

# rAAV-AFP转染人外周血单核细胞来源树突状细胞增强免疫刺激功能

梁政, 罗荣城, 尤长宣, 郑航

## ■背景资料

DC细胞是一类极强的MHC携带细胞, 是体内最强的抗原呈递细胞, 在抗肿瘤免疫方面发挥重要作用, 重组腺相关病毒近来被认为是具有前景的DC治疗抗原载体。

梁政, 罗荣城, 尤长宣, 郑航, 南方医科大学南方医院肿瘤中心 广东省广州市 510515

梁政, 2005年南方医科大学硕士, 主治医师, 主要从事肿瘤生物治疗的研究。

广东省自然科学基金资助项目, No. 037050

通讯作者: 罗荣城, 510515, 广东省广州市, 南方医科大学南方医院肿瘤中心. center@nfhcc.com

电话: 020-87640966

收稿日期: 2006-03-18 接受日期: 2006-04-12

## Infection of rAAV-AFP enhances immunostimulatory effect of human peripheral blood monocyte-derived dendritic cells

Zheng Liang, Rong-Cheng Luo, Chang-Xuan You, Hang Zheng

Zheng Liang, Rong-Cheng Luo, Chang-Xuan You, Hang Zheng, Cancer Center, Nanfang Hospital, Southern Medical University, Guangzhou 510515, Guangdong Province, China

Supported by the Natural Science Foundation of Guangdong Province, No. 037050

Correspondence to: Professor Rong-Cheng Luo, Cancer Center, Nanfang Hospital, Southern Medical University, Guangzhou 510515, Guangdong Province, China. center@nfhcc.com

Received: 2006-03-18 Accepted: 2006-04-12

## Abstract

**AIM:** To study the immunostimulatory effect of rAAV-AFP (recombinant adeno-associated virus expressing  $\alpha$ -fetoprotein antigen) transfection on dendritic cells (DCs) derived from human peripheral blood monocytes.

**METHODS:** Newly isolated dendritic cells were infected with rAAV-AFP. The percentage of viable DCs was observed by trypan blue exclusion every 24 h. After transfection, the alterations of surface markers on mature DCs, including CD80, CD86, CD83, CD40, CD1a, HLA-DR and  $\alpha$ -fetoprotein (AFP), were detected by flow cytometry. Meanwhile,  $^3\text{H}$ -thymidine incorporation method was used to measure the capacity of T-cell proliferation before and after transfection.

The specific killing activity of T cells was evaluated by MTT assay.

**RESULTS:** About 77.7% mature DCs expressed AFP protein. The viable DCs percentages and surface marker expression showed no significant changes after transfection ( $P > 0.05$ ). Transfected DCs still had strong potential of stimulating the proliferation of T lymphatic cells, and there was no significant difference between transfected and non-transfected group ( $P > 0.05$ ). Transfected DCs were capable of inducing specific killing effect on the target cells, and the activity was significantly higher than those in the non-transfected cells when the DCs and T cells were mixed at the ratios of 80:1, 40:1, and 20:1 ( $35.5 \pm 5.5$  vs  $20.6 \pm 4.7$ ;  $28.7 \pm 3.6$  vs  $15.3 \pm 2.5$ ;  $16.2 \pm 2.8$  vs  $9.6 \pm 1.8$ ; all  $P < 0.01$ ).

**CONCLUSION:** AFP gene, which is carried by recombinant adeno-associated virus, can be transferred into DCs with high efficiency. The function of mature DCs is not affected significantly by AFP transfection.

