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抗HER2抗体偶联药物联合抗PD-1单抗对小鼠膀胱肿瘤的作用及机制研究

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[摘要] **目的** 探究抗HER2抗体偶联药物(HER2 antibody-drug conjugate,HER2-ADC)联合抗PD-1单抗(anti-PD-1 antibody,anti-PD-1)对HER2表达膀胱癌的协同治疗效果,并阐明其对肿瘤免疫微环境的调控机制。**方法** 选取40只6~8周龄(体质量18~22 g)的C57BL/6雌性小鼠构建过表达人HER2的MB49膀胱癌细胞C57BL/6小鼠异位肿瘤模型。当肿瘤体积达100 mm^3时,采用随机数字表法分为4组(n=10):对照组(腹腔注射PBS 1.0 mL),anti-PD-1组(腹腔注射anti-PD-1,每只小鼠200 μg/次,间隔3 d给药),HER2-ADC组(腹腔注射HER2-ADC,2.5 mg/kg,1次/7 d)和联合组(HER2-ADC和anti-PD-1给药方案同单药组)。连续给药28 d,期间每3天测量肿瘤体积与体质量,比较各组异位肿瘤生长速度与荷瘤小鼠生存率。停药后持续观察小鼠生存状态至自然死亡,记录中位生存时间(n=5)。药物干预28 d后留取各组部分荷瘤鼠外周血及肿瘤样本(n=5),流式细胞术检测外周血MDSCs细胞($CD11b^+$、$Gr1^+$)和Treg细胞($CD4^+$、$CD25^+$、$FOXP3^+$),免疫组化分析肿瘤内$CD3^+$T、$CD8^+$T、$FOXP3^+$T细胞浸润,全自动生化仪评估肝肾功能:谷丙转氨酶(ALT)、谷草转氨酶(AST)、血尿素氮(BUN)、肌酐(CRE)及组织损伤指标:乳酸脱氢酶(LDH-L)的水平。**结果** 治疗28 d时,联合组肿瘤体积显著低于对照组(P<0.01)及单药组(P<0.01)。单药组(anti-PD-1组中位生存期53 d;HER2-ADC组55 d)及联合组(65 d)均较对照组(41 d)显著延长(P<0.01)。药物干预28 d后,单药组和联合组小鼠外周血中MDSCs细胞和Treg细胞比率低于对照组,联合组较单药组降低(P<0.05)。肿瘤微环境中,联合组$CD3^+$T和$CD8^+$T细胞浸润水平较对照组和单药组明显增加(P<0.05),$FOXP3^+$T细胞浸润水平较对照组和单药组显著减少(P<0.05)。各组血清ALT、AST、BUN、CRE、LDH-L水平差异无统计学意义,且均在正常参考范围内。**结论** HER2-ADC联合anti-PD-1可抑制异位膀胱肿瘤生长,可能通过下调MDSCs/Treg并促进$CD8^+$T细胞浸润发挥协同作用。

[关键词] 膀胱肿瘤;抗HER2抗体偶联药物;抗PD-1单抗;免疫微环境
[中图法分类号] R737.14;R969.4;R979.19 [文献标志码] A

Effect and mechanism of HER2 antibody-drug conjugate combined with anti-PD-1 antibody in mouse bladder cancer models

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[**Abstract**] **Objective** To investigate the synergistic therapeutic effects of HER2 antibody-drug conjugate (HER2-ADC) combined with anti-PD-1 antibody (anti-PD-1) on HER2-expressing bladder cancer and elucidate its regulatory mechanisms on the tumor immune microenvironment. **Methods** Orthotopic

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tumor models were established in 40 female C57BL/6 mice (6~8 weeks old, body mass 18~22 g) using MB49 bladder cancer cells overexpressing human HER2. When tumors reached 100 mm^3, the mice were randomized into (n=10) control (intraperitoneal injection of 1.0 mL PBS), anti-PD-1 (200 μg per mouse every 3 d), HER2-ADC (2.5 mg/kg once weekly), and combination groups (same regimens as above monotherapy). Tumor volume and body mass were measured every 3 d during 28-day treatment. Tumor growth kinetics and survival rates were analyzed. Post-treatment survival was monitored until natural death to determine median survival time (n=5). At day 28, blood and tumor samples (n=5) were collected to detect myeloid-derived suppressor cells (MDSCs; $CD11b^+Gr1^+$) and regulatory T cells (Tregs; $CD4^+CD25^+FOXP3^+$) with flow cytometry, tumor-infiltrating $CD3^+$T, $CD8^+$T, and $FOXP3^+$T cells with immunohistochemical assay, and liver/kidney functions [alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine (CRE)] and tissue damage indicators [lactate dehydrogenase isoenzyme (LDH-L)]. **Results** In 28 d after treatment, the combination group obtained significantly smallest tumor volume than the control group and the 2 monotherapy groups (all P<0.01). The longest median survival was observed in the combination group (65 d, P<0.01), followed by the HER2-ADC group (55 d), anti-PD-1 group (53 d) and control group (41 d). After 28 d of treatment, the combination group exhibited obviously the smallest peripheral proportions of MDSCs/Tregs, most tumor-infiltrating $CD3^+$T/$CD8^+$T cells, and less $FOXP3^+$T cells when compared with the 2 monotherapy groups and control group (all P<0.05). While, the 2 monotherapy groups had smaller MDSCs/Tregs proportions than the control group (P<0.05). No significant differences were observed among the 4 groups in serum ALT, AST, BUN, CRE, or LDH-L levels, and all of them were within normal ranges. **Conclusion** HER2-ADC combined with anti-PD-1 suppresses the growth of orthotopic bladder tumor, probably through their synergic effects on down-regulating MDSCs/Treg and enhancing $CD8^+$T cell infiltration.

