法(chimeric antigen receptor T-cell therapy, CAR-T therapy)目前已在血液系统恶性肿瘤治疗中展现出显著疗效, 并被确立为一种重要的肿瘤免疫治疗策略。然而, 其当前应用主要局限于血液肿瘤领域, 且存在适应症有限及长期疗效与安全性不确定性等问题, 这限制了其更广泛的临床应用。为突破现有局限, 研究者正积极探索改进CAR-T细胞疗法的创新路径, 其中, 靶向非传统T细胞亚群开发新型嵌合抗原受体细胞疗法正成为极具前景的研究方向。该综述旨在系统评述靶向非传统T细胞亚群的嵌合抗原受体细胞的最新研究进展, 分析其在克服传统CAR-T疗法瓶颈方面的独特优势与潜力, 并探讨其当前面临的挑战及未来的发展方向。

关键词 嵌合抗原受体; 调节性T细胞; γδT细胞; 黏膜相关不变T细胞; 自然杀伤T细胞

Research Progress and Prospect of Chimeric Antigen Receptor Therapy Targeting Non-Traditional T Cell Subsets

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Abstract CAR-T therapy (chimeric antigen receptor T-cell therapy) has demonstrated remarkable efficacy in the treatment of hematological malignancies and has been established as an important strategy in tumor immunotherapy. However, its current application is mainly limited to hematological tumors, and it faces issues such as limited indications and uncertainties regarding long-term efficacy and safety, which restrict its broader clinical application. To overcome these limitations, researchers are actively exploring innovative approaches to improve CAR-T cell therapy. Among them, the development of novel chimeric antigen receptor cells targeting non-traditional T cell subsets is emerging as a highly promising research direction. This review aims to systematically review the latest research progress in chimeric antigen receptor cells targeting non-traditional T cell subsets, analyze their unique advantages and potential in overcoming the bottlenecks of traditional CAR-T therapy, and discuss the current challenges and future development directions.

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Keywords chimeric antigen receptor; regulatory T cells; γδT cells; mucosalassociated invariant T cells; natural killer T cells

嵌合抗原受体T细胞疗法(chimeric antigen receptor T-cell therapy, CAR-T therapy)通过改造患者的T细胞, 使其表达一种能够识别并结合肿瘤细胞表面的特定抗原的嵌合抗原受体(chimeric antigen receptor, CAR), 从而引导T细胞精确地识别和杀伤肿瘤细胞。CAR主要由识别肿瘤相关抗原的单链抗体片段、铰链区和T细胞信号转导结构域组成。CAR识别并结合肿瘤抗原, 会触发T细胞的活化、增殖和分化, 进而发挥抗肿瘤作用[1-5]。目前, CAR-T therapy在血液系统恶性肿瘤的治疗中展现出显著疗效。临床研究证实, 该技术对复发/难治性急性B淋巴细胞白血病(B-cell acute lymphoblastic leukemia, B-ALL)、弥漫性大B细胞淋巴瘤(diffuse large B-cell lymphoma, DLBCL)等疾病具有突出效果。标准治疗流程包括: 从患者外周血分离T细胞, 通过病毒载体或非病毒方法导入CAR基因, 体外扩增后回输至患者体内。这些经过改造的T细胞能够在体内持续增殖并发挥抗肿瘤效应[1-7]。

目前传统CAR-T therapy主要聚焦于$CD4^+$ T和$CD8^+$ T细胞, 虽然取得了一系列进展, 但是其仍然具有一定局限性[6-8]。其一, 自体CAR-T细胞制备成本高昂, 且长达两周的生产周期可能延误病情快速进展患者的治疗时机; 其二, 同种异体CAR-T虽具备成本效益高、可及性强及产品质量可控等优势, 但面临细胞耗竭和持久性不足的挑战, 可能增加早期复发风险[9-10]。因此, 相较于传统的$CD4^+$和$CD8^+$ T细胞疗法, 目前越来越多的学者开始关注CAR-T therapy在其他T细胞亚群来源上的应用突破, 包括调节性T细胞(regulatory T cells, Tregs)、γδT细胞(gamma delta T cells)、黏膜相关不变T细胞(mucosal-associated invariant T cells, MAIT)、自然杀伤T细胞(natural killer T cells, NKT)等(图1)。

这些非传统T细胞亚群具有独特的优势, 能够有效预防或减轻自身免疫性疾病, 以及异基因造血细胞或器官移植中的排斥反应, 从而减少对免疫抑制药物的依赖。它们诱导移植物抗宿主病(graft-versus-host disease, GvHD)的风险较低, 且能够通过多种受体识别不同的配体来抑制癌细胞, 可能具有更好的安全性和疗效[11-18]。这些细胞亚群的独特优势和潜力, 有望克服传统CAR-T therapy的局限, 为肿瘤治疗提供新的方向和选择。

1 非传统T细胞亚群CAR疗法的研究进展

1.1 CAR-Treg细胞疗法的研究进展

Treg是传统T细胞的一种常见亚群, 占外周血单核细胞(peripheral blood mononuclear cells, PBMCs)的1%~4%, 高表达IL-2受体α链(CD25)和叉头波P3(forkhead box P3), 同时低表达IL-7受体α链(CD127), 在维持免疫稳态和防止自体免疫中起着关键作用[17-21]。Tregs通过与其他免疫细胞直接相互作用(如通过CTLA-4)或间接通过产生免疫抑制细胞因子(如IL-10、IL-35、TGF-β)来抑制免疫反应。此外, Treg还可以通过与抗原呈递细胞(antigen-presenting cells, APCs)的相互作用, 阻止T细胞的激活并抑制APC的成熟、共刺激分子的表达和细胞因子的分泌[22-25]。

