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Reversing the polarization of tumor-associated macrophages inhibits tumor metastasis

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ABSTRACT

Objective: The M2 phenotype is dominant in tumor associated macrophages (TAM), and plays a key role in promoting tumor growth, invasion and metastasis. Converting TAM polarization from M2 to M1 may contribute to eliciting anti-tumor-specific immune responses and inhibiting tumor metastasis. In this study, the effect of reversing the polarization of TAM on tumor metastasis was investigated.

Methods: Peritoneal macrophages were obtained from BABL/c mice, and M2 polarization was induced by IL-4. In an *in vivo* experiment, BABL/c mice were transplanted with 4 T1 tumor cells. *In vitro* and *in vivo* experimental studies, M2 macrophage polarization was reversed with CpG-DNA or CpG-DNA combined with anti-IL-10R Ab. CD68, MHCII and FRβ molecular expression in macrophages were examined with immunofluorescence staining. The mRNA expression of IL-2, IL-6, IL-13, VEGF and MMP-9 were detected with RT-PCR. VEGF and MMP-9 protein expression of tumors *in situ* was measured by western blot assay. Lung-metastasis of the tumor was observed and assessed by micro-CT.

Results: CpG-DNA and CpG-DNA combined with anti-IL-10R Ab could promote MHCII, IL-2, IL-6 and IL-13 molecular expression, and suppress the expression of FRβ, MMP-9 and VEGF, in both freshly isolated peritoneal macrophages and M2 macrophages. In the CpG-DNA combined with anti-IL-10R Ab injecting group, the percentage of $CD68^+$ $MHCII^+$ cells were significantly higher than that of $CD68^+$ $FR\beta^+$ cells ($P < 0.05$). This was distinct from the result of the control group, which $CD68^+$ $FR\beta^+$ was higher than $CD68^+$ $MHCII^+$ cells ($P < 0.01$). Furthermore, VEGF-A and MMP-9 level in primary tumor tissues in the experimental group was significantly lower ($P < 0.01$), compared to the control group. Moreover, the number of detectable lung-metastasis foci was significantly lower in the experimental group than in the control group ($P < 0.05$).

Conclusion: Reversing the polarization of TAM from M2 to M1 phenotype can inhibit tumor metastasis.

1. Introduction

Macrophages are multifunctional cells that are involved in activities such as dead cell removal, inflammation promotion, antigen presenting, and damaged tissue remodelling [1,2]. Macrophages are heterogeneous cells that exhibit distinct phenotypes and functions. When microenvironment condition varies, macrophages can polarize and differentiate into M1 or M2 cells [3,8,13]. M1 macrophages are cells with immune functions, which contribute to promoting inflammation responses and eliciting specific immune responses [4,5]. However, M2 macrophages are anti-inflammatory cells and suppress effector T cells [5–7]. Recent studies have shown that M2 macrophages are also involved in wound healing or tissue remodelling. As the dominant phenotype in TAMs, M2 macrophages play a key role in promoting tumor growth, invasion and metastasis [9–12]. M2 macrophages can make the endothelium more susceptible for tumor cell invasion by producing proteases, such as matrix metalloproteinases (MMP), that can breakdown the basement membrane around the endothelium [13–15]. By producing vascular endothelial growth factors, M2 also promotes angionesis [16–19]. Under these mechanism, M2 macrophages contribute largely to tumor invasion and metastasis, which usually lead to worse prognosis in both mice and humans [20,21]. We hypothesize that reversing the M2 polarization of TAMs can initiate anti-tumor-specific immune responses, thereby inhibiting tumor metastasis. This method could be a new therapeutic option for cancer, in which the chance of cancer invasion and metastasis can be reduced. CpG-DNA is immunostimulatory DNA sequences which can induce both innate and adaptive cellular immune responses and has great potential clinical applications [35]. It

