

树突状细胞疫苗在人源化裸鼠体内的抗肿瘤免疫保护作用

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Antitumor Protective Immunity Induced by Dendritic Cells Based Vaccine in Humanised Nude Mice Model

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Abstract :Objective To explore antitumor protective immunity induced by dendritic cells based vaccine in humanised nude mice model. **Methods** Immature DCs generated from peripheral blood mononuclear cells were transfected with gastric carcinoma total RNA *in vitro*. A humanized nude mice model was established by intraperitoneal (i. p.) injection of human peripheral blood T-lymphocytes (HuPB TL). The mice were randomly divided into DC_{RNA} group, DCs group and PBS group of 5 mice each. The mice were immunized s. c. with DC_{RNA} or DCs and PBS, respectively, twice, at weekly intervals. Three days after the last immunization mice were challenged s. c. with BGC-823 cells. The CD4⁺ and CD8⁺ T cell subpopulations of human T-lymphocytes from peripheral blood of humanised nude mice were detected by flow cytometry and graft versus host disease (GVHD) were observed. Tumor growth, tumor volume, tumor weight and tumor inhibition rate were observed. Production of cytokine IL-12 and IFN- γ were detected by ELISA kit. **Results** The CD4⁺ and CD8⁺ T cells subpopulations of human T-lymphocytes from peripheral blood of humanised nude mice were found in each group. GVHD was not occurrence in each group. Overall tumor volume in DC_{RNA} group mice was significantly lower than those of DCs group and PBS group ($P < 0.05$). Tumor weight in DC_{RNA} group was significantly lighter than those of DCs group and PBS group ($P = 0.0452$, $P = 0.0004$), but this difference was not statistically significant between DCs group and PBS group ($P = 0.0618$). Inhibition tumor rate for DC_{RNA} group (53.7%) was higher than that for DCs group (25.1%) ($P < 0.05$). Production of cytokine IL-12 and IFN- γ in DC_{RNA} group were significantly increased related to DCs group and PBS group ($P < 0.05$, $P < 0.01$). **Conclusion** A humanised nude mouse model was successfully established by i. p. injection of HuPB TL. DCs based vaccine is capable of inducing enhanced antitumor protective immunity *in vivo*.

Key words :Dendritic cells; Vaccine; Gastric carcinoma; Nude mouse; Humanized; Cellular immunity

摘 要:目的 探讨树突状细胞(DCs)疫苗在人源化裸鼠体内的抗胃癌免疫保护作用。方法 制备DC_{RNA}疫苗;建立人源化裸鼠模型;15只人源化裸鼠随机分为DC_{RNA}组、DCs组和PBS组,每组5只,分别用DC_{RNA}、DCs和PBS皮下免疫注射2次,间隔1周,末次免疫后3天皮下接种胃癌细胞。各组裸鼠外周血人源性CD4⁺T细胞和CD8⁺T细胞的检测和观察GVHD反应。观察裸鼠瘤体积和瘤重。检测细胞因子IL-12和IFN- γ 水平。结果 各组HuPB TL-裸鼠外周血中均检测到人源性CD4⁺T细胞和CD8⁺T细胞,无GVHD反应。DC_{RNA}组瘤体积明显小于DCs组和PBS组($P < 0.05$);DC_{RNA}组瘤重明显小于DCs组和PBS组($P = 0.0452$ 、 $P = 0.0004$),但DCs组和PBS组差异无统计学意义($P = 0.0618$);DC_{RNA}组抑瘤率(53.7%)显著高于DCs组(25.1%)($P < 0.05$)。DC_{RNA}组IL-12和IFN- γ 水平明显高于DCs组和PBS组($P < 0.05$ 、 $P < 0.01$)。结论 HuPB TL腹腔注射,可成功构建裸鼠人细胞免疫功能;DC_{RNA}疫苗在人源化裸鼠体内能诱导产生较强的抗胃癌免疫保护作用。

