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(54) 发明名称

靶向CD123的嵌合抗原受体及其用途

(57) 摘要

本发明提供了特异性结合CD123的单链抗体scFv,其包含选自SEQ ID NO:56、57、58、59、60和61的氨基酸序列。还提供了靶向CD123的嵌合抗原受体,其包含选自SEQ ID NO:65、66、67、68、69和70的氨基酸序列。还提供了编码所述scFv或嵌合抗原受体的核酸分子,包含所述核酸分子的载体和细胞,以及所述抗体、嵌合抗原受体、核酸分子、载体或细胞用于制备治疗表达CD123的肿瘤的药物的用途。

1. 一种靶向CD123的嵌合抗原受体,其氨基酸序列如SEQ ID NO:66所示。
2. 一种核酸分子,其包含编码权利要求1所述的嵌合抗原受体的核苷酸序列。
3. 一种载体,其包含权利要求2所述的核酸分子。
4. 权利要求3所述的载体,其中,所述载体是病毒载体。
5. 权利要求4所述的载体,其中,所述载体是慢病毒载体。
6. 一种细胞,其经权利要求3所述的载体转染,从而包含权利要求2所述的核酸分子或权利要求3所述的载体。
7. 权利要求6所述的细胞,其中,所述细胞为T细胞。
8. 一种药物组合物,其包含权利要求1所述的嵌合抗原受体、权利要求2所述的核酸分子、权利要求3所述的载体或权利要求6所述的细胞,以及可药用载体。
9. 权利要求1所述的嵌合抗原受体、权利要求2所述的核酸分子、权利要求3所述的载体或权利要求6所述的细胞用于制备治疗表达CD123的肿瘤的药物的用途。
10. 权利要求9所述的用途,其中,所述表达CD123的肿瘤是急性髓系白血病。

靶向CD123的嵌合抗原受体及其用途

技术领域

[0001] 本发明涉及具有不同抗原识别域的靶向CD123的scFv序列,基于这些scFv序列设计的嵌合抗原受体(CAR)和CAR-T细胞,以及其用于治疗表达CD123的肿瘤、特别是急性髓系白血病的用途。

背景技术

[0002] 急性髓系白血病(AML)是一种发病率不断上升的恶性肿瘤,主要影响中位年龄为66岁的老年人。美国国家癌症研究所(National Cancer Institute)估计,2018年的新病例将达到19520例,而其中可能会有10670人死于这种疾病[1]。尽管65-74岁的患者在过去连续三十年中,12个月生存率从20%提高到30%,但目前AML的治疗效果仍不令人满意,这些患者的5年生存率<5%[2]。对于较年轻的AML患者,主要的治疗方法是强化化疗,对于复发风险较高的患者(治愈率35-40%),采用异基因造血细胞移植(AHCT),而老年患者的预后仍然很差[2]。复发的AML患者获得第二次缓解的几率小于50%,而在第二次抢救性化疗后,只有16%的患者能实现病情控制[3]。自20世纪70年代引入蒽环类和阿糖胞苷类联合化疗以来,它一直是主要的治疗方法[4,5]。因此,对于此疾病,需要新的治疗策略。

[0003] 嵌合抗原受体T细胞(CAR-T细胞)的概念首先提出于20世纪80年代后期,其结合了抗体特异性和T细胞杀伤效应,形成过继性免疫效应[6,7]。嵌合抗原受体(CAR)分子结构主要包括抗体单链可变片段(scFv)、胞内信号域及连接scFv与胞内信号域的铰链区和跨膜区。近年来,利用抗CD19和抗CD20嵌合抗原受体T细胞(CAR-T细胞)治疗淋巴样恶性肿瘤(B细胞急性淋巴细胞白血病和非霍奇金淋巴瘤)取得了良好的效果[8]。目前,针对AML治疗的CAR-T靶点,有CD123、CD33、CLL1、FLT3等;其中,靶向CD123的CAR-T研究最多。

[0004] CD123是IL-3受体 α 链(IL-3Ra)。CD123与CD131一起组成高亲和力的IL-3受体,与IL-3结合后,能够促进细胞增殖分化,同时抑制造血细胞凋亡[9,10]。CD123的高表达与信号转导及转录激活因子5(STAT5)信号通路的活化相关。STAT5激活通路参与在多种恶性血液病中[11]。CD123主要在造血系统中表达,CD123在正常CD34⁺CD38⁻细胞中低表达或不表达[12]。此外,正常情况下在髓系祖细胞、B淋巴祖细胞中CD123有较高表达,而红系祖细胞和多向分化潜能祖细胞中低表达或不表达[13,14]。CD123在60%-90%以上的AML原始细胞中高表达[15-18]。Bras AE等人[19]检测了455例AML患者,其中约55%的患者AML细胞表达CD123,且阳性率达80%以上。

[0005] 虽然,CAR-T细胞在肿瘤细胞治疗中已取得了较好效果,但是CAR-T的疗效和安全性依然存在较大问题,主要有脱靶效应、细胞因子风暴、神经毒性等[20]。CAR-T细胞的最佳疗效和安全性,取决于多方面因素,其中CAR-T细胞的有效活化具有重要作用。CAR-T细胞的活化程度受CAR分子scFv的亲合力、铰链区长度、共刺激信号域、靶细胞表面抗原密度等多种因素影响。CAR分子的scFv作为与靶细胞表面抗原直接结合的区域,其亲合力对整体CAR-T细胞的活化意义重大。CAR分子scFv亲合力较低时,CAR-T细胞不能得到足够的抗原识别,从而无法达到最佳激活状态;CAR分子scFv亲合力较高时,CAR-T细胞可能会在识别肿瘤细

胞的同时也能够识别抗原密度相对较低的正常细胞,产生严重脱靶效应。

[0006] 因此,为了进一步提高CAR-T细胞的安全性和有效性,保证CAR-T细胞的有效活化,避免脱靶效应,仍需要筛选针对靶点具有适中亲和力的scFv。

发明内容

[0007] 第一方面,本发明提供了一种特异性结合CD123的抗体,其包括重链可变结构域VH和轻链可变结构域VL,所述重链可变结构域包含SEQ ID NO:1 (GYX₁X₂X₃X₄(D)YX₅X₆X₇)的VH-CDR1氨基酸序列,SEQ ID NO:2