[**Key words**] urinary bladder neoplasms; HER2 antibody-drug conjugate; anti-PD-1 antibody; tumor microenvironment

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膀胱癌(bladder cancer,BCa)是泌尿系统最为常见的恶性肿瘤,尽管早期BCa通过手术治疗可达到根治,但仍有不少患者会出现复发或远处转移[1]。晚期BCa患者治疗手段有限,临床预后较差[2]。既往含铂化疗是晚期BCa标准治疗,但疗效已达瓶颈,且部分患者对铂类不耐受[3]。随着靶向精准时代的到来,研究者日益关注BCa的基因变异特征,既往研究发现,93%的BCa患者至少携带1种临床相关基因变异,癌症基因组图谱数据已将BCa列为第三大突变癌症[4]。基于基因变异而带来的精准靶向和免疫治疗正在改写着晚期BCa的治疗格局[5]。针对人表皮生长因子受体2(human epidermal growth factor receptor 2,HER2)基因在BCa中的异常改变,抗HER2靶向治疗为携带此类基因异常的晚期BCa患者带来了新的希望[6-7]。多项临床研究证实抗HER2抗体偶联药物(HER2 antibody-drug conjugate,HER2-ADC)在治疗HER2阳性晚期BCa中显示出显著的临床疗效[8-11]。目前HER2-ADC联合免疫检查点抑制剂(immune checkpoint inhibitors,ICIs)正在晚期BCa中开展临床研究,以探索二者联合治疗的疗效和安全性,然而,确证性的研究结果仍然有限。尤为关键的是,HER2-ADC联合抗PD-1单抗(anti-PD-1 antibody,anti-PD-1)在BCa治疗中能否产生协同增效作用,以及其背后确切的分子机制,目前尚未有研究系统阐明。这一关键科学问题的悬而未决,阻碍了该联合策略在临床上的优化应用。本研究将通过构建HER2过表达的小鼠异位移植瘤模型,在临床前研究中系统考察HER2-ADC联合anti-PD-1的体内抗肿瘤作用及其潜在的协同效应,并深入探究其发挥作用的分子机制,为HER2-ADC联合anti-PD-1治疗HER2阳性晚期膀胱癌的临床应用提供理论基础和实验依据。

1 材料与方法

1.1 实验材料

鼠源膀胱癌细胞株MB49购自中国科学院细胞库;实验小鼠C57BL/6购自中国上海模式生物有限公司;HER2过表达质粒(pcDNA 3.1)构建于上海吉玛制药有限公司;HER2-ADC制备于MedChemExpress公

司;anti-PD-1(克隆号29F.1A12)购自美国BioXcell公司;RPMI1640培养基、胰酶及胎牛血清等常规试剂购自中国武汉普洛赛生物科技有限公司;Anti-ErbB2/HER2抗体(ab134182)、Anti-CD3(ab16669)、Anti-CD4(ab237722)、Anti-CD8(ab217344)、Anti-CD11b(ab24874)、Anti-Foxp3(ab210232)和Anti-Ly6G+Ly6C(ab25377)购于英国Abcam公司;Anti-CD25(No.562606)购于美国BD公司。

1.2 细胞培养与动物饲养

1.2.1 细胞培养 MB49细胞在含有10%胎牛血清、100 U/mL青霉素和100 μg/mL链霉素的RPMI1640培养基中,置于37 ℃,5% CO_2恒温培养箱内。根据细胞状态及生长情况进行换液和传代,用对数生长期MB49细胞进行相关实验。

1.2.2 实验动物 40只6~8周龄,体质量18~22 g的C57BL/6雌性小鼠按照SPF环境要求进行饲养:IVC独立通气笼具系统,温度20~24 ℃,湿度(55±5)%,换气次数15次/h,光照周期12 h∶12 h。饮食为辐照灭菌饲料与酸化饮用水。尸体处理:高压灭菌(121 ℃×30 min)后医疗废弃物转运。所有操作经蚌埠医科大学实验动物伦理委员会审批([2024]KY015),同时实验部分严格遵循国家实验动物管理保护条例,实验数据不违反伦理学条例要求。实验全程采用以下措施减少动物痛苦:所有侵入性操作均在超净台内进行严格无菌操作;肿瘤接种前30 min通过腹腔注射布托啡诺(0.1 mg/kg)进行镇痛;每周进行2次体质量监测评估动物健康状况。