关键词:树突状细胞;疫苗;胃癌;裸鼠;人源化;细胞免疫

中图分类号:R735.2 文献标识码:A
文章编号:1000-8578(2007)06-0420-03

收稿日期:2006-09-11;修回日期:2007-01-31

基金项目:河北省科技厅博士基金项目(04547002D-9);河北省卫生厅重大攻关项目(2003001)

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0 引言

树突状细胞(Dendritic cells, DCs)是体内功能最强的抗原提呈细胞,在诱导特异性抗肿瘤细胞免疫反应中起关键作用。近年来,国外 DCs 疫苗、期临床试验治疗恶性胶质瘤^[1]、肾癌^[2]和结、直肠癌^[3]等,说明 DCs 疫苗是安全可行的。我们前期的实验结果已经证实胃癌总 RNA 转染的 DCs 在体外诱导产生抗胃癌特异性 CTL^[4],在此基础上,我们观察胃癌总 RNA 转染的 DCs(DC_{RNA}疫苗)在人源化裸鼠体内抗胃癌免疫保护作用。

1 材料和方法

1.1 材料

胃癌细胞株 BGC-823,由河北医科大学申海涛博士惠赠。BALB/c(nu/nu)裸鼠购于中国医学科学院实验动物研究所,SPF级,4~5周龄,体重18~20g,均为雌性。饲养于河北省实验动物中心 SPF级动物实验室,笼具、水和垫料均高压消毒,每3天更换1次。RPMI1640培养基购于美国 Gibco 公司,新生牛血清购于杭州四季青生物工程材料有限公司。RPMI1640完全培养基:以 RPMI1640 培养基为基础,加10%灭活的新生牛血清、100U/ml青霉素和100μg/ml链霉素。重组人粒细胞-巨噬细胞集落刺激因子(rhGM-CSF)、重组人白细胞介素-4(rhIL-4)均为美国 Reprtech 公司产品。TRIzol 试剂购于美国 Invitrogen 公司。Ficoll-Paque 淋巴细胞分离液购于瑞典 Amersham Biosciences 公司。尼龙毛购于日本和光纯药工业株式会社。人 IL-12 和 IFN-γ 水平检测 ELISA 试剂盒为深圳晶美生物工程有限公司产品。FITC/PE 标记鼠抗人单抗 CD4、CD8 购于美国 BioLegend 公司。

1.2 方法

1.2.1 细胞 BGC-823 培养和胃癌总 RNA 的提取和鉴定 BGC-823 常规培养、消化传代,取对数生长期细胞用于实验。消化、离心收集细胞,PBS 洗涤3次,按 TRIzol 试剂盒说明书抽提胃癌总 RNA,取少量 RNA 测定 OD_{260}/OD_{280} ,计算总 RNA 含量和纯度 > 1.8;琼脂糖变性电泳可见完整 18S、28S 两条带,表明总 RNA 完整未降解。调细胞 BGC-823 浓度为 1×10^7 /ml。

1.2.2 DCs 的培养及 DC_{RNA}疫苗制备的健康成人外周血(肝素抗凝 50U/ml)行密度梯度离心法分离出外周血单个核细胞(PBMCs),PBS 洗涤3次,在完全培养基,37℃、5%CO₂饱和湿度培养箱培养2h后,吸出未贴壁细胞;留下的贴壁细胞,流式细胞仪(FCM)检测其纯度(>90%),调整细胞密度为 $2 \times$

10^6 /ml,加入6孔板中,每孔加完全培养基、rhGM-CSF100ng/ml 和 rhIL-4 50ng/ml,37℃、5%CO₂饱和湿度培养箱培养,隔天半量换液及添加 rhGM-CSF 和 rhIL-4。参考文献[2]的方法,第5天,将 imDCs 分成2组,1组 imDCs 用 RPMI1640 培养基调整密度为 1×10^7 /ml,加总 RNA 50μg/ml,在 37℃、5%CO₂饱和湿度培养箱中共培养60min 进行转染,转染结束后更换培养基继续培养2天;另1组继续培养2天作为对照组。第7天收集全部悬浮和轻微贴壁细胞,PBS 洗2次,调整其密度为 5×10^6 /ml。