[0008] (X₈X₉X₁₀(X₁₁)X₁₂X₁₃X₁₄X₁₅X₁₆X₁₇X₁₈NX₁₉X₂₀X₂₁KX₂₂X₂₃X₂₄)的VH-CDR2氨基酸序列和SEQ ID NO:3

[0009] (AX₂₅X₂₆X₂₇X₂₈X₂₉X₃₀X₃₁(YD)X₃₂X₃₃X₃₄X₃₅X₃₆X₃₇X₃₈X₃₉X₄₀)的VH-CDR3氨基酸序列;其中X₁选自S、T;X₂选自F、I;X₃选自T、S、M;X₄选自S、D、T、G;X₅选自W、Y、V、A、N;X₆选自M、V、I、W;X₇选自N、H;X₈选自Y、R;X₉选自I、C;X₁₀选自D、N、Y、S;X₁₁选自P、C;X₁₂选自Y、G;X₁₃选自D、N、S;X₁₄选自S、N、D、G;X₁₅选自E、A、G、S;X₁₆选自T、I、S;X₁₇选自H、S、N;X₁₈选自Y、S;X₁₉选自Q、E、P;X₂₀选自K、N、S;X₂₁选自F、L;X₂₂选自D、G、S;X₂₃选自K、R;X₂₄选自A、I;X₂₅选自R、G;X₂₆选自G、S、E;X₂₇选自E、P、R;X₂₈选自G、S;X₂₉选自N、Y、L、W;X₃₀选自W、Y、L;X₃₁选自G、D;X₃₂选自R、E;X₃₃选自Y、S、T;X₃₄选自D、Y、G;X₃₅选自G、Y;X₃₆选自Y、L;X₃₇选自A、G、P;X₃₈选自M、L;X₃₉选自D、A;X₄₀选自Y、C;所述轻链可变结构域包含SEQ ID NO:4 (X₄₁SX₄₂X₄₃X₄₄X₄₅X₄₆X₄₇X₄₈X₄₉(QK)X₅₀X₅₁X₅₂X₅₃W)的VL-CDR1氨基酸序列,SEQ ID NO:5 (X₅₄SX₅₅X₅₆X₅₇X₅₈X₅₉)的VL-CDR2氨基酸序列和SEQ ID NO:6 (X₆₀X₆₁X₆₂X₆₃X₆₄(P)X₆₅TF)的VL-CDR3氨基酸序列;其中X₄₁选自A、S;X₄₂选自Q、K、S;X₄₃选自S、D;X₄₄选自I、V、L;X₄₅选自S、N、D、L;X₄₆选自K、Y、S、D、N;X₄₇选自D、S;X₄₈选自G、S;X₄₉选自D、N;X₅₀选自S、N;X₅₁选自D、Y;X₅₂选自L、I、M;X₅₃选自A、N、H;X₅₄选自G、T、A;X₅₅选自T、N、Q;X₅₆选自L、S、R;X₅₇选自Q、E、A、I、D;X₅₈选自S、P;X₅₉选自G、E;X₆₀选自Q、N;X₆₁选自H、Y、S、F、G;X₆₂选自N、D、H、Y;X₆₃选自K、L、E、R、S;X₆₄选自Y、L、D、S、F、T;X₆₅选自Y、R、W;优选地,所述抗体是scFv。

[0010] 第二方面,本发明提供了一种靶向CD123的嵌合抗原受体,其包含:第一方面所述的抗体;铰链区;跨膜结构域;共刺激结构域;和CD3ζ胞内信号转导结构域。

[0011] 第三方面,本发明提供了一种核酸分子,其包含编码第一方面的抗体或第二方面的嵌合抗原受体的核苷酸序列。

[0012] 第四方面,本发明提供了一种载体,其包含第三方面的核酸分子。

[0013] 第五方面,本发明提供了一种细胞,其经第四方面的载体转染,从而包含第三方面的核酸分子或第四方面的载体。

[0014] 第六方面,本发明提供了一种药物组合物,其包含第一方面的抗体、第二方面的嵌合抗原受体、第三方面的核酸分子、第四方面的载体或第五方面的细胞,以及可药用载体。

[0015] 第七方面,本发明提供了第一方面的抗体、第二方面的嵌合抗原受体、第三方面的核酸分子、第四方面的载体或第五方面的细胞用于制备治疗表达CD123的肿瘤的药物的用途。

[0016] 本发明通过对靶点CD123的CAR分子的scFv进行亲和力筛选以及CAR-T细胞杀伤效应比较,以获得靶点CD123的最佳scFv序列,使得CAR-T细胞具有更优的安全性和有效性。与

现有技术相比,本发明能有效提高靶向CD123的CAR-T细胞对肿瘤细胞系及原代肿瘤细胞的杀伤效率,提高细胞增殖能力。

附图说明

[0017] 图1表示不同scFv的CD123 CAR分子在T细胞中的转导效率。

[0018] 图2表示CD123 CAR-T在培养过程中的总细胞增殖情况。

[0019] 图3表示靶细胞AML原代细胞和THP-1细胞分别刺激下,对CD123 CAR-T细胞分化的影响。

[0020] 图4表示CD123 CAR-T对CD123阳性细胞系(THP-1)及CD123阴性细胞系(K562)的杀伤效率。

[0021] 图5表示CD123 CAR-T对AML原代细胞的杀伤效率。

[0022] 图6表示CD123 CAR-T在AML原代细胞刺激5小时后的细胞因子表达。

具体实施方式

[0023] 本发明提供了一种特异性结合CD123的抗体,其包括重链可变结构域VH和轻链可变结构域VL,所述重链可变结构域包含VH-CDR1、VH-CDR2和VH-CDR3,所述轻链可变结构域包含VL-CDR1、VL-CDR2和VL-CDR3。

[0024] 在一个具体实施方案中,所述VH-CDR1选自由SEQ ID NO:8、9、10、11、12组成的组中的氨基酸序列;所述VH-CDR2选自由SEQ ID NO:14、15、16、17、18组成的组中的氨基酸序列;所述VH-CDR3选自由SEQ ID NO:20、21、22、23、24组成的组中的氨基酸序列;所述VL-CDR1选自由SEQ ID NO:26、27、28、29、30组成的组中的氨基酸序列;所述VL-CDR2选自由SEQ ID NO:32、33、34、35、36组成的组中的氨基酸序列;所述VL-CDR3选自由SEQ ID NO:38、39、40、41、42组成的组中的氨基酸序列。

[0025] 特别地,本发明的特异性结合CD123的抗体包括:

[0026] 1) 包含如SEQ ID NO:8所示的VH-CDR1、如SEQ ID NO:14所示的VH-CDR2和如SEQ ID NO:20所示的VH-CDR3的重链可变结构域;和包含如SEQ ID NO:26所示的VL-CDR1、如SEQ ID NO:32所示的VL-CDR2和如SEQ ID NO:38所示的VL-CDR3的轻链可变结构域;和/或

[0027] 2) 包含如SEQ ID NO:9所示的VH-CDR1、如SEQ ID NO:15所示的VH-CDR2和如SEQ ID NO:21所示的VH-CDR3的重链可变结构域;和包含如SEQ ID NO:27所示的VL-CDR1、如SEQ ID NO:33所示的VL-CDR2和如SEQ ID NO:39所示的VL-CDR3的轻链可变结构域;和/或