**Key Words:** Recombinant adeno-associated virus; Alpha fetoprotein; Dendritic cells; Gene transfection; Hepatocellular carcinoma

Liang Z, Luo RC, You CX, Zheng H. Infection of rAAV-AFP enhances immunostimulatory effect of human peripheral blood monocyte-derived dendritic cells. *Shijie Huaren Xiaohua Zazhi* 2006;14(14):1362-1366

## 摘要

**目的:** 探讨表达甲胎蛋白抗原的重组腺相关病毒rAAV-AFP转染对人外周血单核细胞来源树突状细胞(DC)免疫刺激功能的影响。

**方法:** 用rAAV-AFP转染新分离的DC; 苔盼蓝拒染法检测每天的活细胞比率; 流式细胞仪检测DC表面分子CD80, CD86, CD83, CD40, CD1a, HLA-DR及AFP的表达; 并用 $^3\text{H}$ -TdR掺入法检测转染前后DC刺激自体淋巴细胞增殖能力; MTT法检测DC诱导T细胞的杀伤活性。

**结果:** 成熟DC 77.7%表达AFP蛋白; 转染对活细胞百分率和成熟DC表型无影响, 与未转染组无显著差异( $P>0.05$ ); 转染后DC仍保持较强的刺激自体淋巴细胞增殖的能力, 与未转染组也无显著性差异( $P>0.05$ ), 并且可诱导出特异性杀伤, 效应细胞和靶细胞为80:1, 40:1, 20:1时, 与未转染组相比均有显著差异(35.5 5.5 vs 20.6 4.7; 28.7 3.6 vs 15.3 2.5; 16.2 2.8 vs 9.6 1.8; 均 $P<0.01$ ).

**结论:** rAAV可负载AFP基因在DC中表达, rAAV-AFP转染DC对其功能无明显影响, 免疫功能更强.

**关键词:** 重组腺相关病毒; 甲胎蛋白; 树突状细胞; 基因转染; 肝细胞癌

梁政, 罗荣城, 尤长宣, 郑航. rAAV-AFP转染人外周血单核细胞来源树突状细胞增强免疫刺激功能. 世界华人消化杂志 2006;14(14):1362-1366

<http://www.wjgnet.com/1009-3079/14/1362.asp>

## 0 引言

(HCC) 100 .  
(DC) (AFP) AFP , 2/3 HCC , AFP [1], (TNF) [2], [3-4]. (rAAV) DC . rAAV-AFP DC, DC HCC .

## 1 材料和方法

**1.1 材料** AIM-V RPMI 1640 T Invitrogen ; -2(rhIL-2) ; (rhGM-CSF) ; -4(rhIL-4) Promega ; - $\alpha$ (TNF- $\alpha$ ) ; AB ; PE/FITC CD80, CD86, CD83,

CD40, CD1a, HLA-DR, IgG1 ; FITC AFP Liu Yong ;  $^3\text{H}$ -TdR ; Ficoll (1077 g/L) ; C Kyowa Hakko ; Bel-7402 ; Becton Dickinson FACSCalibur; Backman ; rAAV-AFP

( ) 1  $10^{13}$  /L. DC [5] , :

AIM-V , 6 ( 2.5 mL), 37 , 50 mL/L  $\text{CO}_2$  4 h, rhGM-CSF,  $8 \times 10^5$  U/L, AIM-V 2.5 mL,  $1 \times 10^{11}$  /L rAAV-AFP rAAV-AFP-DC , rAAV-AFP N-rAAV-AFP-DC , 12 h , 3 d , IL-4, 1  $10^6$  U/L, 5 , 6 TNF- $\alpha$ , 20  $\mu\text{g/L}$ , 7 d DC .

### 1.2 方法

#### 1.2.1 rAAV-AFP对DC生长的作用

DC, , DC .

#### 1.2.2 流式细胞仪分析成熟DC的表型

7 d DC,  $10^5$ , 500  $\mu\text{L}$  , PE FITC 4 40 min, PBS 2 , rAAV-AFP DC CD80, CD86, CD83, CD40, CD1a, HLA-DR .

#### 1.2.3 混合淋巴细胞反应(MLR)

2  $10^5$  U/L rhIL-2, 100 mL/L AB RPMI 1640 37 , 50 mL/L  $\text{CO}_2$  . DC , 37 , 50 mL/L  $\text{CO}_2$  30 min , RPMI 1640 , T 7 d rAAV-AFP-DC N-rAAV-AFP-DC 25 mg/L C 37 45 min, PBS 3 ; AIM-V .  $2 \times 10^4$  /

## ■ 研究前沿

基因疫苗因其明确的靶向性, 稳定的产量估计会成为DC疫苗发展的方向, 利用病毒载体介导基因转移, 以其高效和良好的靶向性已成为基因治疗中应用最广泛的方法.