CAR-Tregs在预防和治疗GvHD及自身免疫性疾病方面展现出显著潜力, 多项研究已报道其成功应用[26-36]。动物模型的前临床数据证实, 表达HLA-A2特异性CAR(抗A2 CAR)的工程化Tregs不仅能有效预防GvHD, 其移植排斥保护效果也显著优于多克隆Tregs[27-28]。基于这些积极结果, 目前已有两项临床试验(肾脏移植NCT04817774和肝脏移植NCT05234190)正在评估抗A2 CAR-Tregs的临床转化价值。此外, MOHSENI等[37]进一步研究发现, 经*IL-10*基因修饰的抗A2 CAR-Tregs在转导后能维持稳定的免疫调节表型, 并显著增强对同种异体免疫反应的抑制能力, 这一发现为CAR-Tregs的临床应用提供了重要理论支持。

与GvHD的治疗效果形成鲜明对比, CAR-Tregs在1型糖尿病(type 1 diabetes, T1D)中的应用尚未取得突破性进展。研究表明, 靶向胰岛素或人胰腺内分泌标记物HPi2的CAR-Tregs在异种移植模型中未能有效预防糖尿病发生, 这可能与其持续的基础信号导致细胞扩增能力受损有关[38-39]。然而, IMAM等[29]的研究提供了令人鼓舞的证据: 表达谷氨酸脱羧酶65(GAD65)特异性CAR的Tregs不仅能够在T1D人源化小鼠模型中成功扩增, 还能特异性

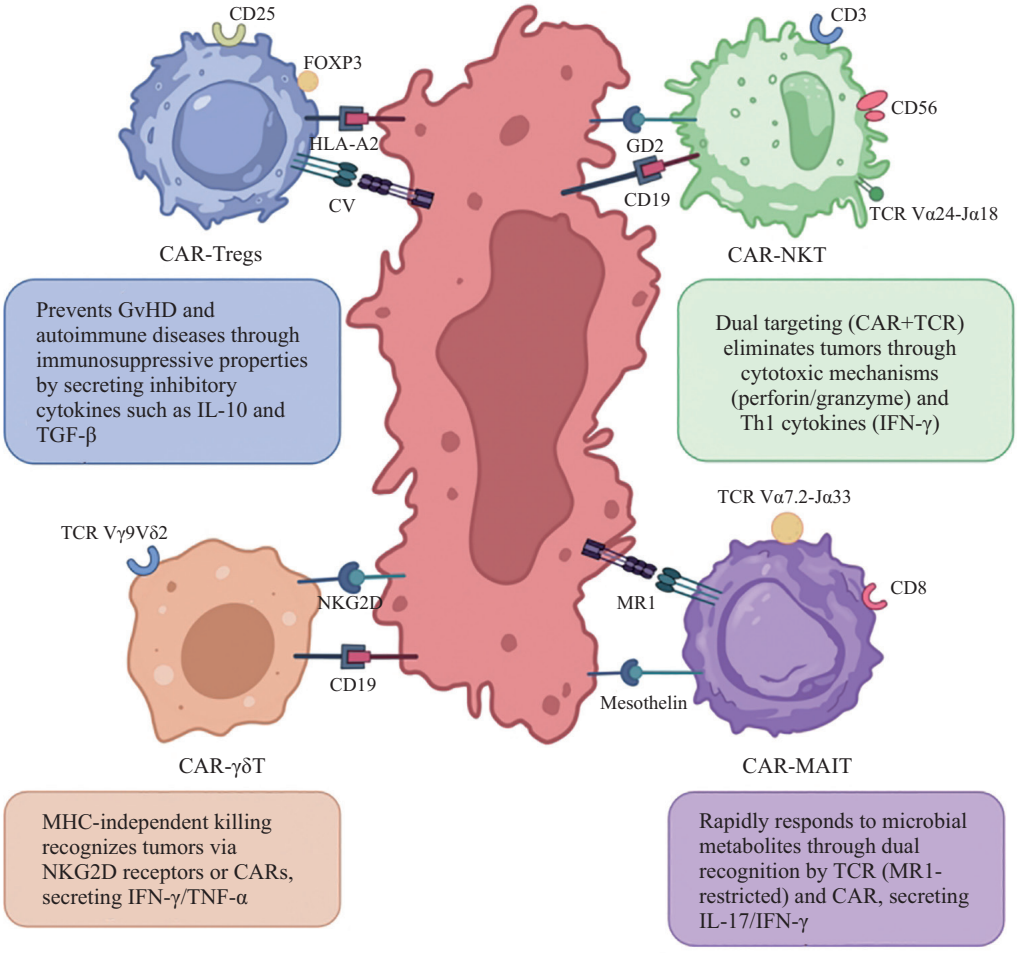

图1 各类靶向非传统T细胞亚群的嵌合抗原受体细胞疗法的作用靶点及其作用机理

Fig.1 Target molecules and mechanisms of action for chimeric antigen receptor cell therapies targeting various non-traditional T cell subpopulations

归巢至胰岛部位, 并表现出部分血糖调控能力。在多发性硬化症(multiple sclerosis, MS)研究领域, 靶向髓鞘少突胶质细胞糖蛋白(myelin oligodendrocyte glycoprotein, MOG)且同时表达FoxP3的CAR修饰$CD4^+$ T细胞, 在小鼠MS模型中显示出显著的治疗效果[30,36]。

