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嵌合抗原受体修饰T细胞疗法：过去、现在和未来

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摘要：嵌合抗原受体修饰T细胞(chimeric antigen receptor-modified T cells，CAR-T)疗法经过30多年的发展已日趋成熟为一种新型治疗方式，特别是CAR-T细胞在血液瘤治疗中取得了巨大成功。目前已经有8款CAR-T产品获得美国食品药品监督管理局(FDA)批准用于血液肿瘤的治疗。除了在血液肿瘤中的应用，CAR-T细胞在实体瘤治疗中的研究更是在如火如荼地进行，但是目前的临床疗效仍不尽如人意，亟待开发出新一代CAR-T细胞疗法。此外，CAR-T细胞疗法的应用也在不断拓展，不仅涵盖了恶性肿瘤治疗，也延伸至感染性疾病、自身免疫性疾病、器官移植排斥、衰老、纤维化等非肿瘤疾病的治疗。本文将主要综述CAR-T细胞疗法的发展历程和近年来的研究进展，特别是CAR-T细胞疗法在实体瘤中的应用，并对该疗法的未来发展作一展望。

关键词：嵌合抗原受体；细胞疗法；恶性肿瘤；非肿瘤疾病

Chimeric antigen receptor-modified T cell therapy: past, present and future

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Abstract: After more than 30 years of development, chimeric antigen receptor-modified T cells (CAR-T) therapy has matured into a novel therapeutic modality. Currently, eight chimeric antigen receptor-modified T cells (CAR-T) products have been approved by the U.S. Food and Drug Administration (FDA) for the therapy of liquid tumors because of great success of CAR-T cells in the treatment of hematological cancers. In addition to hematological malignancies, the studies of CAR-T cell therapy in solid tumors are in full swing, but its therapeutic efficacy remains unsatisfied , and it is urgent to develop a new generation of CAR-T cell therapy. Moreover, the application of CAR-T cell therapy is also constantly expanding, not only covering the treatment of malignant tumors, but also extending to the therapy of infectious diseases, autoimmune diseases, organ transplant rejection, aging, fibrosis and other non-tumor diseases. This paper will review the development of CAR-T cell therapy and the research progresses in recent years, especially CAR-T cell therapy in solid tumors, and give an outlook on the future development of this therapy.

Key Words: chimeric antigen receptor; cell therapy; malignant tumors; non-tumor diseases

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近年来，肿瘤免疫治疗发展迅速，成为继手术、放疗和化疗之后的第四大疗法，已逐渐成为肿瘤一线治疗的主要方式之一。肿瘤免疫治疗通过校正或增强人体免疫系统来达到治疗肿瘤的目的，包括肿瘤疫苗、细胞因子、溶瘤病毒、抗体、免疫检查点抑制剂(immune checkpoint inhibitors，ICIs)和过继细胞治疗(adoptive cell therapy，ACT)等疗法。其中，免疫检查点抑制剂和嵌合抗原受体修饰T细胞(chimeric antigen receptor-modified T cells，CAR-T)在恶性肿瘤治疗中取得了巨大的成功，显示出根治癌症的潜力。作为ACT新贵，CAR-T研究热度逐年递增[1]，逐步涌现出CAR-T以外的嵌合抗原受体修饰NK细胞(CAR-NK)[2]、嵌合抗原受体修饰NKT细胞(CAR-NKT)[3]和嵌合抗原受体修饰巨噬细胞(CAR-M)[4]等不同免疫细胞类型来源的嵌合抗原受体修饰免疫细胞(chimeric antigen receptor-modified immune cells，CAR-ICs)技术。其中，CAR-T细胞在血液瘤的治疗上取得的显著疗效，使CAR-T技术成为各大资本竞相角逐的对象。CAR-T技术发展历程仅30余年，治疗血液瘤的成功是举世瞩目的，但对于实体瘤的治疗迄今仍未获得令人满意的疗效。因此，亟待总结过去和现在的经验教训以开发出新一代CAR-T细胞疗法，特别是要克服CAR-T细胞在实体瘤治疗中诸如肿瘤特异抗原(tumor-specific antigens，TSAs)缺乏、CAR结构设计欠佳、肿瘤浸润受限和抑制性肿瘤微环境(tumor microenvironment，TME)带来的诸多挑战[5]。此外，未来还需进一步拓展CAR-T细胞疗法的非肿瘤疾病应用，包括感染性疾病[6]、自身免疫性疾病[7]、移植排斥[8]、衰老[9]、纤维化[10]等非肿瘤疾病的治疗，续写CAR-T细胞为代表的合成免疫学的新篇章。

