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· 基础研究 ·

靶向B细胞成熟抗原的嵌合抗原受体T细胞的构建及其对肿瘤细胞的杀伤

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[摘要] **目的:**探索通过嵌合抗原受体(chimeric antigen receptor, CAR)-T细胞靶向B细胞成熟抗原(B cell maturation antigen, BCMA)以治疗多发性骨髓瘤(multiple myeloma, MM)的方法。**方法:**构建基于鼠源BCMA scFv的CAR-BCMA分子,包装为慢病毒载体并感染健康人T细胞构建CAR-BCMA-T细胞;构建BCMA阳性细胞系A549-BCMA、A549-BCMAOFP和K562-BCMA作为靶细胞。将CAR-BCMA-T细胞与构建的靶细胞和人骨髓瘤细胞U266共孵育,CCK-8法和流式细胞术检测其对BCMA阳性肿瘤细胞的杀伤能力。构建MM患者来源CAR-BCMA-T细胞并检测其杀伤靶细胞A549-BCMA的能力,并采用ELISA和流式细胞术检测CAR-BCMA-T细胞IFN- γ 的释放水平。**结果:**健康人来源的CAR-BCMA-T经过11 d培养扩增300倍,阳性率达到43%;成功构建BCMA阳性靶细胞。在5:1效靶比下,CAR-BCMA-T对A549-BCMA、K562-BCMA和U266细胞的杀伤率分别在80%、60%和80%左右,显著高于对BCMA阴性细胞的杀伤率,且杀伤力与靶细胞的BCMA表达强度相关。在效靶比20:1时,MM患者来源CAR-BCMA-T细胞对靶细胞A549-BCMA的杀伤率达到95%以上,并且大量分泌IFN- γ 。**结论:**本研究成功构建了健康人及MM患者来源的靶向BCMA的CAR-T细胞,其能够有效特异杀伤BCMA阳性的肿瘤细胞。

[关键词] B细胞成熟抗原;嵌合抗原受体;多发性骨髓瘤; γ 干扰素;杀伤功能

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Construction of anti-BCMA chimeric antigen receptor (CAR-BCMA) modified T cells and its cytotoxicity against tumor cells

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[Abstract] Objective: To explore a novel chimeric antigen receptor (CAR)-T cell treatment to treat Multiple Myeloma (MM) via target B cell maturation antigen (BCMA). **Methods:** A CAR-BCMA molecular was constructed based on mouse originated BCMA scFv, and was packaged into lentiviral vector and transfected into T cells from healthy donors to construct CAR-BCMA-T cells. The BCMA positive cell lines A549-BCMA, A549-BCMAOFP and K562-BCMA were constructed as target cells. Then, the CAR-BCMA-T cells were co-incubated with the constructed target cells and human myeloma U266 cells, and the cytotoxic effects of CAR-BCMA-T cells were evaluated *via* CCK-8 and FACS. Finally, the CAR-BCMA-T cells originated from MM patients were constructed, and its cytotoxicity against A549-BCMA were examined; in addition, the IFN- γ release level in CAR-BCMA-T cells was evaluated by ELISA and FACS. **Results:** After 11 days' incubation, the CAR-BCMA-T cells originated from healthy donors amplified 300 times with a positive rate of 43%. The BCMA positive target cell lines were constructed successfully. Under an effector : target ratio of 5:1, the killing rates of CAR-BCMA-T cells against A549-BCMA, K562-BCMA and U266 were about 80%, 60%, and 80%, respectively, which were significantly

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higher than those against BCMA negative cells; and the cytotoxicity was related to the BCMA expression level in target cells. What's more, at the effector : target ratio of 20:1, the CAR-BCMA-T cells originated from MM patients were demonstrated to exhibit a killing rate of more than 95% against A549-BCMA positive cells, and produced large amount of IFN- γ . **Conclusion:** CAR-BCMA-T cells originated from both healthy and MM donors were successfully constructed, and they can effectively and specifically kill BCMA positive tumor cells.