CAR-Tregs的应用可以扩展到更多自身免疫性疾病, 如自身免疫性肝病(autoimmune liver disease AILD), 以识别合适的靶抗原[40]; 然而, Tregs具有独特的旁观者抑制特性, 这是一种广泛抑制具有不同抗原特异性的T细胞的内在属性, 使得Treg细胞能够在不特定靶向细胞表面抗原的情况下定向到炎症组织[41]。近年研究表明, 去氨基化波形蛋白(citrullinated vimentin, CV)作为一种翻译后修饰的中间纤维蛋白, 在类风湿性关节炎(rheumatoid arthritis, RA)的发病机制中具有重要作用。值得注意的是, CV特异性表达于约50% RA患者的滑膜组织细胞外基质中, 且其富集程度与疾病活动度密切相关。基于CV在炎症部位的特异性分布, RAFFN等[31]团队创新性地开发了靶向CV的CAR, 旨在通过局部免疫调控恢复关节微环境的稳态。这一策略为选择性干预滑膜炎症提供了潜在的治疗突破口。他们观察到, CV特异性CAR-Tregs在RA患者滑膜液的存在下扩增, 表明炎症关节中的CV足以激活这些CAR-Tregs。

CAR-Tregs的治疗应用已扩展至多种疾病领域(表1), 包括血友病A、白癜风、炎症性肠病、哮喘、心血管疾病及衰老相关疾病等[33,42-51]。在基因治疗领域, ARJOMANDNEJAD等[52]创新性地开发了靶向腺相关病毒衣壳(adeno-associated viral vector, AAV)的第三代CAR-Tregs, 成功抑制了宿主T细胞对AAV

表1 靶向非传统T细胞亚群的嵌合抗原受体细胞疗法的相关临床试验

Table 1 Clinical trials of chimeric antigen receptor cell therapy targeting non-traditional T-cell subsets

细胞和参考实验 Cells and references	国家临床试验 NCT	靶点 Target	适应证 Indication	阶段 Phase	状态 Status
Treg, [2]	NCT05234190	HLA-A2	Liver transplant rejection	I/II (ongoning)	Recruiting
	NCT04817774	HLA-A2	Renal transplant rejection	I/II (ongoning)	Active, not recruiting
NKT cells, [5]	NCT03294954	GD2	Neuroblastoma	I (ongoning)	Active, not recruiting
	NCT05487651	CD19	B-cell malignancies	I (ongoning)	Recruiting
	NCT03774654	CD19	B-cell malignancies	I (ongoning)	Recruiting
	NCT04814004	CD19	B-cell malignancies	I (ongoning)	Unknown status
	NCT00840853	CD19	ALL, NHL	I (ongoning)	Active, not recruiting
γδT cells, [5]	NCT04702841	CD7	T-cell malignancies	Early I (ongoning)	Unknown status
	NCT02656147	CD19	B-cell lymphoma, ALL, CLL	I (ongoning)	Unknown status
	NCT05554939	CD19	NHL	I/II (ongoning)	Recruiting
	NCT04107142	NKG2DL	Solid tumors	I (ongoning)	Unknown status
	NCT04796441	CD123	AML	NA (ongoning)	Unknown status

NA: 数据缺失。

NA: not available.

载体的免疫反应——这一现象在基于AAV的基因治疗临床试验中普遍存在。研究证实，这类抗AAV CAR-Tregs不仅能显著抑制效应T细胞的增殖和细胞毒性，还在小鼠模型中实现了以下突破：(1) 维持长期稳定的外源基因表达；(2) 持续分泌免疫抑制性细胞因子；(3) 有效减轻组织炎症反应[52]。值得注意的是，近期启动的I/II期临床试验(NCT05114837)正在评估异基因抗CD19 CAR-Tregs治疗成人复发/难治性急性淋巴细胞白血病(acute lymphoblastic leukemia, ALL)的安全性、耐受性及潜在抗肿瘤效应，标志着该技术向恶性肿瘤治疗领域的重要拓展[53]。

1.2 CAR-NKT细胞疗法的研究进展

NKT细胞是一类独特的$CD3^+CD56^+$ αβT细胞亚群，兼具自然杀伤(natural killer, NK)细胞和传统T细胞的形态与功能特征[54]。其发育始于胸腺皮质中的$CD4^+CD8^+$双阳性(double positive, DP)胸腺细胞阶段，随后与常规T细胞分化途径分离[55-56]。区别于传统T细胞，NKT细胞通过表面TCR识别由单态MHC-I样分子CD1d呈递的脂质和糖脂抗原[57-60]。NKT细胞根据TCR的多样性和抗原特异性可分为两大类。I型NKT细胞，也称为不可变型NKT(invariant natural killer T cells, iNKT)细胞，使用不可变的TCRα链(Vα24-Jα18)，并与有限的TCRβ链库结合，识别CD1d内结合的α-半乳糖基神经酰胺(α-galactosylceramide, α-GalCer)。而II型NKT细胞则表达更多样化的TCR，识别广泛的配体[25,61]。iNKT细胞虽然稀少，但在进化上保守，并且是肿瘤免疫监视中的主要亚群[25,61]。与γδT细胞相似，NKT细胞具有预激活特性，能够在常规αβT细胞完成增殖分化前数日就快速启动免疫防御，这种快速反应能力使其成为抵抗特定病原体感染的重要先天免疫组分。此外，NKT细胞通过分泌多种细胞因子调控αβT细胞的分化和功能，从而有效连接先天性和适应性免疫应答[62-63]。NKT细胞的效应机制包括穿孔素/颗粒酶介导的直接细胞毒作用以及通过死亡受体配体诱导靶细胞凋亡。值得注意的是，这些细胞最重要的体内功能是通过分泌大量细胞因子和趋化因子动态调节免疫反应。它们还表达NK受体，包括NKG2D、NKp33和NKp40[84]。部分NKT细胞群体还表达CD16(γ subunit of Fc receptors, FcγIIIa)，但它们不直接参与抗体依赖的细胞毒性作用(antibody-dependent cell-mediated cytotoxicity, ADCC)[45-46]。