1.2.3 HER2过表达异位膀胱肿瘤模型构建及分组干预 HER2过表达稳转细胞珠构建:MB49细胞6孔板铺板,细胞融合生长至60%~70%时,加入病毒(MOI=10),37 ℃培养24 h后换液,感染72 h后,倒置显微镜下荧光观察感染效率。嘌呤霉素筛选稳转细胞珠,Western blot和qRT-PCR方法验证过表达效率。

本研究采用异位移植法建立膀胱肿瘤模型,具体方法为:接种前1 d给小鼠右侧腋下备皮,制备细胞数为2×10^6的HER2过表达MB49细胞悬液(0.1 mL的PBS与基质胶2∶1混匀),在室温20 ℃环境下抓取固定小鼠,无菌注射器在小鼠右侧腋下注射上述细胞悬液。观察测量肿瘤大小约200 mm^3时进行分组药物干预。肿瘤体积(TV)的计算公式为:$TV=D\times d_1\times d_2$,其中D为肿瘤最长径,d_1和d_2为2个垂直方向上的最大短径[4]。

荷瘤小鼠采用随机数字表法分配至以下4组(n=10),并接受为期28 d的相应治疗:对照组(腹腔注射PBS 1 mL),anti-PD-1组(腹腔注射anti-PD-1,每只小鼠200 μg/次,间隔3 d给药),HER2-ADC组(腹腔注射HER2-ADC,2.5 mg/kg,1次/7 d)和联合组(HER2-ADC和anti-PD-1给药同单药组)。药物干预后28 d,随机采用4%水合氯醛溶液(0.1 mL/10 g)腹腔注射麻醉后行颈椎脱位法处死各组5只荷瘤小鼠,剥离异位膀胱肿瘤,留取眼眶外周血,剩余荷瘤小鼠观察生存时间,根据动物伦理要求建立以下人道终点标准:①肿瘤直径≥20 mm;②体质量下降>20 %持续3 d;③出现活动障碍或溃疡感染。实验期间每日监测肿瘤体积及动物状态,当达到任一终点标准时立即实施二氧化碳吸入安乐死。

1.3 流式细胞仪检测外周血MDSCs细胞和Treg细胞

含EDTA的外周血50 μL加入Anti-mouse CD11b FITC 1 μL,Anti-Mouse Ly6G+LY6C APC 1.5 μL,Anti-Mouse CD25 BV421 1 μL和Anti-Mouse FOXP3-PE 0.63 μL等抗体,混匀,室温避光孵育15 min,加入红细胞裂解液(1×)1 mL裂解8 min,300 g/5 min离心,弃上清,PBS洗涤,300 g/5 min离心,弃上清,PBS 500 μL重悬细胞,上机检测。

1.4 免疫组化检测肿瘤微环境中免疫细胞浸润

异位膀胱肿瘤石蜡切片4 μm,柠檬酸-磷酸氢二钠缓冲液进行抗原修复。3% H_2O_2浸泡15 min,PBS冲洗(3次,5 min/次)。一抗4 ℃孵育过夜,后加入二抗,37 ℃湿盒内放置20 min,PBS冲洗后用DAB工作液显色,苏木精复染,1%盐酸分化、脱水、中性树脂封片,光学显微镜下观察分析。判读标准:直接计数200×随机5个视野下的阳性细胞数,取平均数除以镜下面积,得出细胞密度,单位为细胞/mm^2。

1.5 生物安全性评估

应用Chemray 240自动生化分析仪(中国雷托科学公司)分析评估各药物干预组小鼠外周血血液生化指标,如ALT、AST、BUN、CRE和LDH-L,进行药物安全性评价。

1.6 统计学分析

应用SPSS 25.0统计学软件进行数据处理分析,计量资料用$\bar{x}\pm s$表示。两组间比较:通过Levene检验评估方差齐性。若方差齐(P>0.05)则采用独立样本t检验;若方差不齐(P≤0.05)则采用Welch校正t检验。多组间比较:采用单因素方差分析,若组间差异显著(P<0.05),进一步以Tukey法进行事后两两比较。生存分析:采用Kaplan-Meier法绘制生存曲线,组间差异通过Log-rank检验评估。P<0.05认为差异具有统计学意义。

2　结果

2.1　HER2-ADC联合anti-PD-1减缓小鼠异位膀胱肿瘤生长

为确保研究靶点的临床相关性并为后续药效实验提供基础，建立了稳定表达人HER2的膀胱癌临床前模型。通过慢病毒载体稳定转染技术，在小鼠膀胱癌细胞MB49中实现HER-2基因的过表达。Western blot和qRT-PCR实验证实转染细胞中HER2蛋白和mRNA的稳定高表达（P<0.01）。为了评估不同治疗方案的直接抗肿瘤效果，测量药物干预28 d后各组小鼠异位膀胱肿瘤的体积，结果显示，对照组、anti-PD-1组、HER2-ADC组和联合组异位膀胱肿瘤体积分别为（1 095.7±70.6）、（918.9±47.3）、（881.2±53.7）和（679.6± 109.4）mm^3，与对照组相比，anti-PD-1组、HER2-ADC组及联合组的肿瘤体积均显著减小（P<0.01），与任一单药组相比，联合组的肿瘤体积亦显著减小（P<0.01）。见图1。