[Key words] B cell maturation antigen (BCMA); chimeric antigen receptor; multiple myeloma; IFN- γ ; cytotoxicity

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嵌合抗原受体(chimeric antigen receptor, CAR)T细胞是近年来发展起来的一种新型免疫细胞治疗技术。CAR-T细胞通过胞外的识别区识别肿瘤细胞表面的特异性抗原,之后通过胞内信号转导区激活T细胞的增殖和杀伤靶细胞的能力,从而清除肿瘤^[1-2]。CAR-T细胞胞外识别区大多为单链抗体(single chain variable fragment, scFv),通过抗体特异性和高亲和力的与肿瘤特异性抗原结合。当前靶向CD19的CAR-T在治疗B细胞恶性病变领域取得了突破性成果,但是在其它的肿瘤中由于缺乏合适的靶点,效果并不理想^[3-4]。

多发性骨髓瘤(multiple myeloma, MM)是一种无法治愈的恶性肿瘤,其特点是患者骨髓中浆细胞恶性增生。当前主要使用蛋白酶体抑制剂、免疫调节剂等来缓解多发性骨髓瘤的症状,但是均不能彻底清除肿瘤^[5]。当前一些研究通过靶向CD138、CD38、 κ -轻链、BCMA的CAR-T疗法来治疗MM^[5]。有研究^[6]报道了使用靶向CD138的CAR-T疗法治疗一例MM患者得到了短时间内的部分缓解,另有研究^[7-8]验证了通过靶向CD38分子可以有效杀伤骨髓瘤细胞。

B细胞成熟抗原(B cell maturation antigen, BCMA)是一种表达在浆细胞,浆母细胞和骨髓浆细胞的抗原,其不在B细胞或者造血干细胞上表达^[9]。这种有限的表达细胞类型,使得BCMA可能作为治疗MM的靶点,以开发单抗、抗体偶联药物、双特异性抗体、CAR-T等疗法^[10-12]。本课题设计了一种新型靶向BCMA的CAR分子,将其构建在慢病毒载体,随后构建成CAR-BCMA-T细胞,通过杀伤实验证明了CAR-BCMA-T细胞能够特异性杀伤多种靶细胞,并且大量分泌杀伤和增殖能力相关的细胞因子。

1 材料与方法

1.1 细胞系及其培养

慢病毒包装细胞株Lenti X-293T细胞系购自大连宝生物公司,其培养在DMEM培养基(添加10%胎牛血清和1%青霉素-链霉素溶液)。人慢性髓系白血病细胞K562以及K562-BCMA细胞系由本公司保存,其培养在RPMI 1640培养基(添加10%胎牛血

清)。人骨髓瘤细胞U266细胞系购买自ATCC,其培养在RPMI 1640培养基(添加10%胎牛血清)。PBMC由健康志愿者提供。A549细胞购买自中科院上海细胞库,其培养在DMEM培养基中(添加10%胎牛血清)。

1.2 质粒构建

由上海生工生物工程有限公司合成BCMA scFv序列,将序列由BamHI/NheI双酶切,连接进入载体pHAGE-CAR-CD19-BBZ-IZSGreen中,构建为pHAGE-CAR-BCMA-BBZ-IZSGreen质粒。BCMA带有OFP编码框全长cDNA序列(购买自义翘神州公司),通过常规手段克隆进入pHAGE-MCS-PURO载体中,构建为pHAGE-BCMA-puro, pHAGE-BCMAOFP-puro质粒,用于BCMA阳性靶细胞的制备。

1.3 慢病毒包装与滴度检测

将pHAGE-CAR-BCMA-BBZ-IZSGreen质粒与辅助质粒pMD2.G与psPAX2通过磷酸钙试剂共转染至293T细胞,转染后16h换液,48h收取培养上清即为病毒原液。将病毒原液在超速离心2h,40 000 \times g,将沉淀溶解在X-VIVO-15培养液中。

293T细胞铺到24孔板中,2 \times 10⁵细胞/孔,将纯化后的病毒液稀释100倍,加入各孔分别50、10、2 μ l。感染24h后换液,48h后收取细胞,通过APC-Fab抗体染色,通过流式检测APC阳性细胞比例,计算病毒滴度。病毒滴度(TU/ml)=感染时细胞数量 \times 阳性率 \times 稀释倍数 \times 1 000/加入病毒体积(μ l)。

1.4 CAR-BCMA-T细胞的制备、扩增和表型鉴定

从健康志愿者全血中分离PBMC,重悬于X-VIVO-15中,添加10%FBS,同时加入OKT3/IL-2激活T细胞增殖,激活后第2天加入慢病毒(MOI=5)。第3天通过离心洗去慢病毒,重悬于X-VIVO-15培养基中,加入5%FBS和1 000 IU/ml IL-2继续扩增,每2天进行计数,使细胞密度维持在1 \times 10⁶/ml左右。扩增后第7~10d,取部分细胞进行阳性率检测以及功能检测。通过APC-兔抗鼠Fab(购买自Jackson lab)染色检测CAR阳性率。通过APC-CCR7(购买自BioLegend),PE-CD45RO(BioLegend, US)检测中心记忆细胞表型。

