Mini Review

Effect of natural compounds on human macrophage activation

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Tumor-associated macrophages (TAMs) of M2 phenotype promote tumor proliferation and relate to poor prognosis in patients with glioblastoma. We screened natural compounds possessing inhibitory effect on M2 polarization in human monocyte-derived macrophages. Among 130 purified natural compounds, some natural compounds such as glycyrrhizin, medicarpin and solasodine significantly inhibited the expression of CD163, one of the phenotypic markers of M2 macrophages, as well as suppressed the secretion of IL-10, one of the anti-inflammatory cytokine preferentially produced by M2 macrophages, thus suggesting that glycyrrhizin, medicarpin and solasodine suppress macrophage polarization towards M2 phenotype. Therefore, glycyrrhizin, medicarpin and solasodine may be potentially useful new strategy for the prevention and therapy of tumor.

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Introduction

Macrophages infiltrating in cancer tissues are referred to as tumor-associated macrophages (TAMs) and closely involved in development of tumor microenvironment¹⁻³⁾. TAMs are considered to belong to alternatively activated macrophages (M2) because of their anti-inflammatory functions^{4,5)}. In some kinds of tumors, the presence of TAM is associated with poor prognosis of the patients^{3,6,7)}.

Macrophage subpopulations have different types of receptor expression and cytokine production^{5,8-10)}. Classically activated macrophages (M1 macrophages) have the IL-12^{high}, IL-23^{high}, IL-10^{low} phenotype and produce nitrogen intermediates and inflammatory cytokines such as IL-1 β , tumor necrosis factor- α (TNF- α), and IL-6^{5,8-11)}. In contrast, alternatively activated macrophages (M2 macrophages) have the IL-12^{low}, IL-23^{low}, IL-10^{high} phenotype and have high expression of several receptors such as

class A scavenger receptor (SR-A, CD204), mannose receptor, CD163, dectin-1 and DC-SIGN^{5,8-11}).

We previously demonstrated that CD163 is a useful marker to detect M2 cells on paraffin-embedded surgical specimens¹². In human glioblastoma, the proportion of CD163-positive M2 TAMs are closely involved in tumor cell proliferation and poor prognosis¹³. These observations indicate the significance of macrophage differentiation in tumor development.

In this study, we have prepared 130 purified compounds from natural products, and measured their inhibitory effect on M2 polarization in human monocyte-derived macrophages to screen the candidate agents for cancer immunotherapy.

Materials and methods

1) Cells and cell culture conditions

Peripheral blood mononuclear cells were obtained from healthy volunteer donors. Informed written consent was obtained from healthy donors. CD14⁺ monocytes were purified from peripheral blood mononuclear cells by positive selection via magnetic-activated cell sorting technology (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). Monocytes were cultured in DMEM supplemented with 10% FBS and GM-CSF (10 ng/ml, WAKO, Tokyo, Japan) for 5 days to be differentiated as macrophages.

2) Natural compounds

130 purified natural compounds were prepared by extraction and isolation from natural products such as herbs, vegetables, fruits and natural medicines. The purified natural compounds were dissolved in DMSO to a 10 mM stock solution.

3) Determination of the inhibitory effect of natural compounds on CD163 expression

Human monocyte-derived macrophages (5 x 10⁴ cells per well of a 96-well plate) were incubated with natural compounds (30 μ M) for 24 hr after treatment with IL-10 (30 nM) or TCS for 2 days, followed by the determination of CD163 expression by Cell-ELISA.

4) Cell Enzyme-linked Immunosorbent Assay (Cell-ELISA)

Expression of CD163 on human monocyte-derived macrophages were evaluated with the aid of a Cell-ELISA, as described previously¹². Briefly, each well of a 96-well plate was blocked with Block Ace, and washed three times with PBS containing 0.05% Tween 20 (washing buffer). The wells were incubated with anti-CD163 antibody, AM3K (2 μ g/ml), dissolved in wash-

ing buffer for 1 hr. The wells were then washed with washing buffer three times and react with HRP-conjugated anti-mouse IgG antibody, followed by reaction with ULTRASENSITIVE TMB (Moss INC., MD, USA). The reaction was terminated by the addition of 1 M sulfuric acid, and the absorbance at 450 nm was read by a micro-ELISA plate reader.

5) Determination of the inhibitory effect of natural compounds on IL-10 secretion

Human monocyte-derived macrophages (5 x 10⁴ cells per well of a 96-well plate) were stimulated with LPS from *Escherichia coli* (100 ng/ml) for 24 hr after incubation with corosolic acid (30 μ M) for 24 hr in the presence of tumor cell supernatant (TCS), followed by the determination of IL-10 secretion by means of ELISA kit (eBioscience, San Diego, CA, USA).

