Differential Expression of mRNA in Human Monocytes following Interaction with Human Colon Cancer Cells

TERUKO HONDA[1], HIROYUKI INAGAWA[2] and ISAMU YAMAMOTO[1]

[1]*Department of Medical Technology, School of Life and Environmental Science, Azabu University, 1-17-71 Fuchinobe, Chuou-ku, Sagamihara, Kanagawa 252-5201, Japan;*
[2]*Department of Integrated and Holistic Immunology, Faculty of Medicine, Kagawa University, Miki-cho, Kida-gun, Kagawa 761-0793, Japan*

Abstract. *Monocytes are known to differentiate into tissue-specific macrophages in response to the tissue environment, and it has been suggested that tumor-associated macrophages might promote angiogenesis. Therefore, the factors associated with monocyte differentiation into tumor-associated macrophages may become new targets for cancer therapy. However, these factors remain unclear in human colon cancer. The aim of this study was to identify the factors associated with human monocyte differentiation into tumor-associated macrophages at human colon cancer sites. Materials and Methods: A human monocyte cell line (THP-1) was co-cultured with a human colon cancer cell line (DLD-1) and mRNA expression was analyzed by quantitative real-time PCR. Results: In THP-1 cells, monocyte chemotactic protein (MCP)-1 mRNA expression increased in a time-dependent manner from day 3 after co-culture with DLD-1 cells; furthermore, expression of vascular endothelial growth factor (VEGF)-A, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-8 mRNA was increased from day 5. This increase in mRNA expression in the THP-1 cells was attributable to the presence of the DLD-1 cells. Therefore, MCP-1, VEGF-A, TNF-α, IL-1β, and IL-8 are suggested to be associated with differentiation of human monocytes into tumor-associated macrophages at human colon cancer sites.*

Macrophages are differentiated from monocytes of myeloid origin. Monocytes, after leaving the bone marrow, enter the blood circulation system where they circulate for several days and migrate into various tissues. They then differentiate into tissue-specific macrophages in response to the tissue environment (1).

Macrophages can be divided into M1 and M2 types. M1 type macrophages are believed to exert cytotoxic effects on tumor cells, phagocytose apoptotic/necrotic cell debris, and present antigens to T-cells (2). In contrast, M2 type macrophages are believed to promote angiogenesis and tumor progression (3-6). Numerous macrophages have been demonstrated to be present in tumor tissues. These macrophages are known as tumor-associated macrophages. A high density of tumor-associated macrophages is related to poor prognosis in several types of human cancer (carcinoma of the breast, prostate, cervix, lung, and bladder and malignant melanoma), and that such tumor-associated macrophages are of the M2 type (7-10). Tumor-associated macrophages may promote angiogenesis (11-13). Therefore, the factors associated with monocyte differentiation into tumor-associated macrophages may become new targets for cancer therapy.

Recent studies have revealed that the nuclear factor (NF)-κB signaling pathway in tumor-associated macrophages is important for tumor initiation and growth (14-16). However, the factors associated with monocyte differentiation into tumor-associated macrophages remain unclear in human colon cancer. We examined the differential expression of mRNA in human monocytes following their interaction with human colon cancer cells to identify these factors.

Materials and Methods

Cells and cell co-culture. Human colon cancer DLD-1 cells obtained from the Japan Health Sciences Foundation and human monocyte THP-1 cells obtained from DS Pharma Biomedical were cultured in a 5% CO_2 atmosphere at 37˚C in RPMI-1640 medium (WAKO Pure Chemical Industries, Ltd., Osaka, Japan) containing 10% fetal calf serum supplemented with 100 units/ml each of penicillin and streptomycin (WAKO Pure Chemical Industries, Ltd.). DLD-1 and THP-1 cells were co-cultured using a cell culture insert (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) with a 0.4 μm porous

Correspondence to: Teruko Honda, Department of Medical Technology, School of Life and Environmental Science, Azabu University, 1-17-71 Fuchinobe, Chuou-ku, Sagamihara, Kanagawa 252-5201, Japan. Tel: +81 427547111, Fax: +81 427547611, e-mail: hondat@azabu-u.ac.jp

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membrane to separate the upper and lower chambers. DLD-1 cells were cultured in the upper chamber at 2×10^5 cells/ml, and THP-1 cells were cultured in the lower chamber at 2×10^5 cells/ml. DLD-1 cells, THP-1 cells, and the culture supernatants were collected 0, 1, 3, and 5 days after co-culture.

RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR). Total RNA from DLD-1 and THP-1 cells was isolated by guanidinium thiocyanate-phenol-chloroform extraction with TRIzol Reagent (Invitrogen Corp., Carlsbad, CA, USA) according to the manufacturer's protocol. RNA was quantified by absorbance at 260 nm. cDNA was synthesized using reverse transcriptase with Oligo(dT)20 (TOYOBO Co, Ltd., Osaka, Japan). PCR was performed for 30 cycles with melting at 95˚C for 1 min, annealing at 60˚C for 1 min, and extension at 72˚C for 1 min using gene-specific primers. PCR products were electrophoresed in a 2.0% agarose gel, stained with ethidium bromide, and visualized by ultraviolet irradiation.

Quantitative PCR. mRNA expression was analyzed by quantitative real-time PCR (Model MiniOpticon; Bio-Rad Laboratories, Inc., Hercules, CA, USA). After initial heat denaturation at 95˚C for 3 min, PCR conditions were set at 95˚C for 10 s and 60˚C for 30 s for 40 cycles. Relative quantification was achieved by normalization to the value of the housekeeping gene β-actin. Data were expressed as fold changes in mRNA expression compared with co-culture at day 0.

Enzyme-linked immunosorbent assay (ELISA). The culture supernatants of cells were collected and stored at −20˚C until assay. The monocyte chemotactic protein (MCP)-1 concentration in the culture supernatants of THP-1 cells was measured by ELISA kit (Funakoshi Co, Ltd., Tokyo, Japan) according to the manufacturer's protocol.

Results

mRNA expression in human colon cancer cells and human monocytes. mRNA expression before co-culture was analyzed by RT-PCR. In DLD-1 cells, expression of vascular endothelial growth factor (VEGF)-A, fibroblast growth factor (*FGF*), interleukin (IL)-8, transforming growth factor (TGF)-β, and *NF-κB* mRNA was detected, whereas expression of MCP-1, tumor necrosis factor (TNF)-α, *IL-1β, IL-6, IL-10,* and inducible nitric oxide synthase (*iNOS*) mRNA was not detected (Table I). DLD-1 cells expressed growth- and transcription-related factors and showed up-regulation of proliferation character.

In THP-1 cells, expression of *VEGF-A, FGF, MCP-1, TNF-α, IL-1β, IL-8, TGF-β,* and *NF-κB mRNA* was detected, whereas expression of *IL-6, IL-10,* and *iNOS* mRNA was not detected (Table I). THP-1 cells expressed growth-, chemotaxis-, cytotoxicity-, and transcription-related factors. These data indicated that THP-1 cells had a common character of monocytes/macrophages.

Differential expression of mRNA following interaction between human colon cancer cells and human monocytes. Changes in mRNA expression after co-culture were analyzed by quantitative real-time PCR. In DLD-1 cells, only *VEGF-A* mRNA expression was increased 6.9-fold at day 3 and 11.8-fold at day 5 after co-culture; the increase was time-dependent from day 3 (Figure 1). *FGF, IL-8, TGF-β,* and *NF-κB* mRNA expression remained unchanged in DLD-1 cells after co-culture (Figure 1). These results indicated that the increase in *VEGF-A* mRNA expression in the DLD-1 cells was attributable to the presence of the THP-1 cells. *VEGF-A* is an angiogenesis-related factor. Therefore, it is suggested that human colon cancer cells might induce angiogenesis following interaction with human monocytes.