[0028] 3) 包含如SEQ ID NO:10所示的VH-CDR1、如SEQ ID NO:16所示的VH-CDR2和如SEQ ID NO:22所示的VH-CDR3的重链可变结构域;和包含如SEQ ID NO:28所示的VL-CDR1、如SEQ ID NO:34所示的VL-CDR2和如SEQ ID NO:40所示的VL-CDR3的轻链可变结构域;和/或

[0029] 4) 包含如SEQ ID NO:11所示的VH-CDR1、如SEQ ID NO:17所示的VH-CDR2和如SEQ ID NO:23所示的VH-CDR3的重链可变结构域;和包含如SEQ ID NO:29所示的VL-CDR1、如SEQ ID NO:35所示的VL-CDR2和如SEQ ID NO:41所示的VL-CDR3的轻链可变结构域;和/或

[0030] 5) 包含如SEQ ID NO:12所示的VH-CDR1、如SEQ ID NO:18所示的VH-CDR2和如SEQ ID NO:24所示的VH-CDR3的重链可变结构域;和包含如SEQ ID NO:30所示的VL-CDR1、如SEQ ID NO:36所示的VL-CDR2和如SEQ ID NO:42所示的VL-CDR3的轻链可变结构域。

[0031] 在另一个具体实施方案中,本发明的抗体中的所述重链可变结构域包含选自自由SEQ ID NO:44、45、46、47和48组成的组中的氨基酸序列,或包含与其具有至少85%、至少90%、至少95%、至少99%同一性的氨基酸序列;和/或所述轻链可变结构域包含选自自由SEQ ID NO:50、51、52、53和54组成的组中的氨基酸序列,或包含与其具有至少85%、至少90%、至少95%、至少99%同一性的氨基酸序列。

[0032] 特别地,本发明提供了一种特异性结合CD123的抗体,其包含:

[0033] 1) 如SEQ ID NO:44所示的重链可变结构域和如SEQ ID NO:50所示的轻链可变结构域;和/或

[0034] 2) 如SEQ ID NO:45所示的重链可变结构域和如SEQ ID NO:51所示的轻链可变结构域;和/或

[0035] 3) 如SEQ ID NO:46所示的重链可变结构域和如SEQ ID NO:52所示的轻链可变结构域;和/或

[0036] 4) 如SEQ ID NO:47所示的重链可变结构域和如SEQ ID NO:53所示的轻链可变结构域;和/或

[0037] 5) 如SEQ ID NO:48所示的重链可变结构域和如SEQ ID NO:54所示的轻链可变结构域;

[0038] 在另一个具体实施方案中,本发明提供了一种特异性结合CD123的抗体,其包含:选自自由SEQ ID NO:56、57、58、59、60和61组成的组中的氨基酸序列,或与其具有至少85%、至少90%、至少95%、至少99%同一性的氨基酸序列。

[0039] 优选地,本发明所述抗体为单链抗体scFv;更优选地,所述单链抗体scFv的重链可变结构域和轻链可变结构域由富含甘氨酸和丝氨酸的接头肽链相连,并且重链可变结构域和轻链可变结构域的顺序可互换。

[0040] 本发明还提供了一种靶向CD123的嵌合抗原受体,其包含:本发明所述的抗体;铰链区;跨膜结构域;共刺激结构域;和CD3 ζ 胞内信号转导结构域。

[0041] 在具体实施方案中,所述共刺激结构域选自CD27、CD28、4-1BB、OX-40、CD30、CD40、PD-1、ICOS、LFA-1、CD-2、CD7、LIGHT、NKG2C、B7-H3或其任意组合;优选地,所述共刺激结构域选自4-1BB或CD28,所述4-1BB包含如SEQ ID NO:62所述的氨基酸序列。

[0042] 在一个具体实施方案中,所述CD3 ζ 胞内信号转导结构域包含如SEQ ID NO:63所述的氨基酸序列。

[0043] 在一个具体实施方案中,所述铰链区和跨膜结构域选自IgG1、IgG4、CD8 α 、CD28、IL-2受体、IL-7受体、IL-11受体、PD-1或CD34的铰链区和跨膜结构域。

[0044] 优选地,所述的嵌合抗原受体包含选自自由SEQ ID NO:65、66、67、68、69和70组成的组中的氨基酸序列,或与其具有至少85%、至少90%、至少95%、至少99%同一性的氨基酸序列。

[0045] 本发明还提供了一种核酸分子,其包含编码本发明的抗体或嵌合抗原受体的核苷酸序列。

[0046] 本发明还提供了一种载体,其包含本发明的核酸分子;优选地,所述载体是病毒载体;更优选地,所述载体是慢病毒载体。

[0047] 本发明还提供了一种细胞,其经本发明的载体转染,从而包含本发明的核酸分子

或载体;优选地,所述细胞是T细胞。

[0048] 本发明还提供了本发明的抗体、嵌合抗原受体、核酸分子、载体或细胞用于制备治疗表达CD123的肿瘤的药物的用途;优选地,所述表达CD123的肿瘤是急性髓系白血病(AML)。

[0049] 除非特别指明,本文所述技术方案可以任意组合。

[0050] 本发明进一步通过以下实施例来说明。

[0051] 实施例1:CD123 CAR分子设计及CAR-T制备

[0052] 本实施例以设计具有不同抗原识别域的CD123 CAR分子,及CAR-T细胞制备为例。

[0053] 抗原识别域设计原则:CAR-T-1为对照组,其CD123的scFv序列来自宾夕法尼亚大学报道[21],在本文中命名为scFv-01。CAR-T-2~7为改造组,其scFv来源于小鼠免疫CD123胞外区融合蛋白所得单克隆抗体序列,在本文中命名为scFv-02-07。

[0054] 将不同抗原识别域的CD123 scFv的核苷酸序列通过基因合成的方法合成,然后通过同源重组方法与CD8 α 铰链区、CD8 α 跨膜区、4-1BB和CD3 ζ 胞内区序列连接形成完整的CAR分子,即CAR 1-7。将此CAR核酸片段通过限制性内切酶酶切和连接酶连接的方式,构建到慢病毒转移质粒pLenti6.3/V5 (Thermo Fisher, Waltham, MA, USA) 中。将慢病毒包装质粒pLP/VSVG, pLP1/MDK, pLP2/RSK (Thermo Fisher, Waltham, MA, USA) 与上述获得的转移质粒,用Lipofectamine 3000 (Thermo Fisher, Waltham, MA, USA) 转染至HEK293T细胞,48小时后收集培养基,300g离心去除细胞碎片后,用超速离心机25000rpm离心3小时。将沉淀用1mL生理盐水溶解,即为所需的慢病毒载体。