## ■创新盘点

目前以AFP作为肝癌基因治疗和免疫治疗靶点的DC疫苗中,国内尚无使用腺相关病毒载体的报道。

表 1 DC表型的FACS分析 (% , mean  $\pm$  SD,  $n = 5$ )

DC	CD83	CD86	CD80	CD40	CD1a	HLA-DR
rAAV-AFP-DC	6.90 $\pm$ 2.90	87.72 $\pm$ 6.00	6.97 $\pm$ 2.65	37.21 $\pm$ 9.60	41.22 $\pm$ 6.50	89.02 $\pm$ 8.04
N-rAAV-AFP-DC	7.65 $\pm$ 3.21	90.75 $\pm$ 3.51	7.29 $\pm$ 2.03	40.12 $\pm$ 6.86	43.06 $\pm$ 10.97	88.88 $\pm$ 6.29

10<sup>4</sup>/5 10<sup>3</sup>/2.5 10<sup>3</sup>/, DC 96  
 , 3 , T  
 1 10<sup>5</sup> / , 200  $\mu$ L. 37 , 50 mL/L  
 CO<sub>2</sub> 96 h. 18 h <sup>3</sup>H-TdR,  
 37 MBq/L,  
 . dpm ,  
 SI, SI = ( dpm - dpm  
 )/( dpm - dpm ), 3

## 1.2.4 CTL杀伤效应的测定 AFP

Bel-7402 , T  
 , 20 1 25 mg/L C  
 DC 4 d ,  
 96 ( 1 10<sup>4</sup>  
 / ), 80 1, 40 1, 20  
 1, 10 1 , 5  
 . 37 , 50 mL/L CO<sub>2</sub> 12 h  
 0.5 mg/L MTT, 6 h,  
 100  $\mu$ L, 5 min  
 570 nm A . % = [1- A  
 ]/(T A + A )] 100%,  
 统计学处理 SPSS 10.0  
 , mean SD ,  $\chi^2$  t

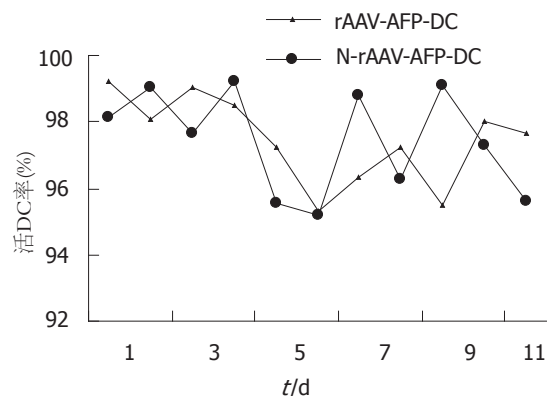


图 1 rAAV-AFP对活DC百分率的影响。

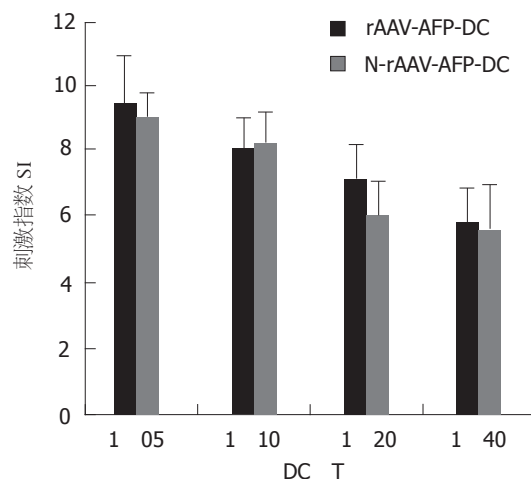


图 2 成熟DC转染rAAV-AFP体外激发自体T细胞增殖情况。

## 2 结果

## 2.1 DC的形态学观察和计数

rAAV-AFP-DC DC  
 , , 3 d  
 , ,  
 , , 7 d ,  
 , N-rAAV-AFP-DC  
 ( $P > 0.05$ , 1).