2.2　HER2-ADC联合anti-PD-1降低小鼠外周血MDSCs和Treg细胞

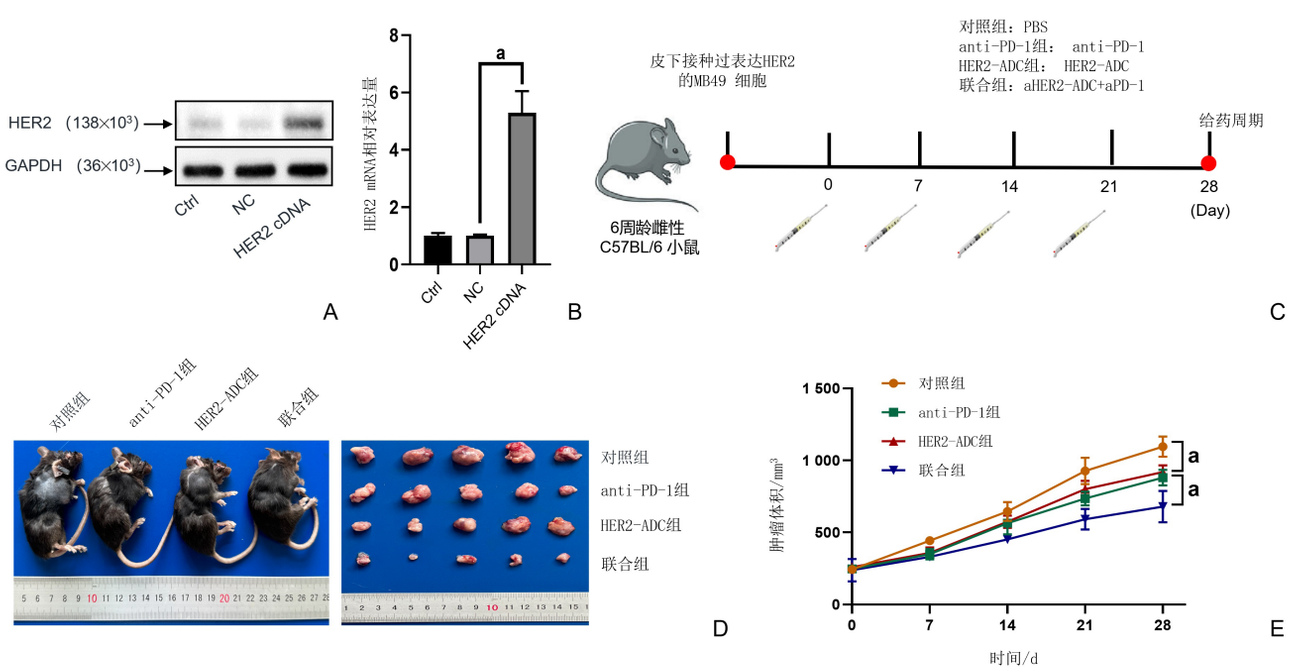
为探究HER2-ADC与anti-PD-1对免疫环境的影响，采用流式细胞术分析药物干预28 d后荷瘤小鼠外周血中髓系衍生抑制细胞（MDSCs）和调节性T细胞（Treg）的比例。药物干预28 d后，与对照组比较，小鼠外周血中MDSCs在HER2-ADC组和联合组显著降低（P<0.05），与HER2-ADC单药组相比，联合治疗组MDSCs比例进一步降低（P<0.05）；与对照组相比，HER2-ADC组和anti-PD-1组Treg细胞比例有所降低，但差异无统计学意义，然而，与对照组及任一单药组相比，联合治疗组中外周血Treg细胞的积累显著减少（P<0.05）。见图2。

2.3　HER2-ADC联合anti-PD-1增强肿瘤微环境中免疫细胞浸润

为评估不同治疗方案对肿瘤组织局部免疫微环境的影响，通过免疫组织化学染色检测关键免疫细胞（$CD3^+$、$CD4^+$、$CD8^+$ T淋巴细胞及$FOXP3^+$细胞）的浸润情况。药物干预后，与对照组相比，联合组肿瘤组织中$CD3^+$ T和$CD8^+$ T细胞浸润水平显著增加（P<0.05）。与HER2-ADC组或anti-PD-1组相比，联合组肿瘤组织中$CD3^+$ T和$CD8^+$ T细胞浸润水平亦显著增加（P<0.05）。$FOXP3^+$T细胞浸润水平较对照组和任一单药组均显著减少（P<0.05）。见图3。