[0055] 从健康志愿者的外周血单个核细胞中,使用CD3/CD28 beads (Thermo Fisher, Cat#40203D),分离出T细胞。将分离纯化后的T细胞以 1.0×10^6 个细胞/mL,接入含有500IU/mL IL-2 (山东金泰生物工程有限公司,中国)的X-VIVO 15培养基 (Lonza, Switzerland) 中培养。在培养48小时后,用带有CD123 CAR分子的慢病毒载体以MOI=0.5感染T细胞,以获得CAR-T-1-7。慢病毒感染24小时后,进行细胞换液,并接入新鲜培养体系X-VIVO 15加500IU/mL IL-2中继续培养。在慢病毒感染5天后,使用移液枪反复吹打培养体系中的细胞并收集至离心管,置于磁力架上去除CD3/CD28 beads。T细胞离心并计数,取部分细胞使用流式细胞仪 (NovoCyte 2060R, ACEA Biosciences, San Diego, CA, USA) 检测各组细胞的CAR分子转导效率。

[0056] 图1所示为T细胞感染慢病毒5天、7天、9天、12天后,各组细胞的CAR分子转导效率。与对照组CAR-T-1相比,CAR-T-2、CAR-T-3、CAR-T-7组的CAR分子转导效率与其接近,CAR-T-4、CAR-T-5、CAR-T-6组明显高于对照组,说明各组CAR-T细胞的CAR蛋白表达情况均较好。

[0057] 实施例2:改造后CD123 CAR-T的体外增殖能力

[0058] 本实施例以检测具有不同抗原识别域的CD123 CAR-T的增殖能力为例。

[0059] 在CD123 CAR-T细胞培养过程中,以慢病毒感染T细胞为培养第二天,之后每隔2至3天,收集CAR-T细胞并离心,加1-2毫升的培养体系重悬,取10微升的细胞稀释一定倍数后,用台盼蓝 (Solarbio, Cat#C0040) 进行染色,倒置显微镜 (Ts2-FL, Nikon, 日本) 下分别计活细胞总数与死细胞总数,计算出各组细胞的增殖情况。

[0060] 图2显示,与对照组CAR-T-1相比,具有不同抗原识别域的CAR-T-2和CAR-T-7的总细胞增殖有明显提高。说明CAR-T-2和CAR-T-7的scFv设计能够改善CD123 CAR-T细胞增殖

能力。

[0061] 实施例3:改造后CD123 CAR-T在靶细胞刺激下的细胞分化情况

[0062] 本实施例以检测具有不同抗原识别域的CD123 CAR-T,分别在两种靶细胞AML原代细胞或THP-1细胞(均表达CD123)的刺激下,其T细胞分化情况为例。

[0063] CAR-T细胞培养至第5天时,各组分别取两份 5×10^5 个细胞于12孔板中,以效靶比(E:T)=5:1分别加入靶细胞AML原代细胞或THP-1细胞。在靶细胞刺激6天后,即CAR-T细胞培养至第11天时,各组分别取 1×10^6 个细胞并离心,100微升DPBS (HyClone, CAT# SH30028.02)重悬;然后每组CAR-T细胞分别加T细胞分化相关荧光抗体进行标记,使用全光谱流式细胞仪(N7-00008-0A, Cyttek Biosciences, Inc. Fremont)进行检测。

[0064] 图3的结果中,CD45RA+CD62L+细胞群表示初始T细胞群和干性记忆T细胞群的组合,CD45RA+CD62L-细胞群为效应T细胞群。具有不同抗原识别域的CAR-T-2和CAR-T-7的CD45RA+CD62L+细胞群占比高于对照组CAR-T-1,且CD45RA+CD62L-细胞群占比低于对照组CAR-T-1。说明改造后的CD123抗原识别域具有更好的CAR-T细胞持久性。

[0065] 实施例4:改造后CD123 CAR-T在体外对细胞系的杀伤效率

[0066] 本实施例以检测具有不同抗原识别域的CD123 CAR-T对表达CD123和不表达CD123的肿瘤细胞系的杀伤效率为例。

[0067] 分别收集并离心表达CD123的细胞系THP-1,以及不表达CD123的细胞系K562,用1毫升生理盐水(河北天成药业有限公司,中国)重悬,加5微升Calcein-AM(浓度 $1 \mu\text{g}/\mu\text{L}$, Cat# C3100MP, ThermoFisher, USA),轻轻混匀,置于37℃培养箱中孵育30分钟,以标记靶细胞。孵育结束后,使用生理盐水清洗2次,加X-VIVO 15重悬并计数。向48孔细胞培养板(Corning Incorporated, Corning, NY, USA)中,每孔加入 1×10^5 个上述标记的靶细胞,并以E:T=10:1的比例加入各组CAR-T细胞,置于37℃、5%CO₂培养箱中孵育5小时。孵育完成后,加2%浓度的Triton-X-100 (Cat#T8787-100ML, Sigma, Germany)裂解阳性对照组,即 1×10^5 个上述标记的靶细胞;每孔分别取100微升杀伤体系上清于酶标板中,使用多功能酶标仪(Varioscan Lux, ThermoFisher)检测荧光值(激发波长:495nm,发射波长:515nm)。

[0068] 图4显示,对于CD123阳性细胞系THP-1,具有不同抗原识别域的CD123 CAR-T中CAR-T-3和CAR-T-5的杀伤效率,较对照组CAR-T-1显著升高。同时,各组改造后CD123 CAR-T和对照组CAR-T-1,对不表达CD123的K562细胞系的杀伤效率,与T细胞组一致。说明各组CD123 CAR-T细胞对靶细胞均有一定杀伤效应,并有较好的特异性,且CAR-T-3和CAR-T-5的scFv设计更有利于CAR-T杀伤肿瘤细胞。

[0069] 实施例5:改造后CD123 CAR-T在体外对AML原代细胞的杀伤效率

[0070] 本实施例以检测具有不同抗原识别域的CD123 CAR-T对AML原代细胞的杀伤效率为例。

[0071] 取3个分别为高抗原密度(即AML 1#, CD123表达率为99.87%, MFI为13298)、中抗原密度(即AML 2#, CD123表达率为75.4%, MFI为7518)、低抗原密度(即AML 3#, CD123表达率为94.58%, MFI为5252)的CD123阳性AML患者骨髓血样(抗原密度水平定义如下,高抗原密度:中位荧光强度(Median Fluorescence Intensity, MFI)大于10000;中抗原密度:MFI大于6000小于10000;低抗原密度:MFI小于6000),将AML患者的骨髓血样本分离单个核细胞,并使用CD34 MicroBeads (Cat#130-100-453, 美天旎, 德国)分选出CD34阳性AML原代细

