

论著

腺病毒介导的T-bet基因转染诱导Th1型淋巴细胞分化

陈祖兵¹,陶剑平^{2△},梁力建¹,刘晓平³,胡文杰¹,李绍强¹

中山大学1附属第一医院肝胆外科, 2医学院微生物教研室, 广东 广州 510080; 3北京大学附属深圳医院肝胆外科, 广东 深圳 518036

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摘要 目的: 构建含有T细胞转录因子T-bet(T-box expressed in T cells)的重组腺病毒Ad.T-bet, 并诱导Th1型淋巴细胞分化。方法: 采用RT-PCR的方法从健康人外周血淋巴细胞的总RNA中克隆T-bet基因, 亚克隆入腺病毒穿梭载体成为pAdtrack-CMV.T-bet, 与腺病毒基因组载体pAdeasy-1同源重组为pAd.T-bet, 经Pac I酶切后转染293细胞, 用Western blotting和RT-PCR的方法鉴定Ad.T-bet; 联合腺病毒和脂质体以感染倍数(m.o.i) 5000感染活化的淋巴细胞, 用ELISA法检测培养液中IFN- γ 含量。结果: 采用RT-PCR方法从健康人外周血淋巴细胞中扩增出T-bet基因, 构建出重组腺病毒Ad.T-bet, Western blotting和RT-PCR均检测出细胞内T-bet蛋白表达; 联合Ad.T-bet和脂质体感染淋巴细胞诱导Th1型细胞因子IFN- γ 持续高表达。结论: 重组腺病毒Ad.T-bet有效诱导Th1型细胞分化, 有望成为改变肿瘤患者免疫抑制状态的有效方法。

关键词 [腺病毒载体](#); [淋巴细胞](#); [基因](#),[T-bet](#)

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Adenovirus-mediated T-bet gene transfers into lymphocyte to induce type-1 T-helper differentiation

CHEN Zu-bing¹,TAO Jian-ping²,LIANG Li-jian¹,LIU Xiao-ping³,HU Wen-jie¹,LI Shao-qiang¹

1Department of Hepatobiliary Surgery, The First Affiliated Hospital, 2Department of Microbiology, Sun Yat-sen Medical College, Sun Yat-sen University, Guangzhou 510080, China; 3Department of Hepatobiliary Surgery, The Shenzhen Hospital of Beijing University, Shenzhen 518036, China

Abstract

AIM: To construct recombinant adenovirus containing transcription factor T-bet (T-box expressed in T cells), and induce type-1 T-helper differentiation of lymphocytes. METHODS: T-bet gene was cloned from total RNA of lymphocyte stimulated with IFN- γ with RT-PCR methods, then subcloned into transfer vector pAdtrack-CMV in BglIII/SalI sites. The new transfer vector pAdtrack-CMV. T-bet was digested with Pme I, subsequently cotransformed into BJ5183 cells with adenoviral backbone plasmid pAdEasy-1. The resultant plasmid pAd. T-bet was linearized by Pac I and transfected into 293 cells with liposome LIPOFECTAMINE 2000 for producing Ad.T-bet. The recombined adenovirus Ad.T-bet was identified through RT-PCR and Western blotting methods. Lymphocytes purified from patients suffering from liver cancer was infected with liposome and Ad.T-bet with multiplicity of infection (m.o.i) 5000, and the concentration of IFN- γ in culture media was evaluated with ELISA methods. RESULTS: T-bet gene was successfully cloned from lymphocytes and incorporated into recombinant adenovirus Ad.T-bet. Lymphocytes infected with Ad. T-bet constantly and strongly secreted Th1 cytokine IFN- γ . CONCLUSION: Recombinant adenovirus Ad.T-bet effectively induces type-1 T-helper differentiation, which is a promising method for restoration of patients' immune reaction against cancer.

Key words [Adenovirus vector](#) [Lymphocytes](#) [Genes](#) [T-bet](#)

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