## 2.2 rAAV-AFP感染DC前体的效率

rAAV-AFP DC , AFP  
 77.7%, AFP DC

## 2.3 成熟DC的表型分析 FACS

, rAAV-AFP

, DC ( $P > 0.05$ , 1).

2.4 MLR测定 rAAV-AFP-DC N-rAAV-AFP-DC  
 DC SI ( $P > 0.05$ , 2),  
 T

## 2.5 CTL活性检测 Bel-7402

, rAAV-AFP-DC N-rAAV-AFP-DC  
 80 1, 40 1, 20 1 CTL  
 ( $P < 0.01$ , 2).

## 3 讨论

(DC)  
 . (HCC)  
 DC

## ■应用要点

本文为rAAV-AFP转染DC的进一步研究提供了可行性依据,也为最终用于临床提供重要参考。

表 2 DC诱导的CTL活性 (% , mean  $\pm$  SD,  $n = 5$ )

DC	效靶比			
	80 : 1	40 : 1	20 : 1	10 : 1
rAAV-AFP-DC	35.5 $\pm$ 5.5 <sup>b</sup>	28.7 $\pm$ 3.6 <sup>b</sup>	16.2 $\pm$ 2.8 <sup>b</sup>	10.5 $\pm$ 2.3
N-rAAV-AFP-DC	20.6 $\pm$ 4.7	15.3 $\pm$ 2.5	9.6 $\pm$ 1.8	9.3 $\pm$ 2.7

<sup>b</sup> $P < 0.01$  vs N-rAAV-AFP-DC组.

AFP HCC

AFP

[6] AFP

AFP

Vollmer *et al*<sup>[7]</sup> T

AFP CTL

AFP HCC

DC, AFP

AFP

T [8-10] DC

AFP-cDNA

DC [11-12]

DC

DC

MHC

(rAAV)

DC

[13]

[14]