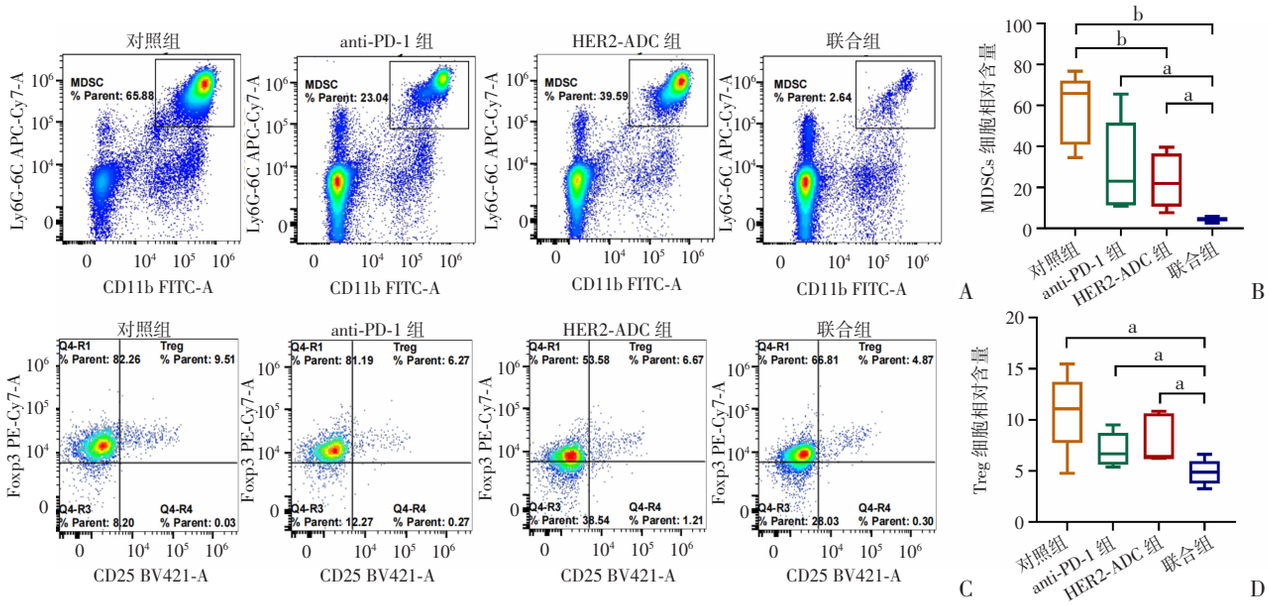
2.4　HER2-ADC联合anti-PD-1在体内的安全性

为评估不同治疗方案的安全性，监测药物干预期间小鼠的体质量变化，并在干预28 d后检测血清生化



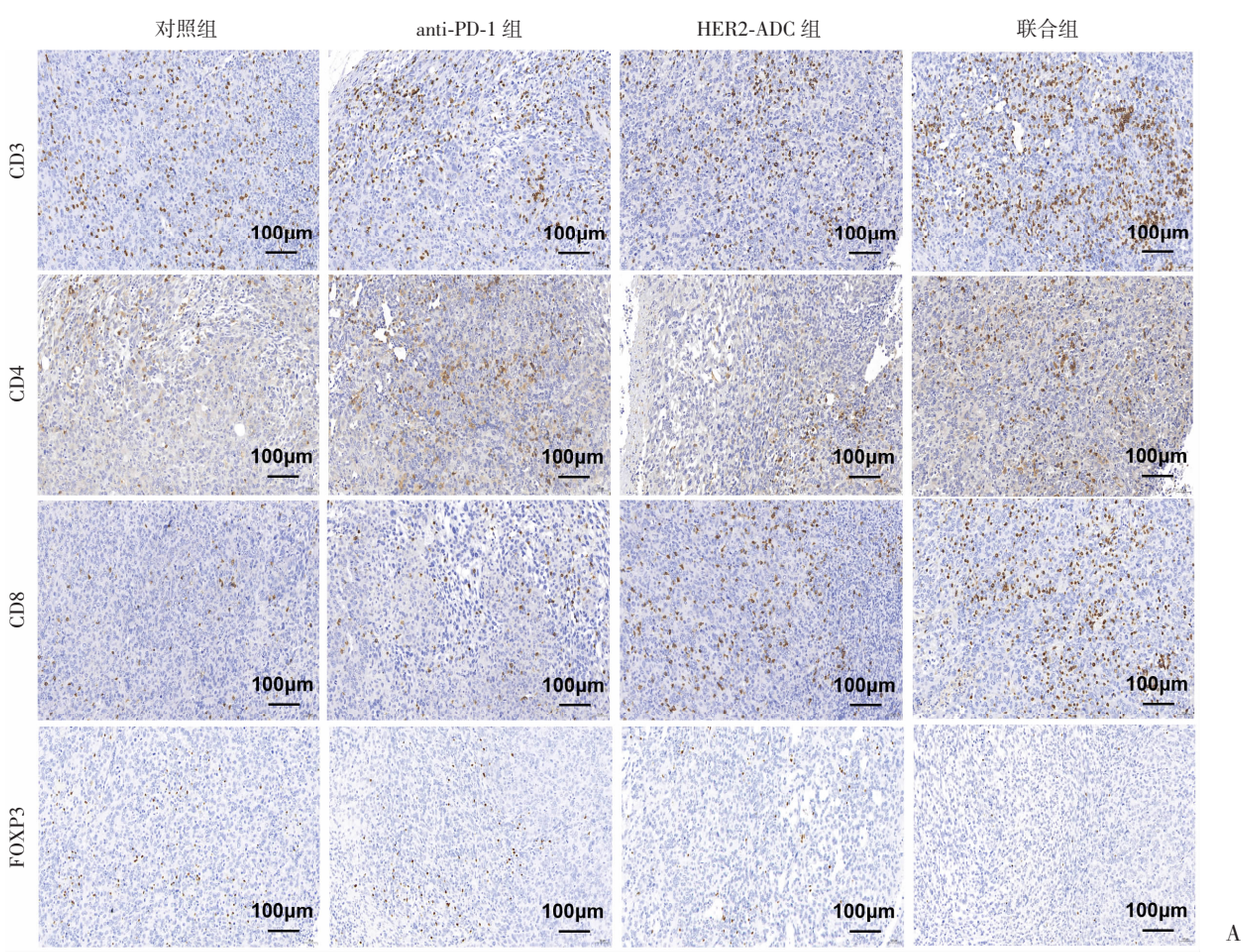
A、B：Western blot和qRT-PCR验证MB49细胞中HER2蛋白和mRNA过表达；C：治疗方案示意图；D：给药28 d后小鼠肿瘤体积对比；E：小鼠移植瘤生长曲线；a：P<0.01