1 CAR-T技术发展历程概述

Kuwana等[11]和Gross等[12]分别于1987年和1989年报道了CAR雏形——采用抗体识别区替换T细胞受体(T cell receptor，TCR)胞外识别区以获得抗体-TCR嵌合分子，使其不再依赖MHC限制性识别，并利用TCR-CD3复合体信号传导通路发挥作用。1991年，Irving等[1]将抗体识别区和CD8铰链、跨膜区和CD3ζ胞质信号传导域融合表达而获得第一代CAR，且能正常传导T细胞活化信号。Finney等[13]则在1998年报道了同时提供CD28共刺激信号(第二信号)和CD3ζ信号(第一信号)的第二代CAR，抗原激活后可产生更高水平的IL-2。2004年，同时提供4-1BB共刺激信号(第二信号)和CD3ζ信号(第一信号)的第二代CAR(当前使用最多的二代CAR)由Imai等[14]报道，相较于一代CAR，具有更强的抗肿瘤活性。Milone等[15]则系统评价了基于CD28或4-1BB共刺激信号的二代CD19 CAR的抗白血病疗效，证实了携带4-1BB共刺激信号域的二代CAR具

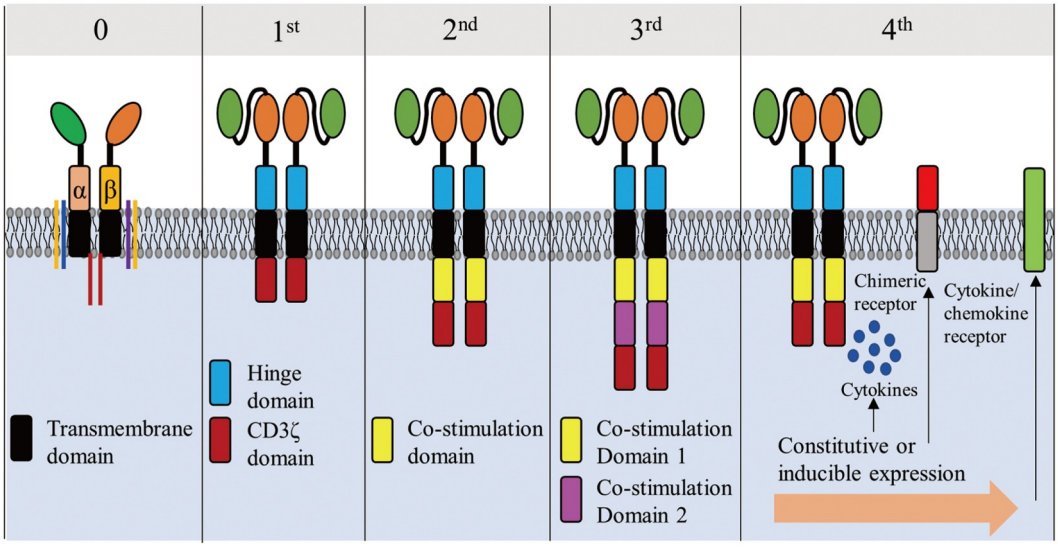

图1 嵌合抗原受体的发展历程

有更优的体内存活能力。三个研究组随后在2009—2010年间同时报道携带CD28和4-1BB共刺激信号域的第三代CAR的抗肿瘤活性进一步增强[16-18]。随着研究的深入，修饰细胞因子、细胞因子受体、趋化因子、趋化因子受体和嵌合激活受体等免疫调节分子的CAR-T逐渐开发出来[19]，以增强抗肿瘤疗效，特别是对癌症占比高达90%以上的实体瘤。

2 CAR-T细胞治疗实体瘤的现状

CAR-T细胞疗法在多种血液瘤治疗中均取得了高缓解率，多款产品已经获批上市，用于治疗B细胞急性淋巴细胞白血病、复发/难治弥漫大B细胞淋巴瘤和复发/难治性多发性骨髓瘤等血液系统肿瘤。近日，我国南京传奇生物和强生公司合作开发的BCMA CAR-T疗法获得美国FDA批准上市，是用于复发/难治性多发性骨髓瘤治疗的第二款BCMA CAR-T细胞产品。然而，CAR-T细胞疗法在实体瘤临床上的疗效至今还令人难以满意。实体瘤CAR-T细胞疗法临床试验目前涉及的癌种包括胶质母细胞瘤、神经母细胞瘤、胰腺癌、肺癌、胆管癌、肉瘤、结直肠癌、肝癌、前列腺癌、黑色素瘤、乳腺癌和肾癌，如表1所示。