2014年，HECZEY等[64]开创性地对人类iNKT细胞进行基因改造，使其表达靶向神经母细胞瘤特异性抗原GD2的CAR，研究比较了不同共刺激域(无共刺激域、单独CD28或4-1BB以及二者组合)对细胞功能的影响。研究发现，无论CAR设计如何，这些工程化iNKT细胞均能通过GD2-CAR和CD1d依赖性TCR双重机制杀伤肿瘤细胞[64]。特别值得注意的是，含有4-1BB共刺激域的CAR构建体(单独或与CD28联合)可将iNKT细胞的细胞因子分泌谱向Th1型极化，显著提升IFN-γ和GM-CSF的产生水平。与

常规CAR-T细胞相比, 抗GD2 CAR-NKT细胞不仅表现出更强的肿瘤趋向性和杀伤能力, 还具有不诱发GvHD的独特优势[64-66]。为此, XU等[67]创新性地将*IL-15*基因整合至抗GD2 CAR表达载体中, 实验证实IL-15能有效改善NKT细胞抵抗低氧肿瘤微环境(tumor mircroenviroment, TME)的抑制作用。在神经母细胞瘤异种移植模型中, 这种改造显著提升了细胞的持久性、肿瘤浸润能力和抗肿瘤活性[68-69]。由于外周血中NKT细胞含量极低(<0.1% T细胞), 其规模化制备面临重大技术挑战。HECZEY团队[70]突破性地建立了符合cGMP标准的NKT细胞分离扩增方案, 最终获得了纯度达96%、CAR表达率为54%的细胞产品, 为后续临床试验(NCT03294954)的开展奠定了基础。这项首次人体研究评估了表达抗GD2 CAR和IL-15的自体NKT细胞在复发/难治性神经母细胞瘤患者中的安全性, 初步结果显示10例受试者中1例达到完全缓解, 1例部分缓解, 3例病情稳定, 且未出现严重不良反应。在另一项重要研究中, TIAN等[71]开发了靶向CD19的CAR-NKT细胞用于B细胞恶性肿瘤治疗, 他们发现具有记忆表型($CD62L^+$)的NKT细胞在抗原刺激下表现出比CD62L-亚群高8倍的体外扩增能力, 且在体内展示出更持久的存活时间而不出现功能耗竭, 这一发现为优化CAR-NKT细胞治疗提供了新的方向[68,71-72]。

1.3 CAR-γδT细胞疗法的研究进展

γδT细胞是一类表达γδ型TCR的先天性淋巴细胞亚群, 占外周血T细胞的0.5%~5.0%, 但在上皮组织中构成主要的淋巴细胞群体[73]。这类细胞在胸腺发育成熟后迁移至淋巴结、外周组织及黏膜部位执行免疫功能[74-75]。与传统的αβT细胞相比, γδT细胞表现出较低的克隆扩增能力和TCR多样性[76-77]。人类γδT细胞具有高度异质性, 根据TCRδ链类型可分为Vδ1、Vδ2、Vδ3和Vδ5等亚群, 其中Vδ2亚群主要与Vγ9链配对形成Vγ9Vδ2 TCR, 而Vδ1亚群则可与多种Vγ链组合[73]。Vγ9Vδ2 T细胞在外周血中占主导地位, 能够识别微生物和宿主来源的磷酸化抗原; 而Vδ1 T细胞主要分布于上皮组织, 其抗原识别谱更为广泛[88]。在功能特性上, Vγ9Vδ2 T细胞可快速分泌TNF-α和IFN-γ等促炎性细胞因子, 而Vδ1 T细胞则倾向于产生胰岛素样生长因子-1(insulin-like growth factor-1, IGF-1), 在组织修复和伤口愈合过程中发挥重要作用[78-81]。值得注意的是, 所有γδT细胞亚群均具有显著的抗肿瘤细胞毒性, 可作为组织驻留或肿瘤浸润性淋巴细胞发挥作用。γδT细胞能够快速扩展, 并通过无主要组织相容性复合体(major histocompatibility complex, MHC)限制的方式识别具有保守分子模式的未加工抗原, 是免疫反应的第一道防线。它们通过分泌穿孔素、颗粒酶、TNF相关的凋亡诱导配体(TNF-related apoptosis-inducing ligand, TRAIL)和FasL等直接杀死肿瘤细胞, 并参与ADCC。此外, γδT细胞还通过NK受体如NKG2D和NKp30等识别和杀伤肿瘤细胞[80,82-83]。