1.2.3 人源化裸鼠模型的建立和 DC_{RNA}疫苗抗胃癌免疫保护作用 将吸出的非贴壁细胞,完全培养基悬浮后轻轻注入尼龙毛柱,37℃水浴1h,冲洗出非贴壁细胞即为人 T 淋巴细胞,FCM 检测其纯度(93%),PBS 洗涤2次,调整细胞密度为 6×10^7 /ml。15只裸鼠腹腔注射人 T 细胞 3×10^7 /只(0.5ml),建立 HuPB TL-裸鼠模型。随机分为 DC_{RNA}组、DCs 组和 PBS 组,每组5只,分别用 DC_{RNA}、DCs (1×10^6 /0.2ml)和 PBS 0.2ml 皮下注射,免疫2次,间隔1周,末次免疫后第3天于裸鼠右腋窝中部外侧皮下接种胃癌细胞 2×10^6 /0.2ml。成瘤后每2天用游标卡尺测量瘤体最长径 a、最短径 b。观察各组裸鼠肿瘤生长速度、瘤体积和瘤重, $TV = 0.52 \times a \times b^{2.5}$ ^[5],抑瘤率(%)^[6] = (对照组平均瘤重-治疗组平均瘤重)/对照组平均瘤重 $\times 100\%$ 。

1.2.4 移植瘤抗宿主病(GVHD)反应和裸鼠人细胞免疫重建成功的检测 观察实验期间裸鼠的体重变化,有无皮疹、腹泻、步态改变等表现;实验结束时处死裸鼠,立即摘眼球将外周血置于含有 EDTA 的抗凝管中并混匀,FCM 检测人源性 CD4⁺ T 细胞和 CD8⁺ T 细胞。

1.2.5 细胞因子 IL-12 和 IFN-γ 水平的检测 采用尼龙毛柱法分离免疫裸鼠的脾 T 淋巴细胞。以 BGC-823 细胞作为刺激细胞,加入 50μg/ml 的 MMC,37℃,灭活30min。将 T 细胞和灭活的 BGC-823 细胞以 10:1 混合,培养48h后收集上清液,采用 ELISA 法检测上清液中 IL-12 和 IFN-γ 水平。

1.3 统计学分析 实验数据用 $\bar{x} \pm s$ 表示,应用 SPSS 13.0 统计软件,采用单因素方差分析, $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 各组裸鼠人细胞免疫重建成功的检测和 GVHD 反应

各组 HuPB TL-裸鼠外周血中均检测到人源性

CD4⁺ T 细胞和 CD8⁺ T 细胞,见图 1。各组裸鼠体重无明显变化,无皮疹、腹泻等。

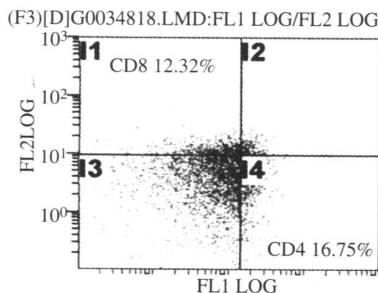


图 1 裸鼠外周血人源性 T 淋巴细胞的流式细胞仪检测结果

2.2 不同组荷瘤鼠肿瘤体积、瘤重及抑瘤率的比较

DC_{RNA} 组瘤体积明显小于 DCs 组和 PBS 组,差异有统计学意义 ($P < 0.05$),见图 2。DC_{RNA} 组瘤重 (0.464 ± 0.156) g 明显小于 DCs 组 (0.750 ± 0.220) g 和 PBS 组 (1.002 ± 0.138) g ($t = 2.371, P = 0.0452; t = 5.776, P = 0.0004$),但 DCs 组和 PBS 组差异无统计学意义 ($t = 2.170, P = 0.0618$);DC_{RNA} 组抑瘤率 (53.7%) 明显高于 DCs 组 (25.1%),差异有统计学意义 ($P < 0.05$)。