虽然CAR-T细胞在神经系统肿瘤、胰腺癌和肉瘤中展现出积极疗效，出现了个别CR患者，但是CAR-T细胞治疗实体瘤的总有效率不尽如人意(10%)[43]，远低于血液瘤治疗的有效率。究其原因，实体瘤的形成不同于血液瘤，实体瘤和血液瘤的内在差异导致其疗效不佳以及潜在的安全性担忧。因此，要将CAR-T细胞疗法从血液瘤转化应用于实体瘤治疗，仍需克服相当多的障碍和阻力，例如TSAs缺乏、CAR结构设计不佳、CAR-T细胞浸润肿瘤受限和免疫抑制性TME等难题[5]。

3 CAR-T细胞治疗实体瘤的主要挑战及其应对策略

3.1 减少CAR-T细胞治疗实体瘤缺乏特异抗原引起的安全性担忧

目前报道的实体瘤靶抗原主要有IL13Rα2、EGFRvIII、CD171、GD2、MSLN、EGFR、CD133、HER2、CEA、PSMA、CEACAM5、c-Met、VEGFR2和GPC3(表1)，几乎所有靶抗原均为肿瘤相关抗原(tumor-associated antigens，TAAs)，在某些正常组织常存在低水平TAAs表达，可能导致CAR-T细胞靶向非肿瘤细胞产生脱靶效应(on-target off-tumor toxicity)而引起严重毒副作用[44,45]，甚至造成患者死亡[46]。

3.1.1 选择合适靶抗原和适宜亲和力抗体

除了前述的传统实体瘤靶点，CAR-T新靶点也日益增加。全面系统地分析潜在靶抗原的组织表达谱，对于研究者选择合适靶抗原以降低off-tumor毒性至关重要。Zhang等[47]利用现有的单细胞测序数据描述了CAR-T靶抗原的正常组织表达谱，发现现有CAR-T靶点在不同的正常组织或细胞中均存在不同程度的表达，为合适靶抗原的选择提供了一定的参考价值。Jing等[48]则利用正常细胞的单细胞测序数据系统分析了520个潜在CAR-T靶点的正常细胞和组织表达谱，并开发了一个便于研究者进行快速检索靶抗原的数据平台——CAR靶标基因单细胞毒性平台(https://hanlab.tamhsc.edu/CARTSC/)，为合适靶点的选择提供了一定的证据。

靶向肿瘤抗原的抗体亲和力是影响CAR-T细胞活性的关键因素。亲和力过高使CAR-ICs可识别低水平TAAs，引发on-target off-tumor毒性的风险大大增加。目前的研究发现，抗体亲和力超过一定阈值(KD<10^{-8} M)对CAR-T抗肿瘤活性的提升作用甚微[49]。Park等[50]证实，抗体亲和力在10^{-6} M的CAR-T的off-tumor毒性较低，同时具备更强的抗肿瘤作用。因此，有必要引入“药物治疗窗”的概念来平衡CAR-T细胞疗法的效应和毒性，方可在可接受毒性的前提下获得治疗有效性，从而实现CAR-T等基因工程免疫细胞的临床转化应用[51]。

3.1.2 引入布尔逻辑门

鉴于TAAs在正常组织低表达的局限性，靶向单一TAA的CAR-T细胞仍然存在off-tumor毒性发生的风险。因此，越来越多的研究者引入布尔逻辑门来调控CAR-T细胞的活性，利用组合抗原识别的“与门(AND gate)”策略同时靶向两个TAAs来实现肿瘤选择性识别。我们和其他团队组合使用靶向TAA-A的一代CAR和识别TAA-B的嵌合共刺激受体，实现了双靶点阳性肿瘤细胞的优势识