胞,用1毫升生理盐水重悬,加10微升Calcein-AM(浓度1 μ g/ μ L,ThermoFisher,USA),轻轻混匀,置于37℃培养箱中孵育30分钟,以标记AML原代细胞。孵育结束后,使用生理盐水清洗2次,加X-VIVO 15重悬并计数。向48孔细胞培养板(Corning Incorporated,Corning,NY,USA)中,每孔加入1 $\times 10^5$ 个上述标记的AML原代细胞,并以E:T=10:1的比例,分别加入CD123 CAR-T细胞,置于37℃、5%CO₂培养箱中孵育5小时。孵育完成后,加2%浓度的Triton-X-100裂解阳性对照组,即1 $\times 10^5$ 个上述标记的AML原代细胞;每孔分别取100微升杀伤体系上清于酶标板中,使用多功能酶标仪(Varioscan Lux,ThermoFisher)检测荧光值(激发波长:495nm,发射波长:515nm)。

[0072] 图5显示,与对照组CAR-T-1相比,具有不同抗原识别域的CAR-T-3对3个高(即AML 1#)、中(即AML 2#)、低(即AML 3#)抗原密度的CD123阳性AML患者的原代细胞均具有显著增强的杀伤效率。说明具有CAR-T-3scFv的CD123 CAR-T细胞,能够有效提高对原代细胞的杀伤效率。

[0073] 实施例6:改造后CD123 CAR-T在靶细胞刺激下的细胞因子表达

[0074] 本实施例以检测具有不同抗原识别域的CD123 CAR-T在靶细胞AML原代细胞刺激下的细胞因子表达水平为例。

[0075] 向48孔细胞培养板(Corning Incorporated,Corning,NY,USA)中,每孔加入1 $\times 10^5$ 个靶细胞AML原代细胞,此AML原代细胞的CD123表达率为99.87%、MFI为13298,并以E:T=10:1的比例加入各组CAR-T细胞,置于37℃、5%CO₂培养箱中孵育5小时。取细胞上清,使用CBA试剂盒(BD Biosciences, San Jose, CA, USA)和流式细胞仪(NovoCyte 2060R, ACEA Biosciences, San Diego, CA, USA)检测各种细胞因子的表达水平。

[0076] 图6显示,与对照组CAR-T-1相比,具有不同抗原识别域的CAR-T-6组,在靶细胞刺激下,IL-2、TNF、IFN- γ 的表达水平明显提高;其余各组CAR-T细胞的细胞因子表达,与对照组CAR-T-1相近。说明CAR-T-6的scFv设计有利于CD123 CAR-T细胞因子的表达。

[0077] 以下表1和表2中为本发明中涉及的具体氨基酸序列。

[0078] 表1示例性抗体的CDR和scFv序列

[0079]

克隆 ID.	SEQ ID NO	氨基酸序列
scFv-01	VH-CDR1	7 GYTFTSYWMN
	VH-CDR2	13 RIDPYDSETHYNQKFKDKA
	VH-CDR3	19 ARGNWDDY
	VL-CDR1	25 ASKSISKDLAW
	VL-CDR2	31 GSTLQSG
	VL-CDR3	37 QHINKYPYTF
	VH	43 QVQLQQPGAELVRPGASVKLSCKASGYTFTSYWMNWWKQRPDQGLEWIGRIDPY DSETHYNQKFKDKAILTVDKSSSTAYMQLSSLTSEDSAVYYCARGNWDDYWGQG TTLTVSS
	VL	49 DVQITQSPSYLAASPGETITINCRASKSISKDLAWYQEKPGKTNKLLIYSGSTLQSG IPSRFSGSGSGTDFTLTISLEPEDEAFMYCQQHINKYPYTFGGGTGLEIK
	scFv	55 QVQLQQPGAELVRPGASVKLSCKASGYTFTSYWMNWWKQRPDQGLEWIGRIDPY DSETHYNQKFKDKAILTVDKSSSTAYMQLSSLTSEDSAVYYCARGNWDDYWGQG

[0080]