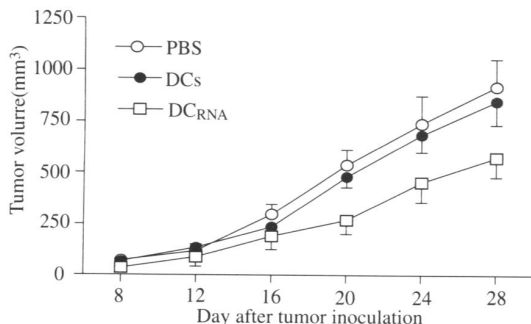


图 2 各组裸鼠皮下移植瘤体积生长曲线

2.3 细胞因子 IL-12 和 IFN- γ 水平的检测

DC_{RNA} 组 IL-12 和 IFN- γ 水平明显高于 DCs 组和 PBS 组,见表 1。

表 1 各组细胞因子 IL-12 和 IFN γ 水平的比较 (pg/ml, n = 5)

组别	IL-12	IFN- γ
PBS 组	24.7 $\pm$ 5.1	57.9 $\pm$ 9.3
DCs 组	279.2 $\pm$ 42.7 *	713.2 $\pm$ 121.8 #
DC _{RNA} 组	387.0 $\pm$ 53.6 Δ	986.4 $\pm$ 163.7

注: * 与 PBS 组比较 $t = 13.23, P < 0.0001$; Δ 与 DCs 组比较 $t = 3.517, P = 0.0079$; # 与 PBS 组比较 $t = 12.00, P < 0.0001$; 与 DCs 组比较 $t = 2.994, P = 0.0172$

3 讨论

裸鼠先天缺乏细胞免疫,为解决研究人体肿瘤

免疫的动物模型不能确切反映人体免疫系统的这个问题,我们借鉴了建立 HuPBL-SCID 鼠嵌合体模型的经验^[7,8],腹腔注射人外周血 T 淋巴细胞 (HuPB TL) 以重建裸鼠的细胞免疫,建立人源化裸鼠模型,即 HuPB TL-裸鼠嵌合体,较好地模拟了人体的免疫微环境。鉴定裸鼠体内人细胞免疫重建成功的最简单方法是 FCM 检测裸鼠外周血或淋巴器官(如脾)人源性 T 细胞特异性标记 CD3⁺ T 细胞或其亚群 CD4⁺ T 细胞和 CD8⁺ T 细胞^[7]。本实验各组裸鼠外周血中均检测到人源性 CD4⁺ T 细胞和 CD8⁺ T 细胞,提示腹腔注射 HuPB TL 可在裸鼠体内成功重建人的细胞免疫系统,且未发现 GVHD。随着 DCs 疫苗策略的不断改进,有必要对疫苗进行临床前动物实验以评价其有效性和安全性, HuPB TL-裸鼠嵌合体模型的建立为 DCs 疫苗研究和在同一系统内比较、评价疫苗疗效奠定了实验基础。

移植瘤可激活 SCID 鼠内重建的人免疫系统发生抗肿瘤免疫应答,疫苗有助于免疫反应^[7]。本实验结果显示,DC_{RNA} 疫苗组荷瘤鼠皮下移植瘤与 DCs 组和 PBS 组相比,其生长速度明显减慢,抑瘤率明显大于 DCs 组,提示重建的人细胞免疫系统已在裸鼠体内发生了抗肿瘤免疫应答,抑制移植瘤生长,DC_{RNA} 疫苗对胃癌生长有明显的抑制作用,能增强荷瘤鼠的抗肿瘤免疫应答。这是由于转染胃癌总 RNA 的 DCs (DC_{RNA} 疫苗) 成熟度增高,能更有效地将肿瘤抗原提呈给 T 细胞,激活肿瘤特异性 CTL 具有杀伤作用,有利于清除肿瘤细胞或远处器官的微转移^[9],同时 CTL 具有记忆效应,再次接触同种抗原时大量活化扩增,能有效抵抗胃癌细胞攻击。DC_{RNA} 疫苗免疫的裸鼠,其 IL-12 和 IFN- γ 水平明显增加,而且 IL-12 有较强的杀伤肿瘤细胞和抗肿瘤转移的作用^[10]。DC_{RNA} 疫苗对人胃癌有较强的免疫保护作用,可能是特异性免疫和非特异性免疫共同作用的结果,为 DC_{RNA} 疫苗应用于胃癌免疫预防的临床研究和预防胃癌术后复发、转移提供了实验依据。