2004年, RISCHER等[76]首次成功将CAR工程化引入γδT细胞, 他们构建的GD2和CD19特异性CAR-Vγ9Vδ2 T细胞在体外实验中展现出抗原特异性的IFN-γ分泌能力和细胞毒性功能[84]。此后, 多项研究聚焦于以第二代单链抗体(scFv)为基础的28ζ CAR修饰γδT细胞, 在血液系统恶性肿瘤和实体瘤模型中证实了这类细胞在体外和体内均具有显著的抗肿瘤活性[73,76,85-86]。目前, 一项评估抗CD19 CAR-γδT细胞治疗血液恶性肿瘤疗效的临床试验(NCT02656147)正在进行中[85]。研究发现, 与传统的CAR-αβT细胞相比, 抗CD19 CAR-γδT细胞不仅能靶向CD19阳性肿瘤细胞, 还能通过内源性γδTCR识别CD19阴性肿瘤细胞, 这种双靶向能力在唑来膦酸(zoledronic acid, ZOL)刺激下进一步增强, 有效避免了肿瘤抗原逃逸导致的治疗失败[87-88]。此外, 另有四项临床试验正在研究靶向CD20、CD7、CD123等肿瘤相关抗原的CAR-γδT细胞在血液系统恶性肿瘤中的应用前景[89-90]。由于γδT细胞具有在肿瘤低氧环境中浸润和功能的天然能力, 使其在实体肿瘤治疗中具有优势。因此, 正在进行的临床试验(NCT04107142)评估了基于NKG2D的CAR-γδT细胞在多种复发/难治性实体肿瘤(如结直肠癌、三阴性乳腺癌、肉瘤等)中的安全性和疗效[91]。*NKG2D*基因CAR使用人类NKG2D的胞外结构域作为抗原识别区, 靶向肿瘤或应激细胞上表达的NKG2D配体[92-93]。Vγ9Vδ2细胞除了具有内源性细胞毒性外, 还可以转化为强大的专业抗原呈递细胞(antigen-presenting cells, APC), 具备交叉呈递肿瘤相关抗原的能力[73]。

1.4 CAR-MAIT细胞疗法的研究进展

MAIT细胞是一类进化上高度保守的类天然T细胞亚群, 主要定位于肝脏和黏膜组织[94-97]。这类细胞在出生时数量稀少, 经过婴儿期逐步积累, 需

要至少六年时间才能达到成人水平[98]。MAIT细胞表达具有高度限制性的αβT TCR, 能够特异性识别由非多态性MHC I类相关分子MR1呈递的非肽类抗原[94-97]。根据表面标志物的表达模式, 人类MAIT细胞可分为不同亚群, 其中外周血中的MAIT细胞主要为CD8阳性或CD4/CD8双阴性表型。值得注意的是, MAIT细胞既可通过TCR依赖性途径, 也可通过非TCR依赖性途径激活, 展现出强大的细胞毒性功能[99-100]。其效应机制包括分泌穿孔素、溶菌素B、TNF相关凋亡诱导配体10(tumor necrosis factor superfamily member 10, TNFSF10)和Fas配体等细胞毒性分子, 同时还能产生干扰素-γ(interferon-gamma, IFN-γ)、肿瘤坏死因子(tumor necrosis factor, TNF)、粒细胞–巨噬细胞集落刺激因子(granulocyte-macrophage colony-stimulating factor, GM-CSF)和白细胞介素-17(interleukin-17, IL-17)等多种促炎细胞因子, 通过这些介质与中性粒细胞、巨噬细胞及其他效应T细胞建立复杂的免疫调控网络[88]。此外, MAIT细胞还表达包括NKG2D、NKp33和NKp40在内的多种自然杀伤细胞活化受体, 这些受体进一步增强了其细胞毒活性[101]。

近年来, 嵌合抗原受体修饰的CAR-MAITs因其独特的抗肿瘤潜力受到广泛关注。初步研究显示, CAR-MAITs在靶向CD19和人表皮生长因子受体2(human epidermal growth factor receptor 2, HER2)等肿瘤相关抗原时, 表现出显著的细胞毒性效应和良好的安全性特征。这些结果为CAR-MAITs疗法在肿瘤免疫治疗领域的应用提供了实验依据, 并为其进一步开发奠定了理论基础。具体而言, 研究表明, CAR-MAITs不仅展现出了与CAR-T细胞相当的细胞毒性, 而且在某些情况下, 其杀伤效果甚至更为突出, 同时保持了更佳的安全性记录[102]。这一发现为CAR-MAITs在癌症治疗中的应用提供了强有力的支持。此外, 一项利用3D肿瘤/TAM/T细胞类器官培养体系的研究进一步揭示了CAR-MAITs在TME中的潜力。该体系模拟了TAM的免疫抑制功能, 结果显示, 抗间皮素CAR-MAITs能够在此环境中保持其抗肿瘤活性[103]。这一能力的维持可能与CAR-MAITs通过其自然杀伤细胞激活受体(NK受体)和T细胞受体(T cell receptor, TCR)直接靶向TAM有关, 从而克服了免疫抑制的障碍[101]。有实验证实我们可以通过慢病毒转导从外周血单核细胞(peripheral blood mononuclear cells, PBMCs)中生成靶向间皮素的CAR-MAIT细胞[104-105]。在一个具有M2极化巨噬细胞的三维类器官培养中, 模拟了免疫抑制的肿瘤微环境, 靶向间皮素的CAR-T细胞的细胞毒性能力大大受到抑制, 而靶向间皮素的CAR-MAIT细胞保持了其对癌细胞的效力, 这可能是由于它们通过NK活化受体和MAIT TCR直接识别TAMs[105]。这些结果表明, CAR工程化的MAIT细胞在实体瘤中对TAMs具有出色的靶向能力, 通过减少来自TAMs和其他相关细胞类型的免疫抑制来增强肿瘤杀伤力。BOHINEUST等[103]生成的CD19 CAR工程化MAIT细胞显示, 这些治疗性MAIT细胞可以在临床前免疫缺陷小鼠模型中移植而不会引发移植物抗宿主病(GvHD), 这与CD19靶向的CAR-T细胞不同。然而, 尽管这些研究为CAR-MAITs的应用前景提供了积极的信号, 但截至目前, 尚未有注册的临床试验来直接验证CAR-MAITs在人体内的疗效和安全性。因此, 未来的研究需要聚焦于设计合理的临床试验方案, 以加速CAR-MAITs从实验室走向临床应用的进程。这一领域的持续发展有望为癌症患者带来新的治疗选择和希望[106]。