表1 实体瘤CAR-T细胞疗法临床试验的疗效

癌种	靶点	CAR-T代次	回输剂量	给药方式	疗效
胶质母细胞瘤	IL13Rα2	1代	$(9.6\sim10.6)\times10^8$	颅内注射	1*PR；2*SD[20]
	IL13Rα2	2代(4-1BB)	2×10^6；1×10^7	颅内注射	1*CR[21]
	EGFRvIII	2代(4-1BB)	$(1.75\times\sim5)\times10^8$	静脉回输	1*SD；9*PD[22]
	EGFRvIII	3代(CD28&4-1BB)	$6.3\times10^6\sim2.6\times10^{10}$	静脉回输	18*NR[23]
	HER2	2代(CD28)	$1\times10^6\sim1\times10^8/m^2$	静脉回输	1*PR；7*SD；8*PD[24]
神经母细胞瘤	CD171	1代	$10^8/m^2$；$10^9/m^2$	静脉回输	1*PR；5*PD[25]
	GD2	1代	$2\times10^7/m^2$；$2.5\times10^7/m^2$；$1\times10^8/m^2$	静脉回输	8*NED；3*CR；4*PD；1*PR；1*SD；2*Tumornecrosis[26]
胰腺癌	MSLN	2代(4-1BB)	$(1\sim3)\times10^8/m^2$	静脉回输	2*SD；4*PD[27]
		四代(7×19)	/	/	1*CR[28]
转移性胰腺癌	EGFR	2代(4-1BB)	$(1.31\sim8.9)\times10^6/kg$	静脉回输	4*PR；8*SD；2*PD[29]
晚期胆道癌和胰腺癌	HER2	2代(4-1BB)	$(1.4\sim3.8)\times10^6/kg$	静脉回输	1*PR；5*SD；5*PD[30]
晚期胆管癌	$EGFR^+$ CD133	2代(4-1BB)	$2.2\times10^6/kg$	静脉回输	1*PR[31]
转移性结直肠癌	CEA	2代(CD28)	$1\times10^5\sim1\times10^8/kg$	静脉回输	7*SD；2*PD；1*NE[32]
	TAG-72	1代	10^8；10^9；10^{10}	静脉回输；肝动脉回输	16*PD[33]
肝癌	GPC3	2代(CD28)	$1.35\times107\sim1.469\times10^8/kg$	静脉回输	2*PR；2*SD；5*PD；4*NE[34]
		四代(7×19)	1×10^6；3×10^6	静脉/瘤内注射	1*PR；2*SD；1*PD[28]
肝癌；胰腺癌；结直肠癌	CD133	2代(4-1BB)		静脉回输	3*PR；14*SD；6*PD[35]
转移性胃肠道肿瘤	CEACAM5	1代	$1\times10^9\sim5\times10^{10}$	静脉回输	7*SD；7*PD[36]
CEA^+肝转移	CEA	2代(CD28)	10^8；10^9；10^{10}	肝动脉回输	1*SD；5*PD[37]
非小细胞肺癌	EGFR	2代(4-1BB)	$(0.45\sim1.09)\times10^7/kg$	静脉回输	2*PR；5*SD；4*PD[38]
前列腺癌	PSMA	2代(CD28)	10^9；10^{10}	静脉回输	2*PR；1*MR；2*NR[39]
乳腺癌	c-Met	2代(4-1BB)	3×10^7；3×10^8	瘤内注射	1*SD；2*PD；3*DOD[40]
肉瘤	HER2	2代(CD28)	$1\times10^4\sim1\times10^8/m^2$	静脉回输	1*PR；4*SD；12*PD；2*NE[41]
小儿横纹肌肉瘤	HER2	2代(CD28)	$1\times10^8/m^2$	静脉回输	1*CR[42]

NED：无疾病迹象(no evidence of disease)；PD：疾病进展(progressive disease)；CR：完全响应(complete response)；PR：部分响应(partial response)；SD：疾病稳定(stable disease)；NR：无反应(no response)；NE：无法评估(not evaluable)；MR：微弱反应(minor response)；DOD：病死(dead of disease)

别[52,53]。另外，Roybal等[54]开发了synNotch-CAR系统，通过synNotch受体识别TAA-A后驱动靶向TAA-B的CAR表达，从而实现单靶点阳性正常组织细胞和双靶点阳性肿瘤细胞的区分。

3.1.3 利用肿瘤微环境信号

实体瘤微环境具有区别于正常组织微环境的独有特征，其利用TME信号调控CAR-T细胞活性的策略更为行之有效，实体瘤的共享特征信号具有普适性，使其不再依赖有限的靶抗原。一个典型的例子是“蒙面CAR(masked CAR)”，携带一种蛋白酶响应可切割掩蔽肽，该掩蔽肽在正常组织环境下可以阻断CAR与靶抗原的结合，进入蛋白酶富集的TME后，释放掩蔽肽，从而识别肿瘤靶抗原并攻击实体瘤[55](图2a)。其次，还可以利用TME富集的IL-4、TGF-β和VEGF分别调控CAR-T的瘤内增殖[56](图2b)和特异识别[57](图2c)。另外一种策略则采用实体瘤缺氧微环境信号来调控CAR表达，使其在缺氧TME下诱导表达，实现CAR-T细胞肿瘤微环境特异识别和活化。Liao等[58]和Kosti等[59]均利用缺氧反应元件(hypoxia response