为了进一步验证来源MM患者T细胞能否构建成CAR-BCMA-T细胞以及杀伤靶细胞能力,从江

苏省中医院获得MM患者血液3份,分别分离PBMC并构建为CAR-BCMA-T细胞。

1.5 靶细胞的构建

将质粒pHAGE-BCMA-puro、pHAGE-BCMAOFP-puro包装为慢病毒,感染A549细胞系,之后通过嘌呤霉素筛选,得到BCMA阳性细胞系A549-BCMA、A549-BCMAOFP。使用该慢病毒感染K562细胞系,通过嘌呤霉素筛选,得到细胞系K562-BCMA。

1.6 CCK-8法、流式细胞术检测CAR-BCMA-T细胞对靶细胞的杀伤能力

基于CCK8的杀伤实验:将CAR-BCMA-T细胞与A549、A549-BCMA、A549-BCMAOFP等靶细胞以不同效靶比共孵育,16 h后将上清去除,通过PBS冲洗掉效应细胞,加入X-VIVO-15/5% FBS/10% CCK-8,37 °C培养1~4 h,检测吸光度 D_{450} 值。杀伤效率=(未杀伤对照-杀伤样品)/(未杀伤对照-空白)×100%。

基于ANNEXIN-V/PI染色的杀伤实验:将CAR-BCMA-T细胞制备成悬液,使用CFSE染色,之后与靶细胞K562、K562-BCMA、U266以不同效靶比混合。4 h后混合细胞离心,重悬于PC-ANNEXIN-V/PI染色液中,10 min后终止染色,流式细胞仪检测凋亡和坏死细胞比例,其中APC和PE双阴性区域细胞为活细胞。杀伤率=(未杀伤组活细胞-杀伤组活细胞)/未杀伤组活细胞×100%。

1.7 ELISA、流式细胞术检测CAR-BCMA-T细胞的IFN- γ 分泌水平

培养上清中细胞因子检测:将MM来源CAR-BCMA-T细胞T20,CAR20,CAR22,CAR24与A549,A549-BCMA等细胞以效靶比5:1共孵育4 h,取上清通过IFN- γ ELISA试剂盒(BD,货号550612,USA),检测IFN- γ 分泌水平,具体操作步骤见说明书。

CAR-BCMA-T细胞内IFN- γ 检测:将CAR-BC-

MA-T细胞与A549,A549-BCMA等细胞以效靶比5:1共孵育4 h,同时将CAR-BCMA-T细胞与PMA(20 ng/ml),离子霉素(ionomycin)(1 μ g/ml)共孵育以作为阳性激活对照。之后离心弃上清,PBS重悬并加入CD3抗体染色,洗去抗体,通过透膜缓冲液使细胞膜通透,加入IFN- γ 抗体染色4 °C,30 min;PBS洗去抗体,进行FACS检测IFN- γ 胞内表达情况。

1.8 统计学处理

本研究中数据统计和作图由软件GraphPad Prism 6完成,计量数据以 $\bar{x}\pm s$ 表示,数据统计使用t检验分析进行,以* $P<0.05$ 或** $P<0.01$ 表示具有统计学意义。

2 结果

2.1 CAR-BCMA-T细胞的成功制备

本研究首先构建了靶向BCMA的CAR分子,其结构为经典第二代CAR分子结构,将CD8引导区、BCMA scFv、CD8 铰链区和跨膜区、4-1BB胞内区和CD3 ζ 胞内区依次串联,具体见图1。