			TTLTVSSGGGSGGGGSGGGGSDVQITQSPSYLAASPGETTINCRAKSSISKDLAW YQEKPGKTNKLLIYSGTLQSGIPSRFSGSGSGTDFTLTSSLEPEDFAMYYCQQHN KYPYTFGGGKLEIK
scFv-02	VH-CDR1	8	GYSFTDYYVH
	VH-CDR2	14	YISCYNGATNYPKFKGKA
	VH-CDR3	20	AGGEGYYGYDGYAMDY
	VL-CDR1	26	ASQDINKYIAW
	VL-CDR2	32	TSTLQPG
	VL-CDR3	38	QYDDLTYF
	VH	44	QVQLKQSGPELVKGTASVKMSCKASGYSTFDYYVHWVRQSHGKSLEWIGYISCY NGATNYPKFKGKATFTVDTSSSTAYMQFKSLTSEDSAVYYCAGGEGYYGYDGYA MDYWGQGTSTVTVSS
	VL	50	DIQLTQSPSSLSASLGGKVITITCKASQDINKYIAWYQHKPGKPRLLIHYTSTLQPGI PSRFSGSGSGGDYFSISNLEPEDIATYYCLQYDDLTYFGGGKLEIK
scFv-03	scFv	56	QVQLKQSGPELVKGTASVKMSCKASGYSTFDYYVHWVRQSHGKSLEWIGYISCY NGATNYPKFKGKATFTVDTSSSTAYMQFKSLTSEDSAVYYCAGGEGYYGYDGYA MDYWGQGTSTVTVSSGGGSGGGGSGGGGSDIQLTQSPSSLSASLGGKVITITCKAS QDINKYIAWYQHKPGKPRLLIHYTSTLQPGIPSRFSGSGSGGDYFSISNLEPEDIA TYYCLQYDDLTYFGGGKLEIK
	VH-CDR1	9	GYTFMTYVIH
	VH-CDR2	15	YCNPNYNDGINYNEKFKGKA
	VH-CDR3	21	ARSPSYGRSYYYGMDY
	VL-CDR1	27	ASQSDYDGDSYMNW
	VL-CDR2	33	ASNLESG
	VL-CDR3	39	QSNEDPYTF
	VH	45	QVQLKQSGPELVKPGASVKMSCKASGYTFMTYVIHVVKQKPGQGLEWFGYCNP YNDGINYNEKFKGKATLTSKSSSTVYMESSLTSEDSAVYYCARSPSYGRSYYY GMDYWGQGTSTVTVSS
scFv-04	VL	51	DIQLTQSPASLAVSLGQRATISCKASQSDYDGDSYMNWYQKPGQPPKVLIIAAS NLESGIPARFSGSGSGTDFTLNIHPVEEEDAATYYCQQSNEDPYTFGGGKLDIK
	scFv	57	QVQLKQSGPELVKPGASVKMSCKASGYTFMTYVIHVVKQKPGQGLEWFGYCNP YNDGINYNEKFKGKATLTSKSSSTVYMESSLTSEDSAVYYCARSPSYGRSYYY GMDYWGQGTSTVTVSSGGGSGGGGSGGGGSDIQLTQSPASLAVSLGQRATISCKA SQSDYDGDSYMNWYQKPGQPPKVLIIAASNLESGIPARFSGSGSGTDFTLNIHP VEEEDAATYYCQQSNEDPYTFGGGKLDIK
	VH-CDR1	10	GYSITSDYAWN
	VH-CDR2	16	YISYSGSSNPNLSKSRI
	VH-CDR3	22	ARGMDY
	VL-CDR1	28	ASSSVSSYLHW
	VL-CDR2	34	TSNLSAG
	VL-CDR3	40	QFHRSPTF
scFv-05	VH	46	DVKLQESGPGLVKPSQSLSLTCTVTGYSITSDYAWNWRQFPGNKLEWMGYISYSG SSNPNLSKSRI SITRDTSKNQFFLQLNSVTTEDATYYCARGMDYWGQGTSTVTVSS
	VL	52	DIVLTQSPAIMSVFLGERVTMTCTASSSVSSYLHWYQKPGSSPKLWIYSTNLAS GVPARFSGSGSGTSYSLTSSMEAEADAATYYCHQFHRSPTFGGGKLEIK
	scFv	58	DVKLQESGPGLVKPSQSLSLTCTVTGYSITSDYAWNWRQFPGNKLEWMGYISYSG SSNPNLSKSRI SITRDTSKNQFFLQLNSVTTEDATYYCARGMDYWGQGTSTVTVSS SGGGSGGGGSGGGSDIVLTQSPAIMSVFLGERVTMTCTASSSVSSYLHWYQK KPGSSPKLWIYSTNLASGVPARFSGSGSGTSYSLTSSMEAEADAATYYCHQFHRS PTFGGGKLEIK
	VH-CDR1	11	GYTFSSYNMH
	VH-CDR2	17	YTPGNGGTNYNQKFKGKA
	VH-CDR3	23	ARERGLYDYDETGYAMDY
	VL-CDR1	29	ASQSISDYLHW
	VL-CDR2	35	ASQSISE
scFv-05	VL-CDR3	41	NGHSFPYTF
	VH	47	QVQLKQSGAELVRSGASVKMSCKASGYTFSSYNMHVWKQTPGQGLEWIGYTPG NGGTNYPKFKGKATLTADTSSSTAYMQISSLTSEDSAVYFCARERGLYDYDETGY YAMDYWGQGTSTVTVSS
	VL	53	DIQLTQSPATLSVTPGDRVSLSCRASQSDYDGDYQKSHESPRLLIKYASQSISE IPSRFSGSGSGDFTLINSVEPEDVGYYFCQNGHSFPYTFGGGKLEIK
	scFv	59	QVQLKQSGAELVRSGASVKMSCKASGYTFSSYNMHVWKQTPGQGLEWIGYTPG NGGTNYPKFKGKATLTADTSSSTAYMQISSLTSEDSAVYFCARERGLYDYDETGY YAMDYWGQGTSTVTVSSGGGSGGGGSGGGGSDIQLTQSPATLSVTPGDRVSLSCR

[0081]

			ASQSIDYLVHWYQQKSHESPRLLIKYASQSISEIPSRFSGSGSGSDFTLINSVEPEDV GVYFCQNGHSFPYTFGGGKLEIK
scFv-06	VH-CDR1	12	GYSFTGYMH
	VH-CDR2	18	RINPYNNATSYNQNFKDKA
	VH-CDR3	24	ARGEGWLLPLAC
	VL-CDR1	30	SSQSLNSSNQKNYLAW
	VL-CDR2	36	ASTRDSG
	VL-CDR3	42	QHYSTPWTF
	VH	48	QVQLKQSGPELVKSGASVKISCKASGYSTGYMHVWKQSHVKSLEWIGRINPYN NATSYNQNFKDKASLTVDKSSSTAYMELHSLTSEDSAVYYCARGEGWLLPLACW GGGTLTVSA
	VL	54	DIVMTQSPSSLAMSVGQKVTMSCKSSQSLNSSNQKNYLAWYQQKPGQSPKLLV YFASTRDSGVPDRFIGSGSGTDFLTISVQAEDLADYFCQQHYSTPWTFGGGKLEIK
scFv-07	scFv	60	QVQLKQSGPELVKSGASVKISCKASGYSTGYMHVWKQSHVKSLEWIGRINPYN NATSYNQNFKDKASLTVDKSSSTAYMELHSLTSEDSAVYYCARGEGWLLPLACW GGGTLTVSAAGGGSGGGSGGGSDIVMTQSPSSLAMSVGQKVTMSCKSSQSL LNSSNQKNYLAWYQQKPGQSPKLLVYFASTRDSGVPDRFIGSGSGTDFLTISVQA EDLADYFCQQHYSTPWTFGGGKLEIK
	VH-CDR1	8	GYSFTDYYVH
	VH-CDR2	14	YISCYNGATNYPKFKGKA
	VH-CDR3	20	AGGEGYYGYDGYAMDY
	VL-CDR1	26	ASQDINKYIAW
	VL-CDR2	32	TSTLQPG
	VL-CDR3	38	QYDDLTYF
	VH	44	QVQLKQSGPELVKTGASVKMSCKASGYSTDYYVHWVRQSHGKSLEWIGYISCY NGATNYPKFKGKATFTVDTSSSTAYMQFKSLTSEDSAVYYCAGGEGYYGYDGYA MDYWGQGTSTVTVSS
scFv-07	VL	50	DIQLTQSPSSLSASLGKVTITCKASQDINKYIAWYQHKPGKPRLLIHYTSTLQPGI PSRFSGSGGGDYFSISNLEPEDATYYCLQYDDLTYFGGGKLEIK
	scFv	61	DIQLTQSPSSLSASLGKVTITCKASQDINKYIAWYQHKPGKPRLLIHYTSTLQPGI PSRFSGSGGGDYFSISNLEPEDATYYCLQYDDLTYFGGGKLEIKGGGGSGGGG SGGGGSQVQLKQSGPELVKTGASVKMSCKASGYSTDYYVHWVRQSHGKSLEWI GYISCYNGATNYPKFKGKATFTVDTSSSTAYMQFKSLTSEDSAVYYCAGGEGYY GYDGYAMDYWGQGTSTVTVSS