2 非传统T细胞CAR疗法的优势与限制

随着免疫治疗的迅速发展, CAR技术在各种非传统T细胞亚群中的应用逐渐展现出其潜力。这些细胞疗法各自具有独特的优势, 同时也面临一定的局限性(表2)。

CAR-Tregs疗法凭借Tregs的免疫抑制特性, 能够有效缓解过度的免疫反应, 预防自身免疫性疾病和移植排斥反应[107-110]。Tregs可以通过调节免疫稳态、减少炎症反应来保护机体免受损伤[107-108,111-112]。此外, Tregs能够在体外扩增, 并在临床试验中展现出良好的安全性和耐受性。然而, 使用大规模的多克隆Tregs可能会带来广泛的非特异性免疫抑制, 增加感染和癌症风险[113-114]。Tregs特征基因的突变引起严重的自身免疫性疾病, 如FOXP3突变。在IL2RA(别称CD25)突变的个体中发生致命性自身免疫性疾病。特定的HLA等位基因与常见的自身免疫性疾病有关, 一些等位基因是自身免疫性易感基因, 另一些是保护性基因[110]。最近的一项研究表明, 在Goodpasture疾病(一种由IV型胶原肽反应性$CD4^+$ T细胞介导的自身免疫性肾脏疾病)中, Tregs结合肽在

表2 各类靶向非传统T细胞亚群的嵌合抗原受体细胞疗法的优缺点及未来发展的挑战

Table 2 Advantages, disadvantages, and future development challenges of chimeric antigen receptor cell therapies targeting various non-traditional T cell subpopulations

细胞类型 Cell type	特点 Features	优点 Advantage	缺点 Disadvantage	主要挑战 Challenge	临床试验进展 Clinical trial progress
CAR-Tregs	The expression of regulatory markers such as FOXP3 relies on cytokines like IL-2 to maintain function	Strong immunosuppressive function, capable of suppressing autoimmune responses, with potential for treating autoimmune diseases and GvHD (graft-versus-host disease)	Difficult to amplify, unstable, and may convert into effector T cells	Non-specific suppression may increase the risk of infection and cancer, while its persistence in the body is limited	Phase I clinical trials primarily for liver and kidney organ transplants, and exploratory studies for type 1 diabetes and multiple sclerosis
CAR-NKT	Recognizes lipid antigens presented by CD1d. Exhibits both innate and adaptive immune functions	Dual-targeted (CAR+TCR). No risk of GvHD, with strong tumor microenvironment penetration capability	Low frequency in peripheral blood, with short persistence *in vivo*. Low amplification efficiency. May induce CRS (cytokine release syndrome)	Enhance persistence within the body. Optimize amplification protocols. Low infiltration efficiency in solid tumors. Challenging to scale up production	Early-phase clinical trials for neuroblastoma and lymphoma
CAR-γδT	Combines NK-like killing with APC function. High tissue penetrability. Strong ability to infiltrate solid tumors	MHC-independent killing. Potent antitumor activity. Significant potential against solid tumors. Low risk of graft-versus-host disease. Capable of cross-activating other immune cells	High heterogeneity (Vδ1/Vδ2 subpopulations exhibit distinct functions), with the Vδ1 subpopulation potentially promoting tumorigenesis. Depletion phenotype control	Subpopulation selection standardization. Overcoming tumor microenvironment suppression. Reducing off-target toxicity	Phase I/II trials for hematologic malignancies (e.g., multiple myeloma). Early-stage studies for solid tumors (e.g., breast cancer)
CAR-MAIT	Expresses MR1-restricted TCR. Secretes cytokines such as IL-17 and IFN-γ. Innate immune memory function	Mucosal tissue enrichment, suitable for mucosa-associated tumors. Rapid response to microbial metabolites (e.g., vitamin B derivatives) against solid tumors (especially liver/colorectal cancer), with significant potential for allogeneic therapy	Scarce in quantity, difficult to amplify, and currently limited in target development	A stable amplification system must be established, and the mechanisms regulating its *in vivo* function must be elucidated. *In vivo* safety and persistence must be validated	No registered clinical trials are currently available. Research is primarily focused on preclinical studies targeting colorectal cancer and lung cancer

具有显性保护性HLA等位基因的个体和共同表达保护性和易感等位基因的个体中显著增加[115]。此外, Tregs的产量有限, 且其在体内的耗竭可能会限制其长期治疗效果[26]。