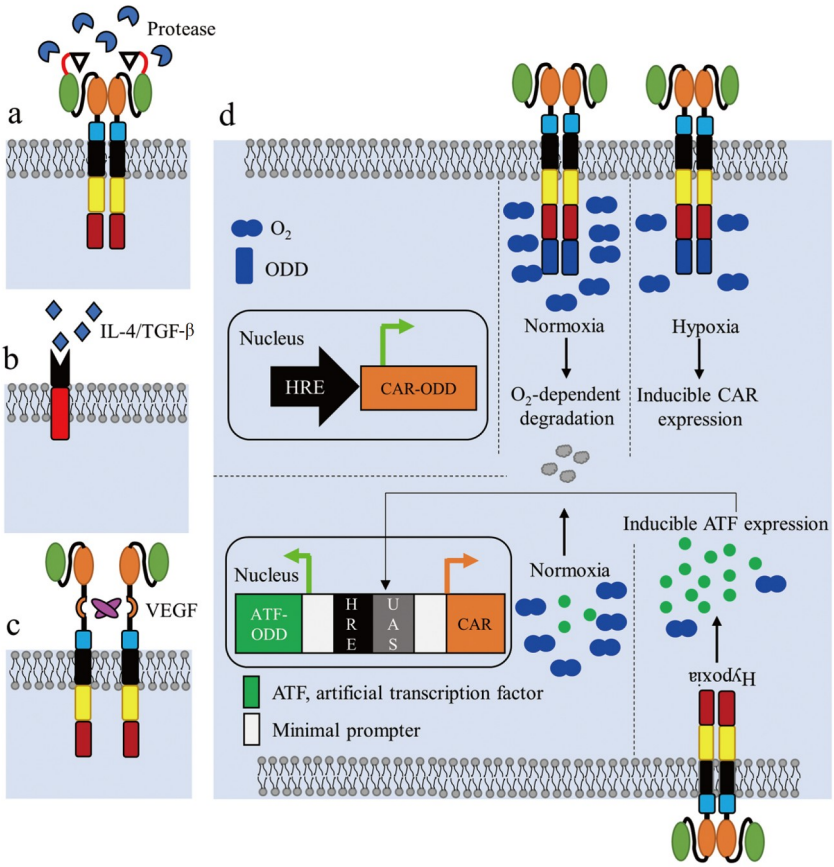

图2　肿瘤微环境信号调控CAR-T细胞安全性策略

element，HRE)和氧依赖降解结构域(oxygen-dependent degradation domain，ODD)同时调控CAR的转录和翻译，实现了缺氧微环境下的精准诱导和肿瘤特异杀伤。进一步开发了缺氧诱导的转录放大系统(HiTA)，不仅可以更加高效地调控CAR-T细胞活性，而且能够用于肿瘤局部释放双特异性T细胞衔接器(bispecific T-cell engagers，BiTE)等抗肿瘤效应分子[60](图2d)。

3.2 优化CAR结构设计和信号传导

3.2.1 CAR结构优化

CAR-T细胞体内长期维持是决定抗肿瘤疗效的关键因素，而CAR结构则是长期维持的重要影响因素。目前较为公认的CAR(二代CAR)主要由胞外识别区、铰链区、跨膜区和胞内信号域组成，第四代CAR则进一步加载了如细胞因子等附属分子。目前的胞外识别区多为单链抗体(single chain antibody fragment，scFv)，从早期的鼠源抗体逐渐转变为全人源抗体，以降低其免疫原性，防止CAR-T细胞免疫排斥[61]。另外，选择合适的抗体(如单域抗体)有助于避免抗体自发交联引起的非抗原依赖强直信号(tonic signaling)，可避免CAR-T细胞耗竭[57]。虽然跨膜区不具备功能，但其长度和氨基酸组成则通过改变CAR构象来改变CAR-T细胞抗肿瘤活性[62]。常用的跨膜区主要是CD28或CD8α跨膜区，相较于CD28跨膜区，CD8α跨膜区的CAR-T分泌更低水平的细胞因子，具有更高的存活能力[63]。胞内信号域由共刺激信号域和CD3ζ信号域组成，为T细胞完全活化的关键所在。目前最为普遍的共刺激信号域源自CD28或4-1BB共刺激分子，携带4-1BB共刺激信号域的CAR-T长期维持能力更强，因为4-1BB活化后可以促进记忆形成和脂肪酸代谢[64]。越来越多的共刺激信号分子被用于CAR的构建，包括CD27、ICOS、OX40等分子，作用也不尽相同[65]。CD3ζ信号域是所有CAR-T使用最多的TCR信号域，含有3个免疫受体酪氨酸激活基序(immunoreceptor tyrosine-based