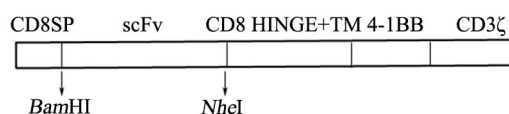
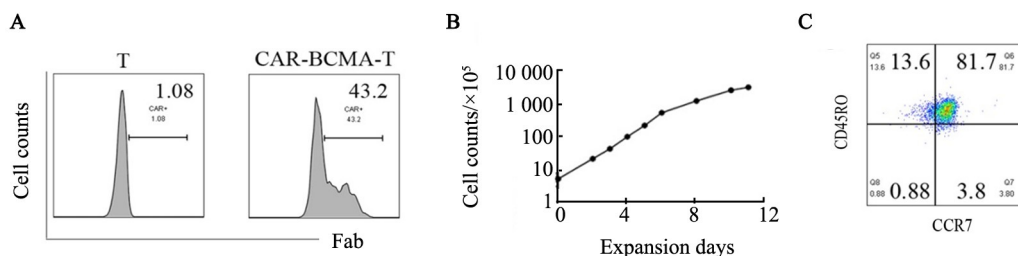


图1 CAR分子结构示意图

Fig.1 The schematics of CAR molecule

将上述分子包装成为慢病毒,使用慢病毒转染T细胞,3 d后通过anti-Fab抗体检测病毒转染效率。结果如图2所示,转染效率为43.2%。经过11 d扩增,细胞增殖达300倍左右。

中心记忆T细胞在体内存活时间更长,其比例与临床效果相关^[13-14]。检测结果显示,经过11 d扩增后中心记忆细胞比例依然保持在80%以上(图2C)。



A: CAR expression efficiencies were determined by FACS 4 d after transduction; B: Expansion curve of CAR-BCMA-T cells;

C: Phenotypes of CAR-BCMA-T cells were determined using CCR7 and CD45RO antibodies by FACS