6) Statistics

All data are representative two or three independent experiments. Data are expressed as means $\pm$ SD. Mann-Whitney's *U*-test was used for two-group comparison. A value of *p* < 0.05 was considered statistically significant.

Results

In the present study, we first developed the assay system for CD163 expression, a M2 phenotype marker, by Cell-ELISA to screen natural compounds possessing inhibitory effect on M2 polarization in human monocyte-derived macrophages. As shown in Fig.1A, Incubation of human monocyte-derived macrophages for 2 days with IL-10 increased CD163 expression. Under the same conditions, we measured the effect of 130 natural compounds on IL-10-induced CD163 expression. As a result, some natural compounds such as glycyrrhizin, medicarpin and solasodine significantly inhibited CD163 expression (Fig. 1B). Next, we measured inhibitory effect of those compounds on CD163 expression and IL-10 secretion in human monocyte-derived macrophages induced by tumor culture supernatant (TCS) of glioblastoma cell line, U373 cells. Stimulation with TCS increased CD163 expression and IL-10 secretion in human monocyte-derived macrophages (Fig.2). Under assay conditions employed, glycyrrhizin, medicarpin and solasodine significantly inhibited TCS-induced CD163 expression (Fig.2A) and IL-10 secretion (Fig. 2B). These data suggest that glycyrrhizin, medicarpin and solasodine significantly inhibit polarization towards M2 macrophages.

Fig.1 Effect of Natural Compounds on CD163 Expression

Human monocyte-derived macrophages (5×10^4 cells per well of a 96-well plate) were incubated with IL-10 for 2 days, followed by determination of CD163 expression by Cell-ELISA as described in the Materials and Methods (A). Human monocyte-derived macrophages (5×10^4 cells per well of a 96-well plate) were incubated with natural compounds ($30 \mu\text{M}$) for 24 hr after treatment with IL-10 (30 ng/ml) for 2 days, followed by determination of CD163 expression by Cell-ELISA as described in the Materials and Methods (B). Data are presented as the mean $\pm$ SD ($n=4$). * $p < 0.01$, ** $p < 0.001$ vs. control.

Fig.2 Effect of Corosolic acid on CD163 Expression and IL-10 Secretion

Human monocyte-derived macrophages (5×10^4 cells per well of a 96-well plate) were incubated with $30 \mu\text{M}$ test compounds (GR: glycyrrhizin, MC: medicarpin, SS: solasodine) for 24 hr after treatment with IL-10 (30 ng/ml) or TCS for 2 days, followed by determination of CD163 expression by Cell-ELISA as described in the Materials and Methods (A). Human monocyte-derived macrophages (5×10^4 cells per well of a 96-well plate) were stimulated with LPS (100 ng/ml) for 24 hr after incubation with $30 \mu\text{M}$ test compounds (GR: glycyrrhizin, MC: medicarpin, SS: solasodine) for 24 hr in the presence of TCS, followed by determination of IL-10 secretion by ELISA as described in the Materials and Methods (B). Data are presented as the mean $\pm$ SD ($n=5$).

Discussion

It is well known that TAMs play an important role in cancer growth. TAMs release many proangiogenic cytokines and growth factors such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), colony stimulation factor-1 (CSF-1), platelet-derived growth factor (PDGF), and basic fibroblast growth factor to promote tumor progression. They also produce arginase-1, IL-10, and transforming growth factor- β (TGF- β), which inhibit the antitumor function of T cells and natural killer cells¹⁻³. In our recent study, we revealed that macrophages account for major share of cells infiltrated in glioblastomas and their polarization toward M2 phenotype is significantly associated to poor prognosis of patients¹³. Therefore, it is speculated that inhibition of macrophage polarization toward M2 phenotype could be a new strategy of anticancer therapy.

In this study, we prepared 130 purified compounds from natural products, and screened their inhibitory effect on M2 polarization of human monocyte-derived macrophages. In this screening, we identified some natural compounds such as glycyrrhizin, medicarpin and solasodine have inhibitory effect on CD163 expression, a marker of M2 phenotype (Fig.1). Furthermore, glycyrrhizin, medicarpin and solasodine inhibited IL-10- and TCS-induced CD163 expression and TCS-induced IL-10 secretion in human monocyte-derived macrophages (Fig.2), thus suggesting that those compounds inhibit M2 polarization of macrophages. Therefore, glycyrrhizin, medicarpin and solasodine may be potentially useful new compounds for anti-tumor therapy.

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