activation motif，ITAM)。研究发现，携带含有1个ITAM的CD28共刺激信号域的CAR-T能够更好地平衡效应和记忆分化程序，使其获得更优的体内维持活性[66]。此外，有研究发现，同时整合CD3ε信号域可以招募磷脂酰肌醇激酶p85来促进CAR-T维持[67]。最后，T细胞完全活化和增殖需要TCR信号、共刺激信号和细胞因子信号的协同。因此，Kagoya等[68]进一步将细胞因子信号域IL-2Rβ/YXXQ整合至二代CAR-T，使其抗肿瘤疗效得以提升。

3.2.2 CAR和TCR信号传导差异

尽管CAR起初设计成模拟TCR信号传导，但是TCR识别靶抗原的能力比CAR至少强百倍以上。Salter等[69]通过比较CAR和TCR活化后的磷酸化蛋白质学变化，发现T细胞信号蛋白CD3δ、CD3ε和CD3γ以及接头分子LAT在CAR-T细胞内均未磷酸化或微弱磷酸化，提示含有CD3ζ信号域的CAR与TCR的信号传导差异显著。为了更好地模拟TCR信号传导，研究者对CAR进一步升级改造成T细胞抗原耦合器(T cell antigen coupler，TAC)、T细胞受体融合构建体(T cell receptor fusion constructs，TRuC)和抗体-TCR(antibody-T-cell receptor，AbTCR)。TAC由抗原识别域、CD3ε结合域和CD4/CD8共受体组成，证实TAC-T细胞具有更好的肿瘤浸润效果和更低的细胞因子分泌，提示其抗肿瘤强效且安全[70]。TRuC是通过将抗原识别域(如单链抗体)直接融合到CD3ε构建获得的，同样表现为低细胞因子分泌、强抗肿瘤活性，甚至优于二代CAR-T[71]。AbTCR则类似于0代CAR(图1)，将TCR可变区置换为抗体可变区，通过CRISPR/Cas9基因编辑系统将AbTCR插入到T细胞原有的TRAC基因座上，从而替换原有的TCR，避免原有TCR和AbTCR的错配，完全模拟TCR信号传导，具有更强的抗原识别能力和抗肿瘤活性[72]。

3.3 增强CAR-T细胞的肿瘤浸润

有效的抗肿瘤CAR-T细胞疗法需要CAR-T细胞首先高效地浸润至肿瘤病灶，其次长期存活和有效扩增，并能维持记忆表型来实现长效抗肿瘤免疫应答。血液瘤所处的血液环境通常不存在T细胞浸润问题，而实体瘤具有丰富纤维组织组成的物理屏障，且内部是微酸、乏氧、低营养、高渗透压和多种免疫抑制性因子构成的不利肿瘤微环境，不利于T细胞的浸润、活化和增殖。因此，人们对如何促进CAR-T细胞进入肿瘤病灶做了很多探索。其中，最为普遍的做法就是修饰趋化因子受体促进CAR-T细胞迁移至对应趋化因子丰富的肿瘤病灶，包括CCR2b、CXCR1/CXCR2、CCR4等趋化受体[5]。其次，异常肿瘤血管新生形成的物理屏障同样会降低CAR-T细胞的浸润，靶向VEGFR-2的CAR-T细胞可以消除异常肿瘤血管，有利于CAR-T细胞的肿瘤浸润，同时切断肿瘤血供来抑制肿瘤生长[73]。另外，靶向肿瘤外围丰富的肿瘤相关成纤维细胞(cancer-associated fibroblasts，CAFs)的FAP CAR-T细胞则通过清除CAFs来促进CAR-T肿瘤归巢[74]。同样的，EIIIB CAR-T作用于肿瘤基质和新生血管，从而促进免疫细胞浸润并有效抑制肿瘤生长[75]。再者，过表达肝素酶(heparinase，HPSE)或透明质酸酶(PH20)的CAR-T可以降解细胞外基质(extracellular matrix，ECM)，从而促进T细胞浸润肿瘤[76,77]。