Table I. *mRNA expression in human monocyte THP-1 cells and human colon cancer DLD-1 cells.*

Gene	THP-1 cells	DLD-1 cells
VEGF-A	+	+
FGF	+	+
MCP-1	+	−
TNF-α	+	−
IL-1β	+	−
IL-6	−	−
IL-8	+	+
IL-10	−	−
TGF-β	+	+
iNOS	−	−
NF-κB	+	+

+ Detected, − not detected. VEGF-A: vascular endothelial growth factor-A, FGF: fibroblast growth factor, MCP-1: monocyte chemotactic protein-1, TNF-α: tumor necrosis factor-α, IL-1β: interleukin-1β, IL-6: interleukin-6, IL-8: interleukin-8, IL-10: interleukin-10, TGF-β: transforming growth factor-β, iNOS: inducible nitric oxide synthase, NF-κB: nuclear factor-κB.

In THP-1 cells, *MCP-1* mRNA expression increased 5.3-fold at day 3 and 28.0-fold at day 5, *VEGF-A* mRNA expression increased 5.0-fold at day 5, *TNF-α* mRNA expression increased 11.2-fold at day 5, *IL-1β* mRNA expression increased 8.6-fold at day 5, and *IL-8* mRNA expression increased 29.1-fold at day 5 after co-culture (Figure 2). Moreover, *MCP-1* mRNA expression in THP-1 cells increased in a time-dependent manner from day 3 after co-culture, and *VEGF-A, TNF-α, IL-1β,* and *IL-8* mRNA expression in THP-1 cells increased at day 5 after co-culture. *FGF, TGF-β,* and *NF-κB* mRNA expression remained unchanged in THP-1 cells after co-culture (Figure 2). The increase in *MCP-1, VEGF-A, TNF-α, IL-1β,* and *IL-8* mRNA expression in the THP-1 cells was attributable to the presence of the DLD-1 cells. Therefore, it is suggested that MCP-1, VEGF-A, TNF-α, IL-1β, and IL-8 might be associated with human monocyte differentiation into tumor-associated macrophages at human colon cancer sites. In addition, these experiments showed that *MCP-1* mRNA expression was detected in THP-1 cells but not in DLD-1 cells. Human monocytes may possibly be recruited by