图2 CAR-BCMA-T细胞制备和扩增的鉴定

Fig.2 Construction of CAR-BCMA-T cells and verification of its expansion

2.2 CAR-BCMA-T有效杀伤BCMA阳性靶细胞

为了进一步验证CAR-BCMA-T细胞对于BC-

MA阳性肿瘤细胞的杀伤能力,基于BCMA阴性肿瘤细胞A549,K562构建了靶细胞A549-BCMA、A549-

BCMOFP、K562-BCMA。流式检测结果如图3所示, A549和K562细胞不表达BCMA, 但是A549-BC-

MA和A549-BCMA-OFP、K562-BCMA和U266细胞呈BCMA阳性, 且前者表达强度均高于后者。

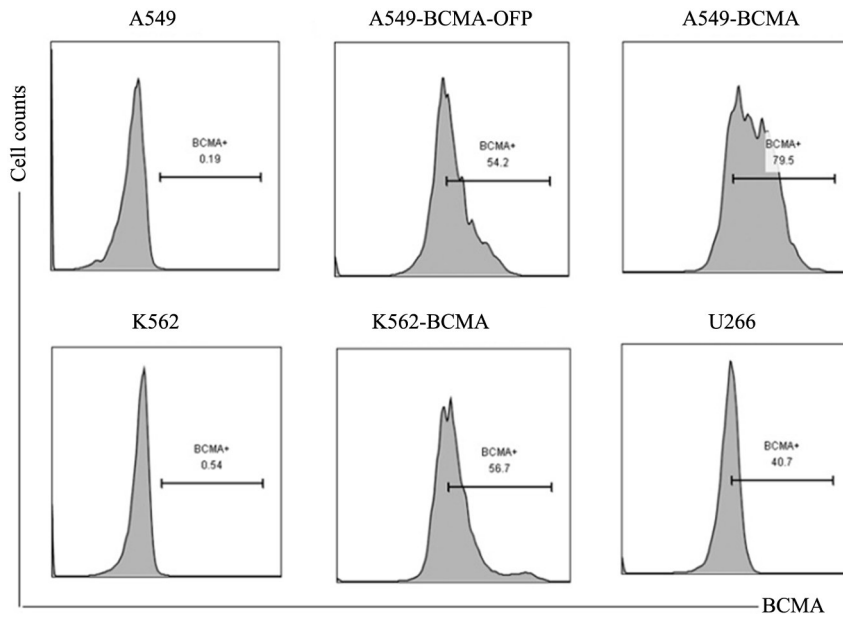


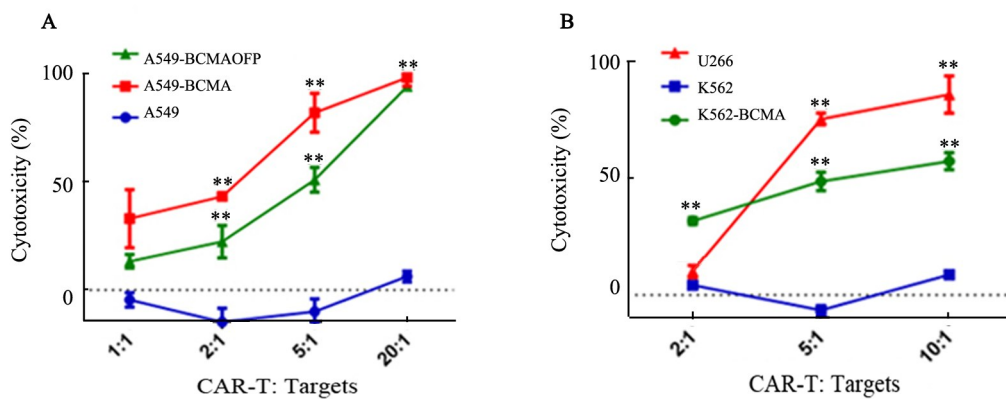
图3 流式细胞术检测不同靶细胞BCMA表达

Fig. 3 Expression of BCMA in various target cell lines by FACS

2.3 CAR-BCMA-T细胞可特异性杀伤BCMA阳性肿瘤细胞

CCK-8实验结果如图4A所示, CAR-BCMA-T在20:1比例下有效杀伤靶细胞A549-BCMA和A549-BCMAOFP, 但是不杀伤BCMA阴性细胞A549。在5:1效靶比下, CAR-BCMA-T杀伤A549-BCMA比例为80%左右, 杀伤A549-BCMA-OFP细胞为50%左

右, 这一结果与BCMA在两个细胞上面的表达水平相一致。CAR-BCMA-T细胞能够有效杀伤K562-BCMA和U266细胞(图4B)。在5:1效靶比时, CAR-BCMA-T杀伤K562-BCMA效率为60%, 杀伤U266效率为80%。以上结果说明由健康志愿者PBMC制备的CAR-BCMA-T细胞能够有效杀伤BCMA阳性肿瘤细胞, 且与BCMA表达强度相关。



**P<0.01 vs A549 cells group

A: The CAR-BCMA-T cells (effector) were co-incubated with A549 (BCMA negative control), A549-BCMA (target), A549-BCMAOFP cells (target) with indicated ratios. The cytotoxic effect was measured by CCK-8 method; B: The CAR-BCMA-T cells (effector) were co-incubated with K562 (BCMA negative control), K562-BCMA (target), U266 cells (target) with indicated ratios. The cytotoxic effect was measured by ANNEXIN-V/PI staining and FACS. The data were analyzed by student *t* test (target vs. control).

图4 CAR-BCMA-T细胞能有效杀伤BCMA阳性肿瘤细胞

Fig. 4 CAR-BCMA-T could effectively kill BCMA positive tumor cells

2.4 MM患者PBMC制备的CAR-BCMA-T细胞能够有效杀伤靶细胞

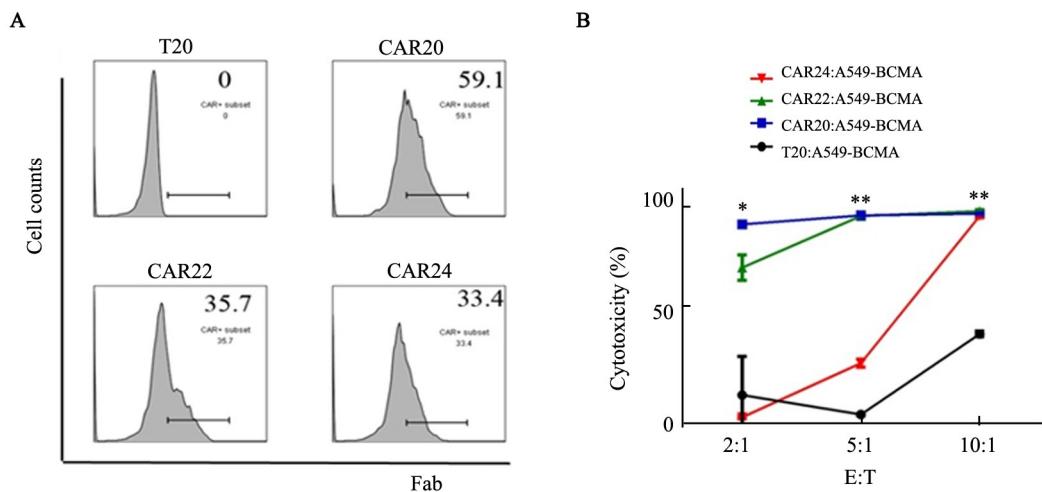
3例患者的CAR-BCMA-T细胞CAR20、CAR22、CAR24阳性率分别为34%、35%、59%。杀伤靶细胞A549-BCMA发现三者均可以有效杀伤靶细胞,在效靶比20:1时,杀伤靶细胞的效率均达到95%以上,但是T细胞不能杀伤A549-BCMA细胞或者杀伤效率比较低,结果详见图5。