3.4 抵御免疫抑制性肿瘤微环境

如前所述，实体瘤微环境是一个极其不利的环境，除了恶劣的理化因素外，还充斥着多种免疫抑制性细胞，包括肿瘤相关巨噬细胞(tumor-associated macrophages，TAMs)、髓系来源抑制细胞(myeloid-derived suppressor cells，MDSCs)和调节性T细胞(regulatory T cells，Tregs)，以及IL-4、TGF-β、腺苷等抑制性分子[5]。

3.4.1 靶向免疫抑制性细胞亚群

Rodriguez-Garcia等[78]利用CAR-T细胞靶向删除FRβ+TAMs，进而促进机体内源性抗肿瘤免疫应答，并增强针对肿瘤自身的MSLN CAR-T疗效。其次，还可以通过放疗、化疗、靶向药和免疫检查点阻断剂等辅助治疗来重塑肿瘤微环境以便CAR-T浸润肿瘤和对抗TME。肝癌靶向药索拉非尼(Sorafenib)被发现可以通过重编程TAMs以促进炎症因子IL-12的分泌来提升GPC3 CAR-T细胞的抗肝癌疗效[79]。多靶点受体酪氨酸激酶抑制剂舒尼替尼(Sunitinib)可以抑制STAT3信号传导和诱导MDSCs凋亡，与CAIX CAR-T联用后抗肿瘤活性增强[80]。PARP抑制剂奥拉帕尼(Olaparib)可以破坏SDF1α/CXCR4趋化信号轴引起的MDSCs招募，从

而增强EGFRvⅢ CAR-T的抗肿瘤应答[81]。针对Tregs对CAR-T细胞的多种抑制机制，研究者相应地利用Tregs靶向抗体(GITR/4-1BB)、CCR4 CAR-T、过表达炎性因子(IL-12、IL-18等)以及干扰抑制性分子受体的表达等多种策略来抵御Tregs的免疫抑制作用[5]。

3.4.2 增强CAR-T细胞持久性

为了促进免疫抑制性TME内CAR-T细胞的维持，越来越多的研究将细胞因子(IL-7、IL-12、IL-15、IL-18、IL-21和IL-23等)表达在CAR-T细胞上，使其获得T细胞增殖和存活的第三信号，而这些细胞因子在TME内通常是不足甚至缺乏的[5]。Ma等[82]发现，T细胞活化后上调IL-23受体和IL-23亚单位p19，但不表达IL-23亚单位p40。因此，在CAR-T细胞上仅需过表达p40亚单位；接触靶抗原活化后产生的p19与p40形成IL-23，既能降低细胞因子的毒副作用，又能通过自分泌IL-23信号促进CAR-T细胞增殖。此外，组成性激活的细胞因子受体(C7R)亦可用来持续传导T增殖相关细胞因子IL-7的信号，使CAR-T细胞获得长期存活和维持的能力[83]。有研究表明，除了表达细胞因子或细胞因子受体外，在CAR-T细胞上直接修饰不依赖抗原刺激的共刺激分子OX40，可显著增强CAR-T细胞的增殖活性和杀伤活性，并降低其凋亡和耗竭水平[84]。

此外，近年的研究表明，CAR-T细胞产品的记忆性T细胞(memory T cell，T_{CM})和干细胞样记忆性T细胞(stem cell-like memory T cell，T_{SCM})，因具有长期存活、自我更新和分化为效应性T细胞(effector T cell，T_{eff})的能力，为CAR-T细胞长期维持和免疫监视的关键因素，对于肿瘤控制具有更显著的作用和治疗意义[85,86]。因此，在离体培养CAR-T细胞过程中，研究者们对如何增加自我更新的记忆性CAR-T生成和维持做了诸多探索。Xu等[85]利用重组细胞因子IL-7和IL-15替换传统培养用的IL-2来增加细胞回输产品的CAR-T_{SCM}细胞($CD8^+CD45RA^+CCR7^+$)，并证实该亚群比例是CAR-T细胞回输6周内体内扩增的关键因素。Gattinoni等[87]和Sabatino等[88]在细胞因子IL-7和IL-21的培养基础上进一步添加糖原合成酶激酶-3β(glycogen synthase kinase-3β，GSK-3β)激活Wnt信号来阻止T_{eff}发育分化并高效诱导具有自我更新能力且抗肿瘤活性更佳的CAR-T_{SCM}。除了激活Wnt信号外，研究者利用工程化饲养细胞体外激活Notch信号来诱导CAR-T_{SCM}的生成，并证实Notch-FoxM1信号轴在CAR-T_{SCM}的线粒体生成中发挥了重要的作用[89]。另外，适当的代谢重编程也可以生成长效的记忆性T细胞。线粒体分裂抑制剂(Mdivi-1)或融合启动子(M1)已经报道用于重塑线粒体代谢来生成记忆性T细胞[90]，而PPARδ的激动剂GW501516则通过激活过氧化物酶体增殖物激活受体(peroxisome proliferator-activated receptor，PPAR)来增强T细胞记忆相关的脂肪酸氧化来提升ACT的持久性[91]。