Liu *et al*<sup>[15]</sup> rAAV

BA46, DC

DC

CTL

AFP

DC, DC

T, AFP

rAAV DC

T

77.7% DC

AFP

DC

AFP

DC

rAAV

DC

DC

AFP

CTL

AFP

DC

rAAV

AFP

#### 4 参考文献

- 1 Semeniuk DJ, Boismenu R, Tam J, Weissenhofer W, Murgita RA. Evidence that immunosuppression is an intrinsic property of the alpha-fetoprotein molecule. *Adv Exp Med Biol* 1995; 383: 255-269
- 2 Ozoren N, El-Deiry WS. Cell surface Death Receptor signaling in normal and cancer cells. *Semin Cancer Biol* 2003; 13: 135-147
- 3 Dudich E, Semenkova L, Gorbatoeva E, Dudich I, Khromykh L, Tatulov E, Grechko G, Sukhikh G. Growth-regulative activity of human alpha-fetoprotein for different types of tumor and normal cells. *Tumour Biol* 1998; 19: 30-40
- 4 王兴旺, 胥彬. 甲胎蛋白对H-22腹水肝癌小鼠脾淋巴细胞增殖的影响. *上海免疫学杂志* 1995; 15: 327-329
- 5 Liu Y, Chiriva-Internati M, Grizzi F, Salati E, Roman JJ, Lim S, Hermonat PL. Rapid induction of cytotoxic T-cell response against cervical cancer cells by human papillomavirus type 16 E6 antigen gene delivery into human dendritic cells by an adeno-associated virus vector. *Cancer Gene Ther* 2001; 8: 948-957
- 6 贺平, 刘彬彬, 叶胜龙, 汤钊猷. 人肝癌、癌旁组织中AFPmRNA的差异性分析. *中国肿瘤生物治疗杂志* 1998; 5: 163-166
- 7 Vollmer CM Jr, Eilber FC, Butterfield LH, Ribas A, Dissette VB, Koh A, Montejo LD, Lee MC, Andrews KJ, McBride WH, Glaspy JA, Economou JS. Alpha-fetoprotein-specific genetic immunotherapy for hepatocellular carcinoma. *Cancer Res* 1999; 59: 3064-3067
- 8 谢裕安, 罗小玲, 梁安民, 匡志鹏, 吴继宁. 肝癌细胞抗原致敏树突状细胞体外诱导特异性细胞毒T淋巴细胞. *世界华人消化杂志* 2004; 12: 1479-1481
- 9 Butterfield LH, Meng WS, Koh A, Vollmer CM, Ribas A, Dissette VB, Faull K, Glaspy JA, McBride WH, Economou JS. T cell responses to HLA-A\*0201-restricted peptides derived from human alpha fetoprotein. *J Immunol* 2001; 166: 5300-5308
- 10 Grimm CF, Ortmann D, Mohr L, Michalak S, Krohne TU, Meckel S, Eisele S, Encke J, Blum HE, Geissler M. Mouse alpha-fetoprotein-specific DNA-based immunotherapy of hepatocellular carcinoma leads to tumor regression in mice. *Gastroenterology* 2000; 119: 1104-1112
- 11 张利旺, 张红梅, 贾军, 任军. 以AFP为靶点的肝癌树突状细胞免疫治疗的实验研究. *现代肿瘤医学* 2005;

#### ■名词解释

重组腺相关病毒 (rAAV): 一种活病毒载体, 具有稳定表达 定点整合 安全性较高, 且不表达任何病毒自身蛋白基因等优势, 是介导基因转移和基因治疗常用的载体系统之一, 他因其位点特异性的整合能力 自然缺陷以及无致病原性, 被认为是最有前景的DC治疗抗原载体。

#### ■同行评价

本文采用rAAV-AFP(表达甲胎蛋白抗原的重组腺相关病毒)转染人外周血单核细胞来源树突状细胞(DC), 并检测转染对成熟DC表型DC的刺激自体淋巴细胞增殖的能力以及CTL反应的影响, 有一定学术及实用价值。

- 13: 736-739
- 12 Zeng B, Lu FG, Liu XW, Yang DY, Fang WY, Wang J, Liao AJ, Shi W. Construction of eukaryotic expression vector of mouse alpha-fetoprotein cDNA and its expression in dendritic cells and in vitro antitumor effect on hepatoma. *Aizheng* 2005; 24: 1332-1337
- 13 Zaiss AK, Liu Q, Bowen GP, Wong NC, Bartlett JS, Muruve DA. Differential activation of innate immune responses by adenovirus and adeno-associated virus vectors. *J Virol* 2002; 76: 4580-4590
- 14 Enger PO, Thorsen F, Lonning PE, Bjerkvig R, Hoover F. Adeno-associated viral vectors penetrate human solid tumor tissue *in vivo* more effectively than adenoviral vectors. *Hum Gene Ther* 2002; 13: 1115-1125
- 15 Liu Y, Chiriva-Internati M, You C, Luo R, You H, Prasad CK, Grizzi F, Cobos E, Klimberg VS, Kay H, Mehta JL, Hermonat PL. Use and specificity of breast cancer antigen/milk protein BA46 for generating anti-self-cytotoxic T lymphocytes by recombinant adeno-associated virus-based gene loading of dendritic cells. *Cancer Gene Ther* 2005; 12: 304-312

电编 张敏 编辑 潘伯荣

ISSN 1009-3079 CN 14-1260/R 2006

• 消息 •

## 国际肝胆胰协会中国分会第二届全国学术研讨会 暨第三届全国普通外科主任论坛通知

本刊讯

2006-10

2004-12

Jim Tooli

Büechler

Broelsch

2006

10

500-800

chenxp@medmail.com.cn,

1095

( ), : 430030;

: 027-83662599.