参考文献:

[1] Caruso DA, Orme LM, Neale AM, et al. Results of a phase I study utilizing monocyte-derived dendritic cells pulsed with tumor RNA in children and youth adults with brain cancer[J]. Neuro-oncol, 2004, 6(3): 236-246.
[2] Su Z, Dannull J, Heisra, et al. Immunological and clinical responses in metastatic renal cancer patients vaccinated with tumor RNA-transfected dendritic cells[J]. Cancer Res, 2003, 63(9): 2127-2133.

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- Cancer Lett,2005,224(2):243-252.
- [3] Wu J, Xia HH, Tu SP, et al. 15-Lipoxygenase-1 mediates cyclooxygenase-2 inhibitor-induced apoptosis in gastric cancer[J]. Carcinogenesis,2003,24(2):243-247.
- [4] Liavonchanka A, Feussner I. Lipoxygenases: occurrence, functions and catalysis[J]. J Plant Physiol,2006,163(3):348-357.
- [5] Kuhn H, Walther M, Kuban RJ. Mammalian arachidonate 15-lipoxygenases structure, function, and biological implications[J]. Prostaglandins Other Lipid Mediat,2002,68-69:263-290.
- [6] Jampilek J, Dolezal M, Opletalova V, et al. 5-Lipoxygenase, leukotrienes biosynthesis and potential antileukotrienic agents[J]. Curr Med Chem,2006,13(2):117-129.
- [7] Matsuyama M, Yoshimura R, Mitsuhashi M, et al. 5-Lipoxygenase inhibitors attenuate growth of human renal cell carcinoma and induce apoptosis through arachidonic acid pathway[J]. Oncol Rep,2005,14(1):73-79.
- [8] Hennig R, Ding XZ, Tong WG, et al. 5-Lipoxygenase and leukotriene B₄ receptor are expressed in human pancreatic cancers but not in pancreatic ducts in normal tissue[J]. Am J Pathol,2002,161(2):421-428.
- [9] Hoque A, Lippman SM, Wu TT, et al. Increased 5-lipoxygenase expression and induction of apoptosis by its inhibitors in esophageal cancer: A potential target for prevention[J]. Carcinogenesis,2005,26(4):785-791.
- [10] Chen X, Li N, Wang S, et al. Aberrant arachidonic acid metabolism in esophageal adenocarcinogenesis, and the effects of sulindac, nordihydroguaiaretic acid, and alpha-difluoromethylornithine on tumorigenesis in a rat surgical model[J]. Carcinogenesis,2002,23(12):2095-2102.
- [11] Ding XZ, Tong WG, Adrian TE. Multiple signal pathways are involved in the mitogenic effect of 5(S)-HETE in human pancreatic cancer[J]. Oncology,2003,65(4):285-294.
- [12] Shureiqi I, Wojno KJ, Poore JA, et al. Decreased 13-S-hydroxyoctadecadienoic acid levels and 15-lipoxygenase-1 expression in human colon cancers[J]. Carcinogenesis,1999,20(10):1985-1995.
- [13] Shureiqi I, Wu Y, Chen D, et al. The critical role of 15-lipoxygenase-1 in colorectal epithelial cell terminal differentiation and tumorigenesis[J]. Cancer Res,2005,65(24):11486-11492.
- [14] Shureiqi I, Xu X, Chen D, et al. Nonsteroidal anti-inflammatory drugs induce apoptosis in esophageal cancer cells by restoring 15-lipoxygenase-1 expression[J]. Cancer Res,2001,61(12):4879-4884.
- [15] Hsi LC, Xi X, Lotan R, et al. The histone deacetylase inhibitor suberoylanilide hydroxamic acid induces apoptosis via induction of 15-lipoxygenase-1 in colorectal cancer cells[J]. Cancer Res,2004,64(23):8778-8781.
- [16] Hsi LC, Xi X, Wu Y, et al. The methyltransferase inhibitor 5-aza-2'-deoxycytidine induces apoptosis via induction of 15-lipoxygenase-1 in colorectal cancer cells[J]. Mol Cancer Ther,2005,4(11):1740-1746.
- [17] Nixon JB, Kim KS, Lamb PW, et al. 15-Lipoxygenase-1 has anti-tumorigenic effects in colorectal cancer[J]. Prostaglandins Leukot Essent Fatty Acids,2004,70(1):7-15.
- [18] Chang TL, Peng X, Fu XY. Interleukin-4 mediates cell growth inhibition through activation of Stat1[J]. J Biol Chem,2000,275(14):10212-10217.
- [19] Spanbroek R, Hildner M, Kohler A, et al. IL-4 determines eicosanoid formation in dendritic cells by down-regulation of 5-lipoxygenase and up-regulation of 15-lipoxygenase 1 expression[J]. Proc Natl Acad Sci USA,2001,98(9):5152-5157.
- [20] Takata S, Matsubara M, Allen PG, et al. Remodeling of neutrophil phospholipids with 15(S)-hydroxyeicosatetraenoic acid inhibits leukotriene B₄-induced neutrophil migration across endothelium[J]. J Clin Invest,1994,93(2):499-508.