3.4.3 拮抗免疫检查点和可溶性抑制性分子的抑制

免疫检查点对CAR-T细胞的抑制作用已经得到证实，包括PD-1、CTLA-4、LAG-3、TIGIT、TIM3等。自分泌免疫检查点阻断抗体(αPD1/PD-L1)、PD-L1 CAR、PD1显性负性受体(dominant negative receptor，DNR)、PD1嵌合转换受体(chimeric switch receptor，CSR)以及免疫检查点干扰(PD1 KD/KO)均在CAR-T上有所尝试，并证实其拮抗免疫检查点的抑制作用[5]。此外，腺苷受体A2AR干扰(A2AR KD/KO)[92]、靶向TGF-β的CAR[93]、IL-4/TGF-β嵌合转换受体[56]等针对可溶性抑制性分子的策略同样可以拮抗免疫抑制性TME，从而提升抗肿瘤疗效。

4 CAR-T细胞治疗的未来发展方向

综上所述，针对实体瘤的CAR-T细胞疗法，人们已经进行了大量探索，临床前研究结果虽提示其具有巨大的应用前景，但迄今为止仍未获得令人满意的临床疗效。因此，CAR-T细胞治疗实体瘤的未来发展方向仍然需要克服肿瘤靶抗原、CAR结构设计及其信号传导、肿瘤浸润和免疫抑制性肿瘤微环境等带来的主要挑战。因此，未来需要：(1)利用多组学技术加强肿瘤新靶点的发现与鉴定，例如整合转录组学和蛋白质组学数据以鉴定治疗乳腺癌的CAR-T新靶点[94]；(2)利用普适性的肿瘤微环境信号调控TAAs或泛表达抗原的CAR-T细胞以提升其安全性，例如肿瘤微环境富集的腺苷和ATP；(3)全面系统地比较不同CAR结

构和模拟TCR的构建体以确定最优的嵌合激活受体，推进临床应用；(4)利用全基因组筛选和多组学技术进一步解析CAR-T细胞抗肿瘤活性的内在调控机制，从而开发出更为强大的CAR-T细胞疗法[95,96]；(5)进一步解析T细胞肿瘤浸润和肿瘤微环境免疫抑制机制，以开发强效浸润和抵御免疫抑制性微环境的CAR-T细胞疗法，例如G蛋白调节子1(regulator of G protein signaling 1，RGS1)参与调控乳腺癌抗肿瘤T细胞浸润的机制解析可为抗肿瘤CAR-T的开发提供参考[97]。

除了实体瘤CAR-T技术的开发应用外，还需进一步拓展CAR-T等基因工程化免疫细胞的非肿瘤适应症。例如，CAR-T细胞可用于感染性疾病(HIV[6]、HBV[98]、HCV[99]、SARS-CoV-2[100])、自身免疫性疾病[7]、移植排斥[8]、衰老[9]、纤维化[10]等非肿瘤疾病的治疗。

5 结语

CAR-T技术的发展历程已有30余年，已经在血液瘤治疗中取得了辉煌成绩，全球已有8款血液瘤CAR-T产品获批上市。然而，CAR-T细胞疗法至今仍未在实体瘤中实现突破。近日，科济药业自主开发的CLDN18.2 CAR-T药物成为全球首个且唯一一个进入到确证性Ⅱ期临床试验的用于治疗实体瘤的CAR-T细胞候选产品，该疗法目前在胃癌/食管胃结合部腺癌患者中获得的客观缓解率高达61.1%，远远优于常规治疗的4%~8%以及PD1抗体的11%。因此，我们有理由相信，随着对CAR-T技术的进一步了解，不断克服肿瘤靶抗原、CAR结构设计及其信号传导、肿瘤浸润和免疫抑制性肿瘤微环境所带来的种种挑战，将会有更多的实体瘤患者获益，最终实现CAR-T细胞治疗实体瘤的临床应用。

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