[0082] 表2示例性嵌合抗原受体序列

[0083]

克隆 ID	SEQ ID NO	氨基酸序列
4-1BB	62	KRGRKLLYIFKQPFMRPVQTTQEEDGCSCRFEEEEGGCEL
CD3 ζ	63	RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEM GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDG LYQGLSTATKDTYDALHMQALPPR
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CAR-4	67	MLLLVTSLLLCELPHPAFLLIPNWSHPQFEKGGGSGGGGSGDVKLQESGPG LVKPSQSLSLTCTVTGYSITSDYAWNWRQFPGNKLEWGMGYSYSGSSNSNP SLKSRISITRDTSKNQFFLQLNSVTTEDATYYCARGMDYWGQGSTVTVSSG GGGSGGGGSGGGGSDIVLTQSPAIMSVFLGERVTMTCTASSVSSSYLHWYQ QKPGSSPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISSMEAEDAATYYCHQ FHRSPRTFGGGTKEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYWAPLAGTCGVLLLSLVITLYCKRGRKLLYIFKQPFMRPVQT TQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREE YDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR RGKGGHDGLYQGLSTATKDTYDALHMQALPPR
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[0086] 参考文献:

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- [0001] 序列表
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[0812]	Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Gly Gly Glu Gly Tyr Tyr
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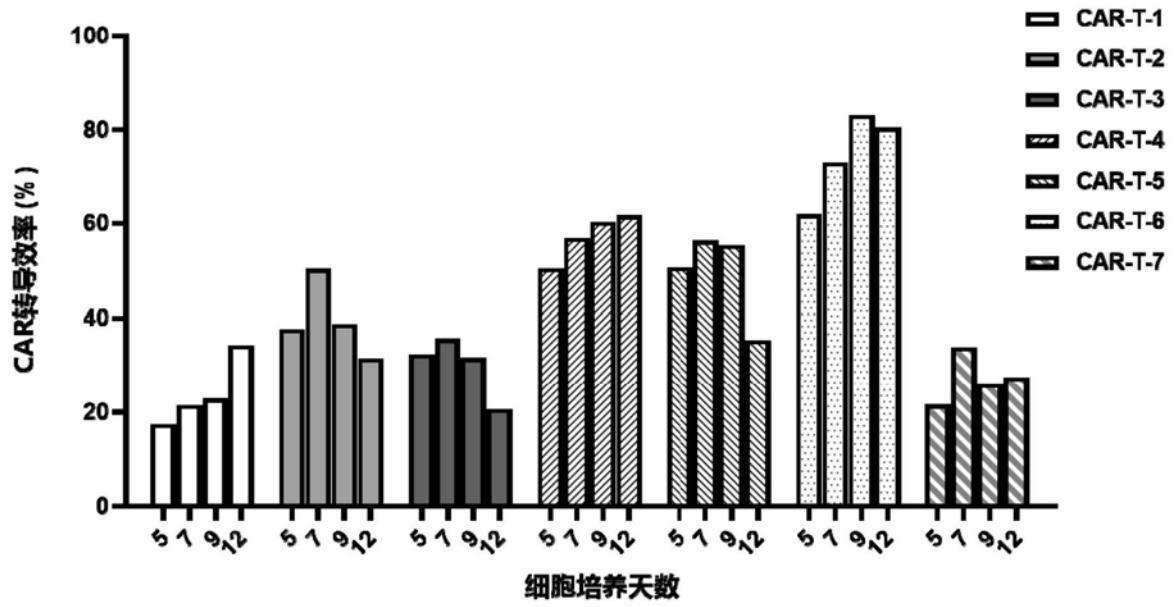


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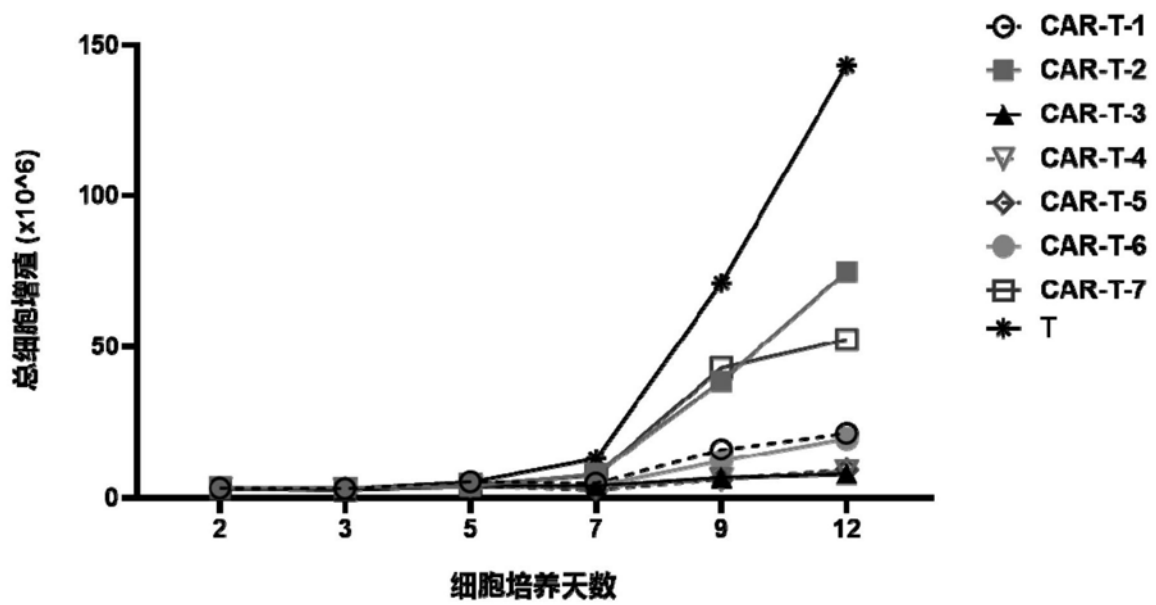