图1　小鼠异位膀胱肿瘤模型构建及药物干预（n=10，$\bar{x}\pm s$）



A:流式细胞术检测各组小鼠外周血白细胞中MDSCs细胞的百分比;B:MDSCs细胞的定量分析;C:流式细胞术检测各组小鼠外周血$CD4^+$ T细胞群中Treg细胞的百分比;D:Treg细胞的定量分析; a: $P<0.05$, b: $P<0.01$

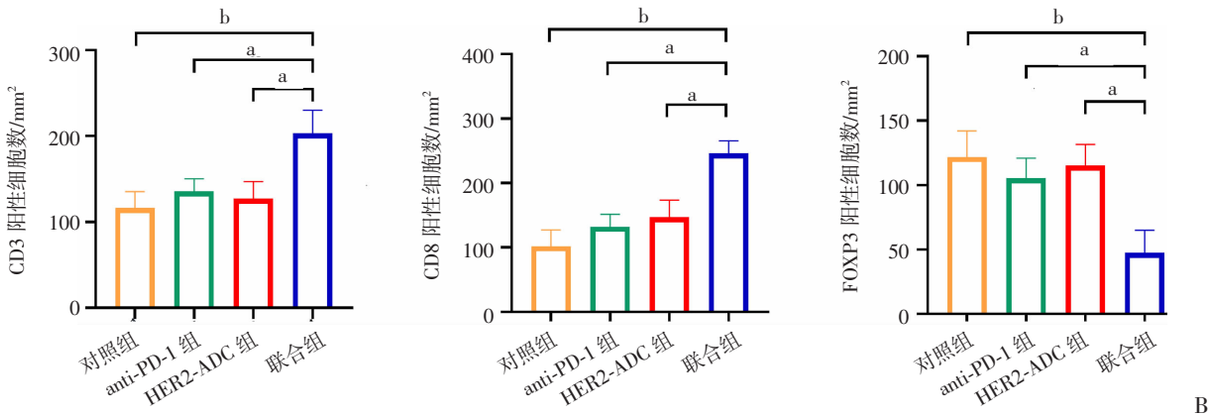
图2　流式细胞仪检测外周血MDSCs和Treg细胞　($n=5$, $\bar{x}\pm s$)



A:免疫组化染色检测$CD3^+$ T细胞、$CD4^+$ T细胞、$CD8^+$ T细胞和$FOXP3^+$ T细胞

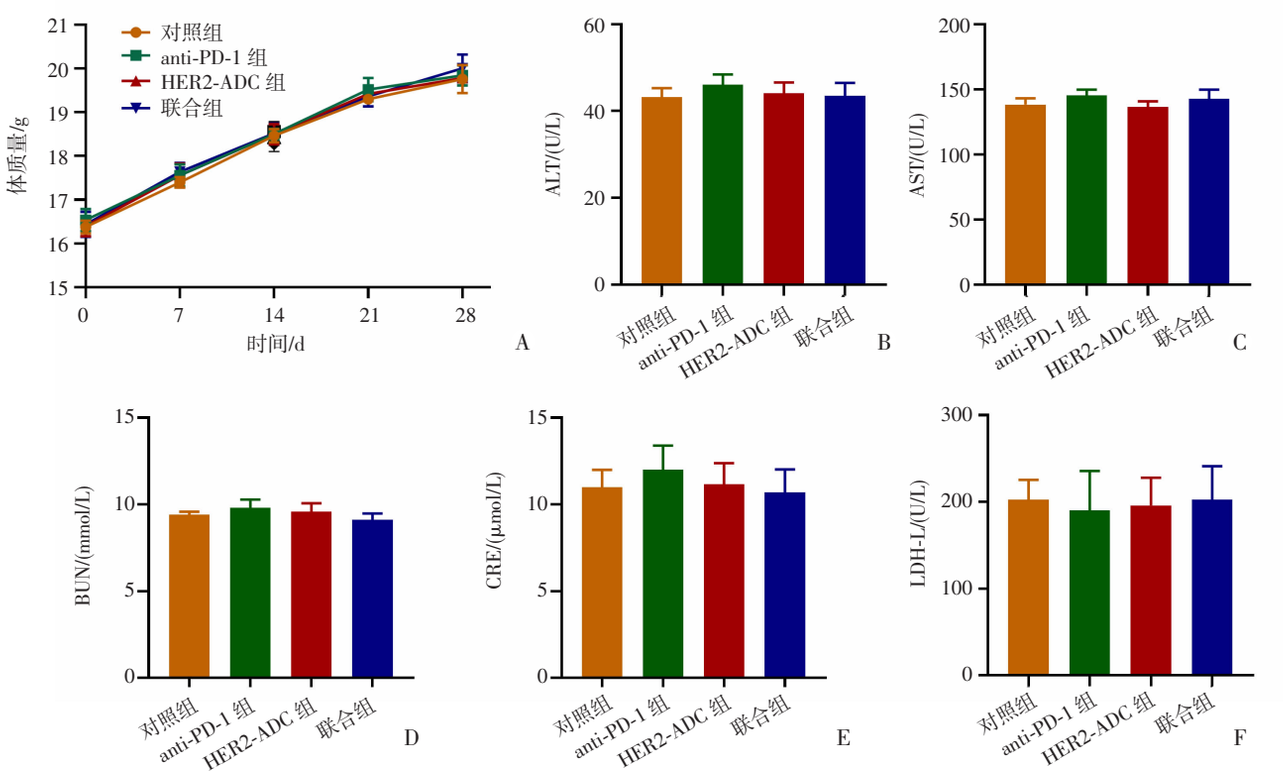
图3　免疫组化检测肿瘤组织中免疫细胞浸润水平($n=5$, $\bar{x}\pm s$)