CAR-NKT细胞疗法展现出显著的抗肿瘤潜力, 其作用机制主要包括两方面: 一是通过直接的细胞毒性作用杀伤肿瘤细胞; 二是通过激活αβT细胞实现间接的肿瘤清除[116]。NKT细胞具有自然的肿瘤靶向能力, 能够通过CD1d受体识别肿瘤细胞并消除肿瘤相关免疫抑制细胞, 如TAM和骨髓来源抑制性细胞(myeloid-derived suppressor cells, MDSCs)[101,106,117,138]。然而CAR-NKT细胞的功能障碍, 特别是在IFN-γ的产生方面, 降低了NKT细胞基于肿瘤微环境(TME)的免疫治疗质量[118]。另外, NKT细胞在外周血中的频率较低, 这使得其扩增和工程化过程中面临一定挑战[119-120]。虽然通过α-GalCer刺激可以有效扩增NKT细胞, 但其数量和质量仍然受到限制, 因此如何优化扩增和纯化方法仍是一个亟

待解决的问题[119-120]。NKT细胞治疗进展的另一个障碍是这些细胞在其来源处数量较少。因此，在NKT细胞的分离和选择性扩增方面的进展可以为基于NKT细胞的免疫治疗打开新的窗口。由于免疫细胞数量少、功能障碍以及免疫细胞在TME中的低浸润，这两个最近的问题在癌症患者中已成为非常严重的危机[121-123]。其他障碍包括肿瘤细胞中CD1d表达量的减少[123]，输注后α-GalCer的无反应性[118]，以及在培养基中用于过继转移的NKT细胞和自体DC细胞的长期处理和分化，这导致一些患者放弃治疗或不再符合治疗条件[92,118]。

CAR-γδT细胞疗法在多抗原识别和强大的细胞毒性活性方面具有显著优势。γδT细胞能够识别多种肿瘤相关抗原，减少肿瘤通过单一抗原丧失而逃逸的风险[124]。此外，γδT细胞还具有较强的免疫调节作用，能够激活其他免疫细胞，如树突状细胞、$CD8^+$ T细胞和NK细胞，进一步增强抗肿瘤免疫反应[84,125-126]。然而，CAR-γδT细胞也存在一定的挑战，如其可能在体外扩增过程中出现免疫耗竭，影响治疗效果[127]。虽然一些研究通过修改共刺激受体来减少这一现象，但仍需进一步优化细胞工程技术以提高其疗效。与αβT细胞相比，γδT细胞在外周血单核细胞(PBMCs)中占比相对较小，需要大量扩增以达到临床相关的细胞数量[85,126]。此外，一旦扩增并输给患者，γδT细胞的持久性明显低于αβT细胞[84]，这可能会削弱机体长期抗肿瘤反应的维持。对于$V\delta1^+$ T细胞，已经描述了两种免疫抑制表型。第一种涉及γδ17 T细胞，其分泌IL17并诱导髓样抑制性细胞(MDSCs)的招募以及巨噬细胞产生TGF-β[76,84,126]。第二种表型包括γδT调节细胞(Tregs)，通过分泌IL-10和TGF-β促进肿瘤生长[76,126]。最后，$V\delta2^+$ T细胞对AICD、滋养信号和耗竭的易感性似乎限制了其治疗效力。一种方法是使用$V\delta1^+$ T细胞。另一种方法是应用共刺激嵌合受体[84,127]。

CAR-MAIT细胞疗法凭借其广泛的组织分布和强大的细胞毒性活动，在癌症治疗中展现了潜力[128]。该细胞群体在肝脏及肠道等恶性肿瘤高发部位具有天然的组织定位优势，同时其不依赖于MHC分子呈递的抗原识别机制为异体来源的通用型细胞治疗提供了理想平台[128]。值得注意的是，MAIT细胞能够快速启动免疫反应，并且在经过合适激活后，可以转变为强大的抗肿瘤效应细胞[129]。然而，该疗法的临床转化面临重要挑战。首先，MAIT细胞在人类外周血中的频率较低，且其扩增过程可能受到限制[130-131]。其次，在慢性疾病微环境中MAIT细胞易出现功能耗竭现象，这可能影响其长期治疗效果。这些因素共同构成了CAR-MAIT细胞疗法发展过程中需要解决的关键科学问题[130-131]。目前，还没有临床试验启动以探索CAR-MAIT细胞在治疗中的应用。关于CAR-MAIT细胞的体内临床前研究仍然稀少，其在治疗中的潜在应用的许多方面尚未完全理解。详细的安全性特征是什么、肿瘤浸润能力如何以及CAR-MAIT细胞在体内是否保持其效应记忆亚群是尚未解答的关键问题。这些CAR-MAIT细胞的体内持久性尚未得到广泛研究。

3 非传统T细胞CAR疗法的未来方向

随着肿瘤免疫疗法的快速发展，靶向非传统T细胞亚群的嵌合抗原受体细胞疗法展现出显著的临床潜力，但其广泛应用仍面临诸多挑战。未来的研究将需要在关键方向上进行深入探索，以推动这一领域的进步。

非传统T细胞亚群(如Tregs、NKT、γδT细胞和MAIT细胞)在外周血中的低丰度特性为其临床应用带来了实质性障碍。当前亟需解决的关键科学问题主要集中于体外扩增技术的优化，包括开发新型培养体系与功能性添加剂，以提高细胞扩增效率的同时维持其原始表型和功能特性，并有效避免免疫耗竭现象的发生。此外，通过改进基因编辑策略来确保细胞在扩增过程中的功能稳定性也是未来研究的重要方向。这些技术突破将直接推动非传统CAR-T细胞疗法从基础研究向临床转化的进程。