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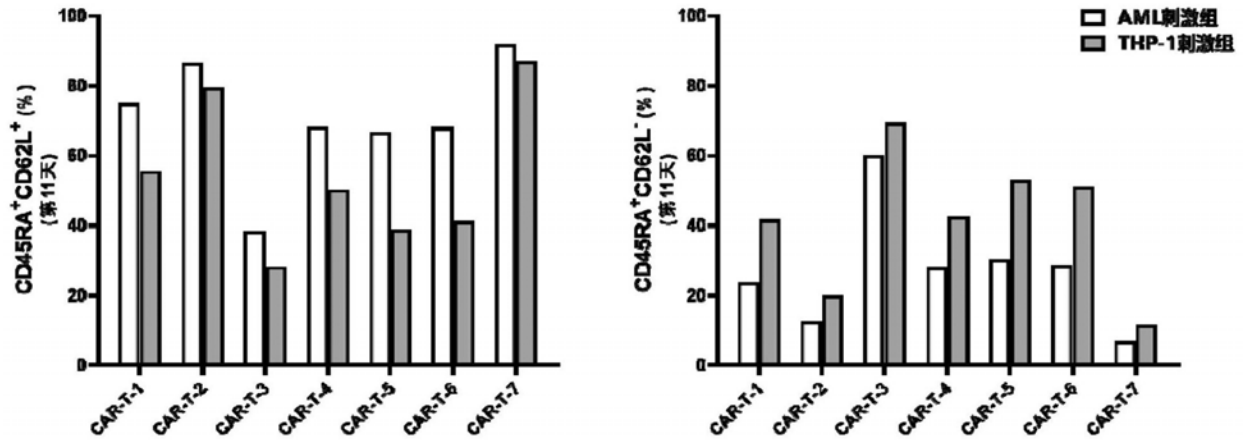


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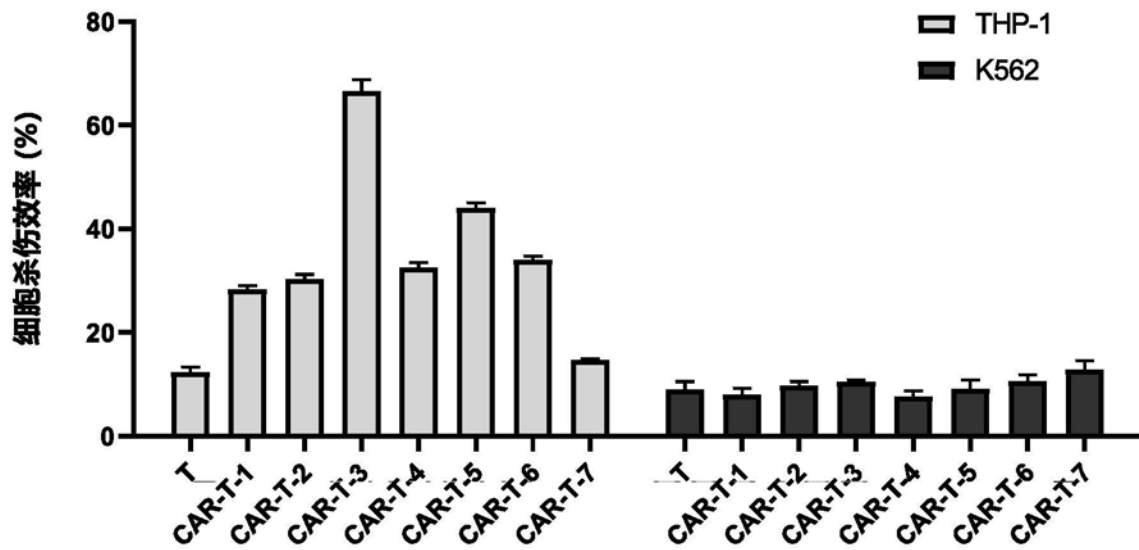


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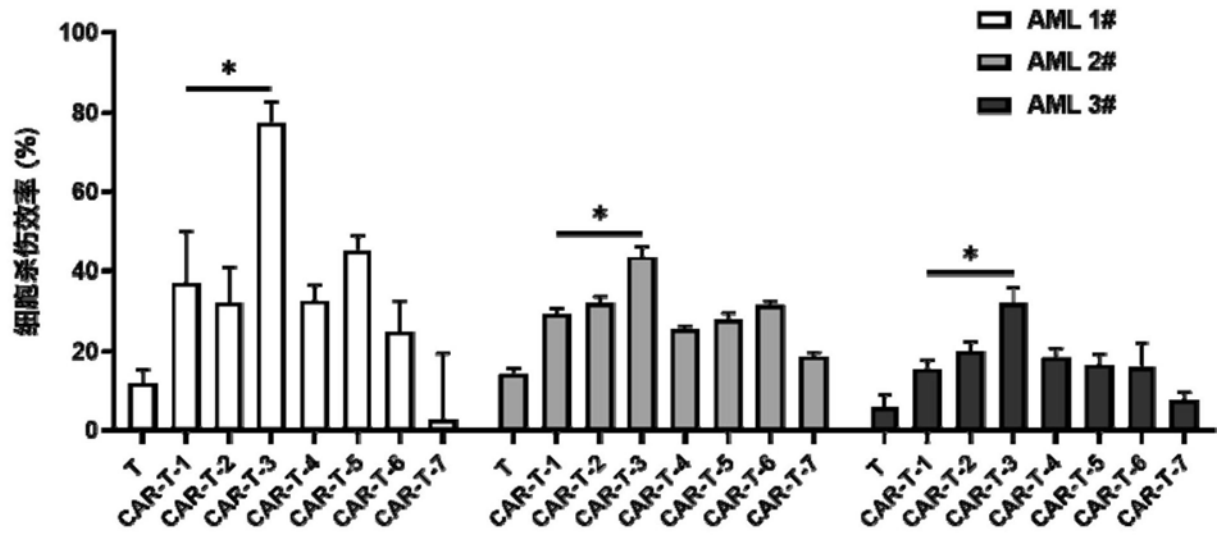


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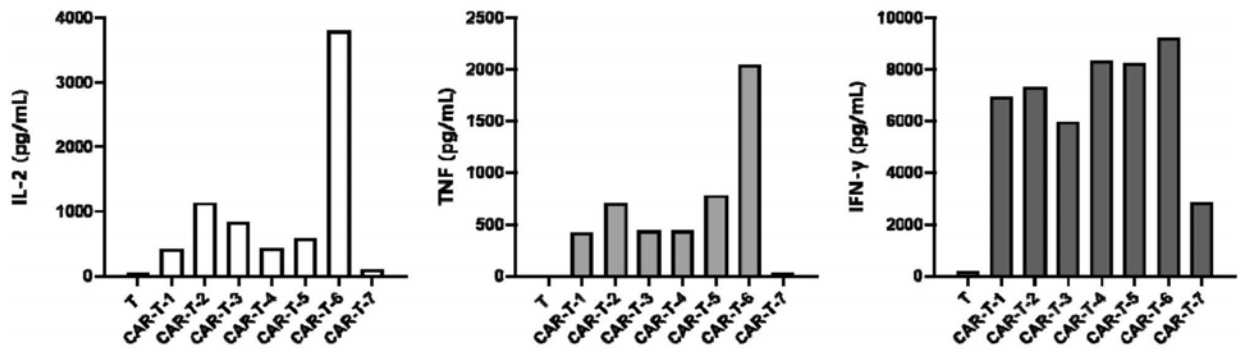


图6