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- [3] Morse MA, Nair SK, Mosca PJ, et al. Immunotherapy with autologous, human dendritic cells transfected with carcinoembryonic antigen mRNA[J]. Cancer Invest,2003,21(3):341-349.
- [4] 谢绍建, 闫庆辉, 单保恩, 等. 转染自体胃癌细胞总 RNA 的树突状细胞诱导个体化免疫治疗的体外实验[J]. 细胞与分子免疫学杂志,2006,22(1):92-95.
- [5] Pennati M, Binda M, DE Cesare M, et al. Ribozyme-mediated down-regulation of survivin expression sensitizes human melanoma cells to topotecan in vitro and in vivo[J]. Carcinogenesis,2004,25(7):1129-1136.
- [6] 潘一峰, 张阳德, 王吉伟, 等. PLGA 纳米载体介导的端粒酶 hTERT 反义核酸对裸鼠肝癌移植瘤的影响[J]. 中国现代医学杂志,2006,16(5):664-665.
- [7] 梁朝霞, 程琪, 陈怀增, 等. 人免疫重建荷人人乳头瘤病毒 16 阳性宫颈癌-严重联合免疫缺陷鼠模型的建立及其免疫学特性研究[J]. 中华医学杂志,2004,84(17):1465-1469.
- [8] 黄嘉凌, 刘燕艳, 刘然义, 等. 腹腔注射人淋巴细胞建立人肝癌 PBL-SCID 嵌合模型[J]. 中华肝胆外科杂志,2005,11(2):121-124.
- [9] 赵峰, 周清华, 陆燕蓉, 等. 突变 k-ras 基因修饰的树突状细胞疫苗的抗肿瘤作用[J]. 中华实验外科杂志,2005,22(10):1229-1230.
- [10] Huttner KG, Breuer SK, Paul P, et al. Generation of potent anti-tumor immunity in mice by interleukin-12-secreting dendritic cells[J]. Cancer Immunol Immunother,2005,54(1):67-77.

[编辑:刘红武;校对:马福元]