尽管非传统T细胞亚群在某些肿瘤类型(如肝癌、胃肠道肿瘤等)中展现出较好的抗肿瘤效果，但如何改善这些细胞在肿瘤微环境中的渗透性和生存能力，依然是一个关键问题[104]。肿瘤微环境通常存在免疫抑制因素，如TAMs、骨髓来源抑制性细胞(MDSCs)等，非传统T细胞在这一环境中的功能可能会被抑制[25,57,89]。传统的Treg细胞，可能通过产生可溶性介质或直接的细胞间相互作用，抑制针对其他抗原的效应T细胞——这一现象被称为旁观者抑制[132-133]。如果在即将进行的临床研究中得到验证，旁观者抑制可能与大多数自身免疫性疾病特别相关，因为这些疾病通常对多种自身抗原具有反应

性[109,133]。在T1D中, 工程化表达自体反应性TCR的Treg细胞被证明能够诱导旁观者抑制, 因为通过特异性抗原的局部激活导致对另一T1D自身抗原的响应T细胞的增殖受到抑制[109,111,134]。因此, 从工程化Treg细胞向内源性T细胞转移抑制状态可能是自我维持的, 这表明长期维持工程化Treg细胞可能并不总是必要的。因此, 未来的研究应着力于通过优化CAR设计、调控免疫微环境或联合使用其他免疫治疗(如免疫检查点抑制剂、靶向治疗等)来增强这些细胞的抗肿瘤效能。

非传统T细胞亚群在肿瘤免疫治疗中的应用价值不仅体现在直接的细胞毒作用, 更在于其独特的免疫调节功能。具体而言, CAR修饰的CAR-Tregs通过维持免疫稳态来防止过度的炎症反应[135-137], 而CAR工程化的CAR-NKT和CAR-γδT则能够特异性识别肿瘤相关抗原并清除免疫抑制性细胞群体, 从而有效逆转肿瘤微环境的免疫抑制状态[116,124]。然而, 肿瘤细胞可通过抗原丢失、免疫检查点分子上调等逃逸机制规避这些细胞的杀伤作用。针对这一挑战, 未来的研究重点应聚焦于开发新型多靶点CAR构建体以及优化联合治疗策略, 通过同时靶向多个肿瘤相关抗原和阻断免疫抑制通路, 显著增强治疗细胞对抗肿瘤免疫逃逸的能力, 最终实现更全面和持久的抗肿瘤效果。

非传统T细胞CAR疗法的安全性问题是制约其临床应用的关键因素之一。尽管这类细胞具有较低的免疫原性特征, 但在临床治疗过程中仍可能诱发细胞因子释放综合征(cytokine release syndrome, CRS)等免疫相关不良反应[113]。特别是CAR修饰的CAR-Tregs因其固有的免疫抑制功能, 可能导致过度免疫抑制状态, 进而削弱患者的抗感染和抗肿瘤免疫监视能力[114]。针对这些潜在风险, 建立完善的安全性评估体系至关重要, 包括开发实时监测生物标志物、设计可调控的CAR表达系统以及优化剂量方案等策略。未来的研究重点应当是在确保治疗效果的同时, 通过精准调控CAR细胞的活性和持久性, 实现疗效与安全性的最佳平衡, 从而推动这类创新疗法向临床转化。

当前, CAR-T治疗在血液系统恶性肿瘤治疗中已取得显著进展, 但在实体瘤治疗领域仍面临重大挑战。值得关注的是, 基于非传统T细胞亚群(包括CAR修饰的自然杀伤T细胞、γδT细胞以及黏膜相关不变T细胞)的新型免疫疗法, 在肝脏和胃肠道等特定解剖部位实体瘤的治疗中展现出独特优势[138-139]。这些细胞类型因其固有的组织趋向性和MHC非限制性识别机制, 能够更好地克服实体瘤微环境障碍。然而, 实体瘤治疗仍须应对多重生物学屏障, 包括致密的肿瘤基质结构、高度免疫抑制的微环境特征以及显著的肿瘤内异质性。针对这些挑战, 未来研究应着重于以下方向: 开发具有增强组织穿透能力的CAR结构设计, 构建能够抵抗免疫抑制信号的智能型CAR系统, 以及优化细胞产物使其更好地适应肿瘤微环境特性。这些创新策略将有望突破当前实体瘤CAR细胞疗法的瓶颈。

4 总结与展望

本篇综述所讨论的靶向非传统T细胞亚群的嵌合抗原受细胞疗法在面对肿瘤时所展现的作用与潜力均基于产生含有scFv的CARs。值得注意的是, 近期研究开发出一种采用VHH二聚体作为抗原识别结构域的新型CAR-T细胞[140]。与常规抗体相比, 这类纳米抗体在抗原结合亲和力和特异性方面表现出相当的性能[141]。同时因其独特的结构优势——缺乏可变轻链而无需复杂的折叠组装过程或连接肽优化——展现出显著的技术优势。特别是在实体瘤治疗领域, 纳米抗体能够识别和结合传统scFv难以企及的隐蔽表位, 这一特性使其成为CAR设计中极具前景的抗原识别元件替代品。基于这些突破性发现, 我们认为纳米抗体技术将为突破实体瘤治疗瓶颈提供新的解决方案。

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