2.5 CAR-BCMA-T细胞能大量分泌IFN-γ

分析CAR-BCMA-T细胞杀伤靶细胞过程中细

胞因子IFN-γ的分泌能力,结果如图6所示,在BCMA阳性靶细胞刺激下,不同患者来源CAR-BCMA-T细胞IFN-γ释放增加了50~1 000倍。

在与A549或者不和靶细胞共孵育时,CAR-BCMA-T细胞内没有明显的IFN-γ表达,比例仅为1%~2%;然而与靶细胞A549-BCMA或者直接被PMA/Ionomycin激活时,IFN-γ表达比例达到30%以上,说明CAR-BCMA-T的杀伤能力可以特异性的被BCMA激活。说明CAR-BCMA-T细胞构建成功,且能大量分泌杀伤相关细胞因子IFN-γ(图6)。



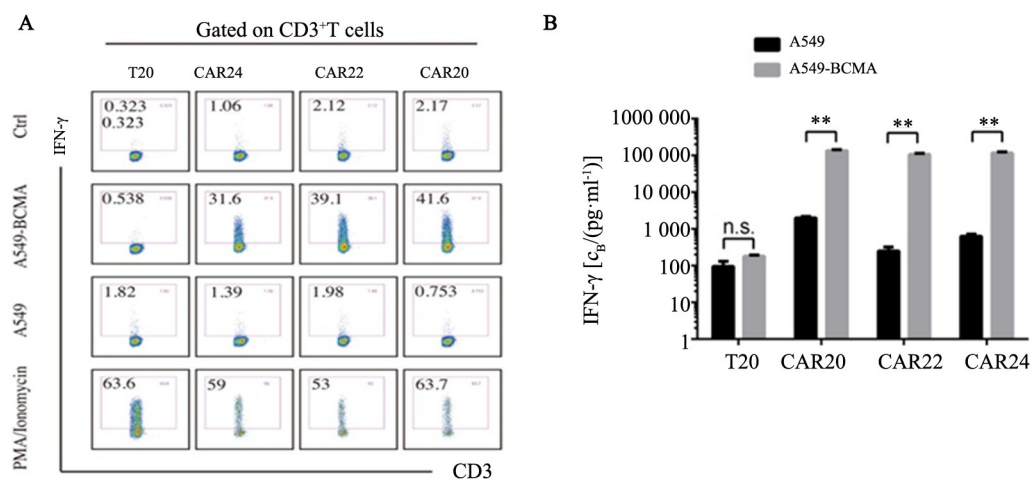
*P<0.05, **P<0.01 vs CAR-BCMA-T cell groups

A: The CAR+ transduction efficiencies were determined using FACS;

B: The cytotoxicity of CAR-BCMA-T cells against A549-BCMA cells; The data were analyzed by student *t* test

图5 MM患者PBMC制备CAR-BCMA-T及其杀伤功能

Fig. 6 Construction of CAR-BCMA-T cells with PBMC from MM patients and its cytotoxicity



*P<0.05, **P<0.01

A: The CAR-BCMA-T cells constructed with PBMC from MM patients were co-incubated with A549, A549-BCMA or PMA/Ionomycin for 4 h, among which the PMA/Ionomycin was served as positive control. Then the cells were permeabilized and stained with CD3 and IFN-γ antibodies, and analyzed by FACS; B: The IFN-γ expression levels in CAR-BCMA-T cells upon co-incubation with A549 or A549-BCMA cells. The data were analyzed by student *t* test

图6 MM患者PBMC构建CAR-BCMA-T细胞及其功能检测

Fig. 6 The CAR-BCMA-T cells constructed with PBMC from MM patients and the detection of its function