图3B续下页



B:CD3⁺ T细胞、CD8⁺ T细胞和FOXP3⁺ T细胞免疫组化染色结果的定量分析;a:P<0.05,b:P<0.01

续图3 免疫组化检测肿瘤组织中免疫细胞浸润水平(n=5, $\bar{x}\pm s$)



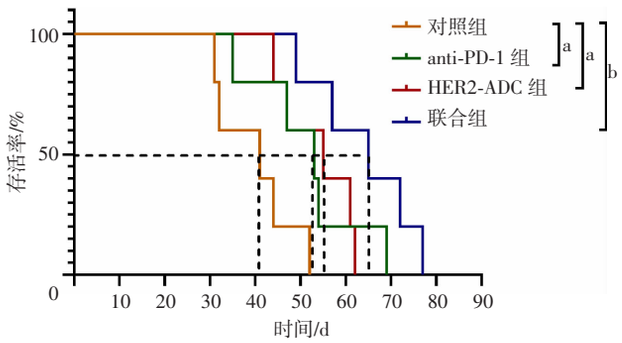
A:小鼠体质量变化;B~F:ALT、AST、BUN、CRE和LDH-L水平的定量分析

图4 各组小鼠体质量变化及血清生化结果 (n=5, $\bar{x}\pm s$)

指标(ALT, AST, BUN, CRE, LDH-L)。药物干预期间各组小鼠体质量变化无明显差异(图4A)。药物干预28 d后,与对照组相比,anti-PD-1组、HER2-ADC组和联合组小鼠血清中ALT、AST、BUN、CRE和LDH-L水平有波动。各组间比较,所有检测的血清生化指标(ALT, AST, BUN, CRE, LDH-L)差异均无统计学意义。所有组别的检测值均处于该品系小鼠的常规正常参考范围内。见图4。

2.5 HER2-ADC联合anti-PD-1延长荷瘤小鼠生存时间

为评估不同治疗方案的远期疗效,持续监测荷瘤小鼠的生存情况。单药组(anti-PD-1组:53 d;HER2-ADC组:55 d)和联合组(65 d)荷瘤小鼠中位总生存时间较对照组(41 d)均延长(P<0.05)。与对照组相比,联合组生存期差异具有统计学意义(P<0.01)。尽管联合组中位生存时间较单药组延长了10~12 d,但Log-rank检验显示差异无统计学意义(图5)。



a: $P<0.05$, b: $P<0.01$

图5 各组荷瘤小鼠生存曲线(n=5, $\bar{x}\pm s$)

3 讨论

3.1 HER2-ADC与anti-PD-1联合治疗的协同抗肿瘤效应

抗HER2抗体偶联药物与免疫检查点抑制剂联合使用的协同效应受到临床研究的密切关注,然而许多临床研究仍在进行中,高级别循证证据相对有限[12]。本研究通过构建HER2过表达小鼠异位膀胱肿瘤模型,观察到HER2-ADC联合anti-PD-1干预荷瘤小鼠,较单药干预对异位移植瘤生长抑制作用更为明显。在评估远期疗效时,联合治疗组小鼠的中位生存期较单药组延长10~12 d,这种生存期延长的趋势值得关注,其未达显著性可能部分归因于本研究的样本量限制(n=5)[13]或相对较短的给药周期未能充分激发免疫治疗的长期记忆效应[14]。虽差异无统计学意义,但提示潜在生存获益,实验结果与膀胱癌领域早期临床探索趋势相符。例如,在RC48-C014 Ⅰb/Ⅱ期研究中,HER2-ADC维迪西妥单抗(RC48)联合特瑞普利单抗(抗PD-1)治疗晚期尿路上皮癌患者,显示出有前景的客观缓解率(73.2%)和疾病控制率(90.2%)[15]。其他研究也提示该联合策略在膀胱癌新辅助治疗中的潜力[16-17],HER2-ADC联合免疫在其他实体瘤中的早期临床试验也发现可改善抗肿瘤效果[18]。这些初步临床数据为本研究的临床前协同效应提供了支持性背景。