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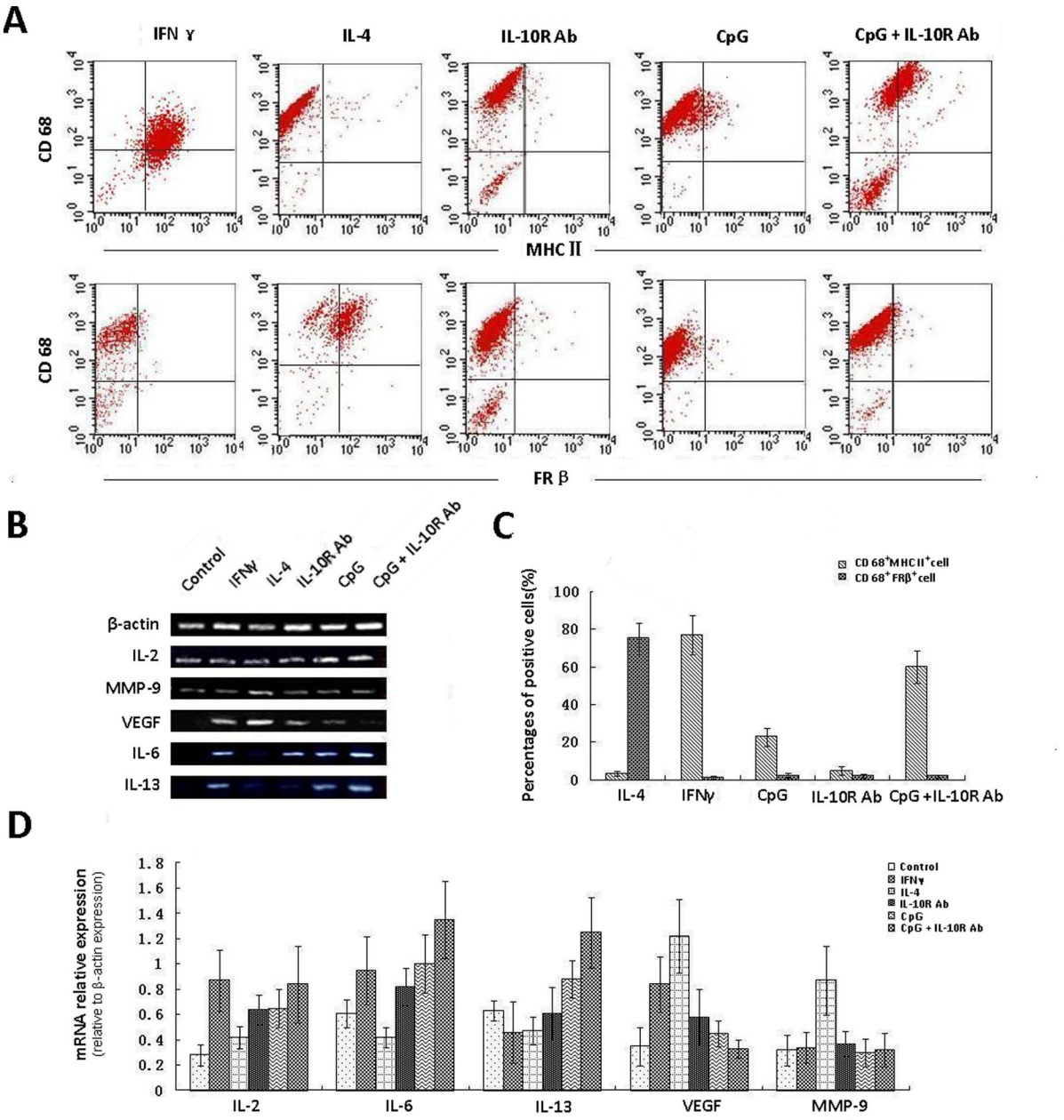

Fig. 1. Effect of CpG-DNA, anti-IL-10R Ab and CpG-DNA combined with anti-IL-10R Ab on macrophage polarization. Macrophages were cultured separately in medium containing IFNγ(100 IU/ml), IL-4(10 IU/ml), anti-IL-10R Ab(1.2 μg/ml), CpG (4 μg/ml) or CpG combined with anti-IL-10R Ab (CpG 4 μg/ml, IL-10RAb 1.2 μg/ml) for 72 h. (A) Flow cytometry analysis of CD68, MHCII and FRβ molecules expression on the macrophages. Macrophages were double stained by CD68-PE and MHCII-FITC, CD68- PE and FRβ- FITC. (B) RT-PCR detection of mRNA expression of IL-2, IL-6, IL-13, VEGF and MMP-9. (C) Percentage of $CD68^+$ $MHCII^+$ and $CD68^+$ $FR\beta^+$ cells. (D) Comparison of relative mRNA expression of genes IL-2, IL-6, IL-13, VEGF and MMP-9. Values are mean ± SD of three assays.

is reported that CpG–DNA combined with anti-IL-10R Ab could promote M1 macrophages polarization [28]. This study demonstrated that CpG-DNA or CpG-DNA combined with IL-10R Ab may promotes a M2-to-M1 macrophage reverse polarization, and the intratumor injection of CpG-DNA combined with anti-IL-10R Ab could inhibit tumor metastasis.

2. Materials and methods

2.1. Mice and Cell lines

BABL/c mice were obtained from Dalian Medical University. The mouse 4 T1 cell line was purchased from ATCC.

2.2. Cytokines and mAbs

The mAbs, rabbit anti-mouse VEGF-A, MMP-9 and FITC-labeled anti-mouse MHCII were purchased from eBioscience (USA). The mAbs, PE-labeled anti-mouse CD68, FITC-labeled anti-mouse FRβ and isotype-matched antibodies were purchased from Biolegend (USA). The mouse anti-mouse IL-10R Ab was purchased from Santa Cruz Biotechnology (USA). IL-4 and IFN-γ were purchased from Dakewe (USA). CPG-DNA was purchased from Peprotech (USA). Fetal bovine serum (FBS) was purchased from Sijiqing (China). The RPMI-1640 culture medium was purchased from Gibco (USA). The Total RNA Extractor kit was purchased from Sangon (China).