3 讨论

最初第一代靶向CD19的CAR-T疗法在患者中并未取得很好的疗效,随着免疫学和T细胞共刺激机制的揭示,新开发的第二代CAR-T在白血病领域取得突破性进展,在一些临床研究中取得了60-90%的患者完全缓解^[1]。但是,CAR-T疗法在其它癌症中并未取得明显突破,一个很重要的原因是缺少理想的靶点^[15-16]。BCMA仅表达于浆细胞表面,以及在多发性骨髓瘤患者的恶性肿瘤表面。重要的是,BCMA不表达在造血干细胞表面。这使得BCMA成为一个近乎理想的MM靶点^[11-12]。最近研究^[12, 17-18]也表明了靶向BCMA的CAR-T能够在体内有效清除骨髓瘤细胞。本研究提供了一种新型的靶向BCMA的CAR分子的构建方法,验证了CAR-BCMA-T细胞能够有效、特异地杀伤BCMA阳性骨髓瘤细胞,MM患者来源的血液能够有效构建为CAR-BCMA-T细胞,并且杀伤BCMA阳性靶细胞,分泌IFN- γ 。

本研究采用的CAR分子结构为第二代结构,共刺激分子为4-1BB。在多个研究中证明4-1BB为信号域的CAR分子相较于CD28为信号域的CAR分子具有更好的体内增殖能力和清除肿瘤能力^[19-21],所以,本研究所采用的CAR可能会优于报道^[9]的以CD28为共刺激域的CAR分子。为了证明CAR-BCMA-T细胞杀伤BCMA阳性靶细胞的能力和特异性,本研究构建了多种BCMA表达的细胞模型,包括A549-BCMA、A549-BCMAOFP、K562-BCMA等。CCK-8实验验证了CAR-BCMA-T细胞杀伤BCMA靶细胞的能力,且杀伤水平与BCMA表达强度正相关,说明了CAR-BCMA-T细胞杀伤靶细胞的BCMA特异性。流式细胞术显示CAR-BCMA-T细胞能够有效杀伤骨髓瘤细胞U266。本研究中的CAR-BCMA-T细胞在10:1时杀伤靶细胞能力达到90%以上。而SMITH等^[18]报道的CAR-BCMA-T在效靶比为20:1其杀伤靶细胞比例大约在60%左右。

为了进一步证明CAR-BCMA-T细胞构建的可行性,本研究使用MM患者血液样品构建了CAR-BCMA-T细胞。3例患者均构建为了CAR-BCMA-T细胞,转染效率达到35-50%,能够有效杀伤BCMA阳性靶细胞,且在BCMA刺激下大量分泌杀伤相关细胞因子IFN- γ ,分泌量达到100 000 pg/ml以上,远高于CARPENTER等^[9]报道的分泌量。

综上,本研究报道了一种靶向BCMA的CAR-T细胞构建方法,并且在多种水平证明了其可行性和有效性,下一步本研究拟进行动物实验验证CAR-BCMA-T在体内的杀瘤效果。

[参考文献]