3.2 联合治疗重塑免疫抑制微环境的双通路机制

HER2-ADC为靶向特异性抗原的单克隆抗体和小分子细胞毒药物通过连接子偶联而成,兼具强大细胞毒作用和抗体药物的肿瘤靶向性[19-20]。其联合免疫治疗的潜在机制多样,包括Fc效应、启动免疫原性细胞死亡(ICD)、树突状细胞(DC)的成熟、增强T细胞浸润、强化免疫记忆以及表达免疫调节蛋白,如PD-L1和主要组织相容性复合体(MHC)[21-25]。本研究发现HER2-ADC联合免疫治疗显著降低MDSCs和Treg细胞水平,可能是其协同增效的机制之一。MDSCs和Treg是肿瘤微环境中关键的免疫抑制细胞[26-27],MDSCs通过ROS、IL-10和TGF-β等因子抑制$CD8^+$ T细胞并刺激Treg增殖,是免疫治疗耐药的关键因素[28]。因此,减少并解除MDSCs/Treg的免疫抑制是当前靶向治疗的主要策略[29]。本研究考察了不同干预组肿瘤组织样本中免疫细胞浸润情况,发现联合组$CD3^+$和$CD8^+$T淋巴细胞浸润水平显著增加,这可能是其协同增效的另一机制。在小鼠乳腺癌模型中维迪西妥单抗可增强免疫治疗疗效,伴随T淋巴细胞浸润增加和免疫记忆形成[30]。在CT26-HER2模型中,T-DXd可增加肿瘤浸润DC和$CD8^+$T细胞数量,并上调PD-L1/MHC Ⅰ表达[31]。上述表明HER2-ADC可通过调控肿瘤微环境增强免疫治疗疗效。

目前对于已知免疫治疗反应良好的肿瘤类型,HER2-ADC与ICIs联合使用可能带来的协同益处尚不确定,临床数据表明与单独使用免疫治疗的疗效数据相比,联合治疗显著提高了缓解率[32-33]。本研究在膀胱肿瘤模型中发现HER2-ADC联合anti-PD-1具有协同抑瘤作用,并显著降低外周血MDSCs/Tregs,其双重协同机制为源头阻断:HER2-ADC清除$HER2^+$细胞,减少促MDSC/Treg因子(如IL-6/VEGF),抑制其分化;功能抑制:anti-PD-1阻断后,活化$CD8^+$ T细胞经IFN-γ/STAT1通路抑制MDSC功能并诱导Treg凋亡。这一"源头阻断-功能抑制"双机制与Kornberg团队在黑色素瘤模型中的发现一致[34],提示跨瘤种免疫调控通路的普适性。

3.3 联合治疗有效且安全

在推进HER2-ADC与anti-PD-1联合治疗的临床转化过程中,安全性是核心考量因素。本研究虽未评估长期用药的潜在风险,如自身免疫反应或器官累积毒性,但在28 d治疗周期内,联合组未表现出对肝脏、肾脏和心脏等主要器官的明显毒性,联合治疗在短期应用中耐受性良好,为其临床推进提供重要支持。实验结果表明,联合治疗不仅具有良好的治疗效果,还表现出较高的安全性。相较于临床常见免疫联合方案相关的肝毒性(如抗CTLA-4疗法)或间质性肺炎(如PD-1/PD-L1单药),本联合策略在动物模型中展现出更优的安全性特征。其优势主要体现在两方面:其一,HER2-ADC通过HER2靶向递送机制聚焦细胞毒作用,减少对正常组织的损伤;其二,联合治疗可能通过协同效应降低单药剂量需求,从而规避剂量限制性毒性(如ADC相关的骨髓抑制)。然而,本研究仍存在

以下局限性:当前实验仅采用固定剂量给药,未来需通过剂量爬坡实验确定最佳治疗窗;此外,长期安全性尚未评估,需在延长治疗周期(>28 d)的模型中监测免疫相关不良事件;同时,由于未在临床样本中验证免疫标志物变化,需进一步在膀胱癌患者队列中考察HER2表达异质性(免疫组化评分1+~3+)对疗效及安全性的影响。

综上所述,本研究证实HER2-ADC联合anti-PD-1在膀胱癌模型中具有协同抗肿瘤作用,初步揭示了其通过降低外周血免疫抑制细胞(MDSCs、Tregs)比例并促进肿瘤微环境中效应T淋巴细胞浸润的可能机制,该发现为克服膀胱癌免疫治疗耐药提供了新的理论依据,未来需进一步评估长期治疗的安全性及疗效,并通过前瞻性临床研究优化联合策略。

利益冲突 本文所有作者声明不存在利益冲突

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