- [1] SADELAIN M, RIVIERE I, RIDDELL S, et al. Therapeutic T cell engineering[J]. *Nature*, 2017, 545(7655): 423-431. DOI: 10.1038/nature22395.
- [2] JUNE C H, SADELAIN M. Chimeric antigen receptor therapy[J]. *N Engl J Med*, 2018, 379(1): 64-73. DOI: 10.1056/NEJMra1706169.
- [3] JUNE C H, O'CONNOR R S, KAWALEKAR O U, et al. CAR T cell immunotherapy for human cancer[J]. *Science*, 2018, 359(6382): 1361-1365. DOI: 10.1126/science.aar6711.
- [4] MCHAYLEH W, BEDI P, SEHGAL R, et al. Chimeric antigen receptor T-cells: the future is now[J]. *J Clin Med*, 2019, 8(2). pii: E207. DOI: 10.3390/jcm8020207.
- [5] ORMHOJ M, BEDOYA F, FRIGAULT M J, et al. CARs in the lead against multiple myeloma[J]. *Curr Hematol Malig Rep*, 2017, 12(2): 119-125. DOI: 10.1007/s11899-017-0373-2.
- [6] TIAN C, YANG H, ZHU L, et al. Anti-CD138 chimeric antigen receptor-modified T cell therapy for multiple myeloma with extensive extramedullary involvement[J]. *Ann Hematol*, 2017, 96(8): 1407-1410. DOI: 10.1007/s00277-017-3029-3
- [7] DRENT E R, GROEN W, NOORT W A, et al. Pre-clinical evaluation of CD38 chimeric antigen receptor engineered T cells for the treatment of multiple myeloma[J]. *Haematologica*, 2016, 101(5): 616-625. DOI: 10.3324/haematol.2015.137620.
- [8] DRENT E, THEMELI M, POELS R, et al. A Rational strategy for reducing on-target off-tumor effects of CD38-chimeric antigen receptors by affinity optimization[J]. *Mol Ther*, 2017, 25(8): 1946-1958. DOI: 10.1016/j.yymthe.2017.04.024.
- [9] CARPENTER R O, EVBUOMWAN M O, PITTALUGA S, et al. B-cell maturation antigen is a promising target for adoptive T-cell therapy of multiple myeloma[J]. *Clin Cancer Res*, 2013, 19(8): 2048-2060. DOI: 10.1158/1078-0432.CCR-12-2422.
- [10] COQUERY C M, ERICKSON L D. Regulatory roles of the tumor necrosis factor receptor BCMA[J]. *Crit Rev Immunol*, 2012, 32(4): 287-305. DOI: 10.1615/CritRevImmunol.v32.i4.10.
- [11] TAI Y T, ANDERSON K C. Targeting B-cell maturation antigen in multiple myeloma[J]. *Immunotherapy*, 2015, 7(11): 1187-1199. DOI: 10.2217/imt.15.77.
- [12] LEE, L. D. BOUNDS, J. PATERSON, et al. Evaluation of B cell maturation antigen as a target for antibody drug conjugate mediated cytotoxicity in multiple myeloma[J]. *Br J Haematol*, 2016, 174(6): 911-922. DOI: 10.1111/bjh.14145.
- [13] GOMEZ-EERLAND R, NUIJEN B, HEEMSKERK B, et al. Manufacture of gene-modified human T-cells with a memory stem/central memory phenotype[J]. *Hum Gene Ther Methods*, 2014, 25(5): 277-287. DOI: 10.1089/hgtb.2014.004.
- [14] GARGETT T, BROWN MP. Different cytokine and stimulation conditions influence the expansion and immune phenotype of third-generation chimeric antigen receptor T cells specific for tumor antigen GD2[J]. *Cytotherapy*, 2015, 17(4): 487-495. DOI: 10.1016/j.jcyt.2014.12.002.
- [15] LI F, ZHANG T, CAO L, et al. Chimeric antigen receptor T cell based immunotherapy for cancer[J]. *Curr Stem Cell Res Ther*, 2018, 13(5): 327-335. DOI: 10.2174/1574888X13666180420110239.

- [16] CASTELLARIN M, WATANABE K, JUNE C H, et al. Driving cars to the clinic for solid tumors[J]. *Gene Ther*, 2018, 25(3): 165-175. DOI: 10.1038/s41434-018-0007-x.
- [17] BU D X, SINGH R, CHOI E E, et al. Pre-clinical validation of B cell maturation antigen (BCMA) as a target for T cell immunotherapy of multiple myeloma[J]. *Oncotarget*, 2018, 9(40): 25764-25780. DOI: 10.18632/oncotarget.25359.
- [18] SMITH E L, STAEHR M, MASAKAYAN R, et al. Development and evaluation of an optimal human single-chain variable fragment-derived BCMA-targeted CAR T cell vector[J]. *Mol Ther*, 2018, 26(6): 1447-1456. DOI: 10.1016/j.ymthe.2018.03.016.
- [19] ZHAO Z M, CONDOMINES S J, VAN DER STEGEN C, et al. Structural design of engineered costimulation determines tumor re-
jection kinetics and persistence of CAR T cells[J]. *Cancer Cell*, 2015, 28(4): 415-428. DOI: 10.1016/j.ccell.2015.09.004.
- [20] LI G, BOUCHER JC, KOTANI H, et al. 4-1BB enhancement of CAR T function requires NF- κ B and TRAFs[J]. *JCI Insight*, 2018, 3(18). pii: 121322. DOI: 10.1172/jci.insight.121322.
- [21] ZHONG Q, ZHU Y M, ZHENG L L, et al. Chimeric antigen receptor-T cells with 4-1BB co-stimulatory domain present a superior treatment outcome than those with CD28 domain based on bioinformatics[J]. *Acta Haematol*, 2018, 140(3): 131-140. DOI: 10.1159/000492146.

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