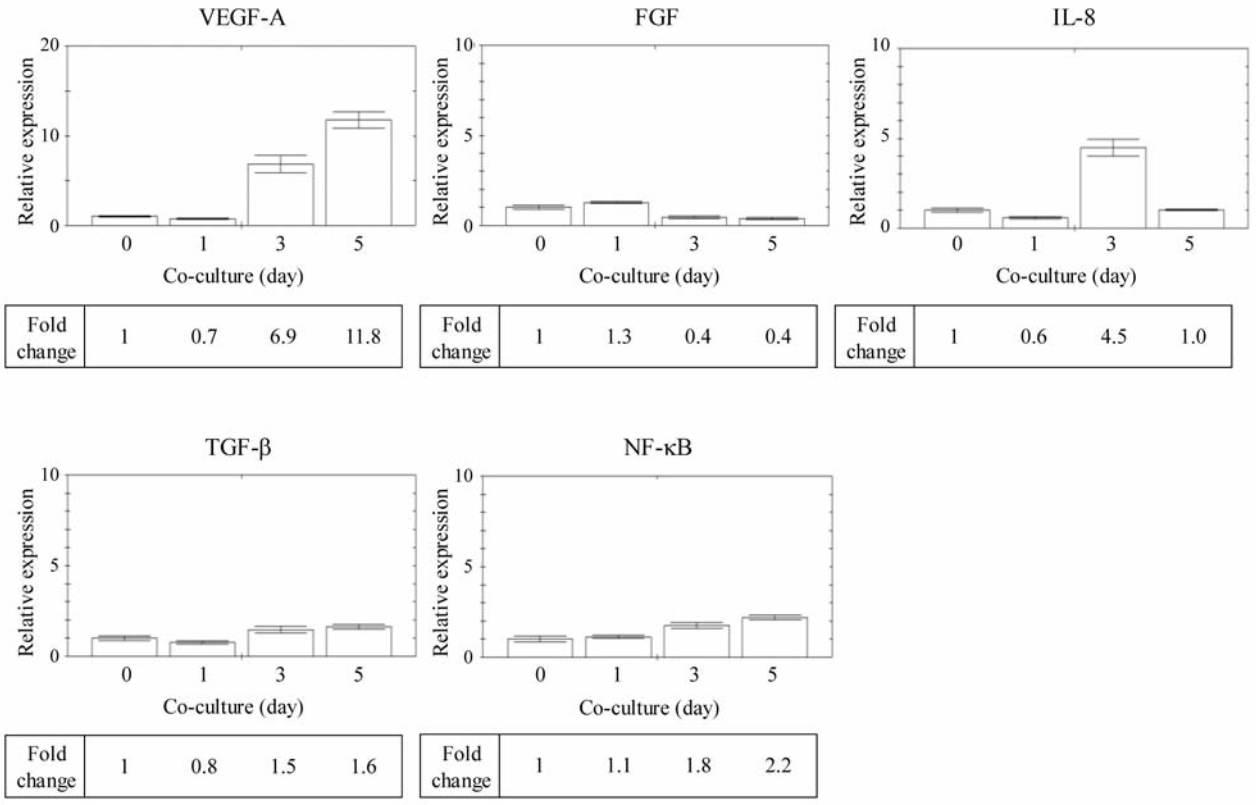

Figure 1. *Differential expression of mRNA in DLD-1 cells. After co-culture with THP-1 cells mRNA expression was analyzed by quantitative real-time PCR. Relative quantifications were achieved by normalization to the value of the housekeeping gene β-actin. Data are expressed as fold-change in mRNA expression compared with co-culture at day 0.*

human monocyte-derived MCP-1 in human colon cancer. Moreover, VEGF-A, TNF-α, IL-1β, and IL-8 are known angiogenesis-related factors. Human monocytes may possibly promote angiogenesis following interaction with human colon cancer cells.

MCP-1 protein expression in culture supernatants. MCP-1 mRNA expression in THP-1 cells increased in a time-dependent manner from day 3 after co-culture with DLD-1 cells. The MCP-1 concentration in the culture supernatants of THP-1 cells was measured to confirm protein expression by ELISA. MCP-1 protein expression was not detected at 0, 1, and 3 days (<31.3 pg/ml), but was detected at day 5 (53.2 pg/ml) after co-culture (Table II). It was confirmed that MCP-1 was produced in human monocytes following interaction with human colon cancer cells.

Table II. *MCP-1 protein expression in the culture supernatants.*

Co-culture (day)	MCP-1 (pg/ml)			
	0	1	3	5
THP-1 cells	<31.3	<31.3	<31.3	53.2

Discussion

Monocytes in the peripheral circulation are believed to be recruited to the tumor site by the cancer cell-derived chemotactic cytokines MCP-1, colony-stimulating factor (CSF)-1, granulocyte-macrophage colony-stimulating factor (GM-CSF), and VEGF (6-7, 17-21). Moreover, it is reported that macrophages are recruited to the tumor site by cancer cell-derived MCP-1 (6). In the present study, we showed that *MCP-1* mRNA expression was detected in THP-1 cells but not in DLD-1 cells. Thus, expression of *MCP-1* mRNA in human cancer cells may differ with cancer type, and human monocytes may possibly be recruited by human monocyte-derived MCP-1 in human colon cancer.

VEGF-A, TNF-α, IL-1β, and *IL-8* mRNA expression in THP-1 cells was increased at day 5 after co-culture. In addition, VEGF-A, TNF-α, IL-1β, and IL-8 are known angiogenesis-related factors. Activated macrophages have

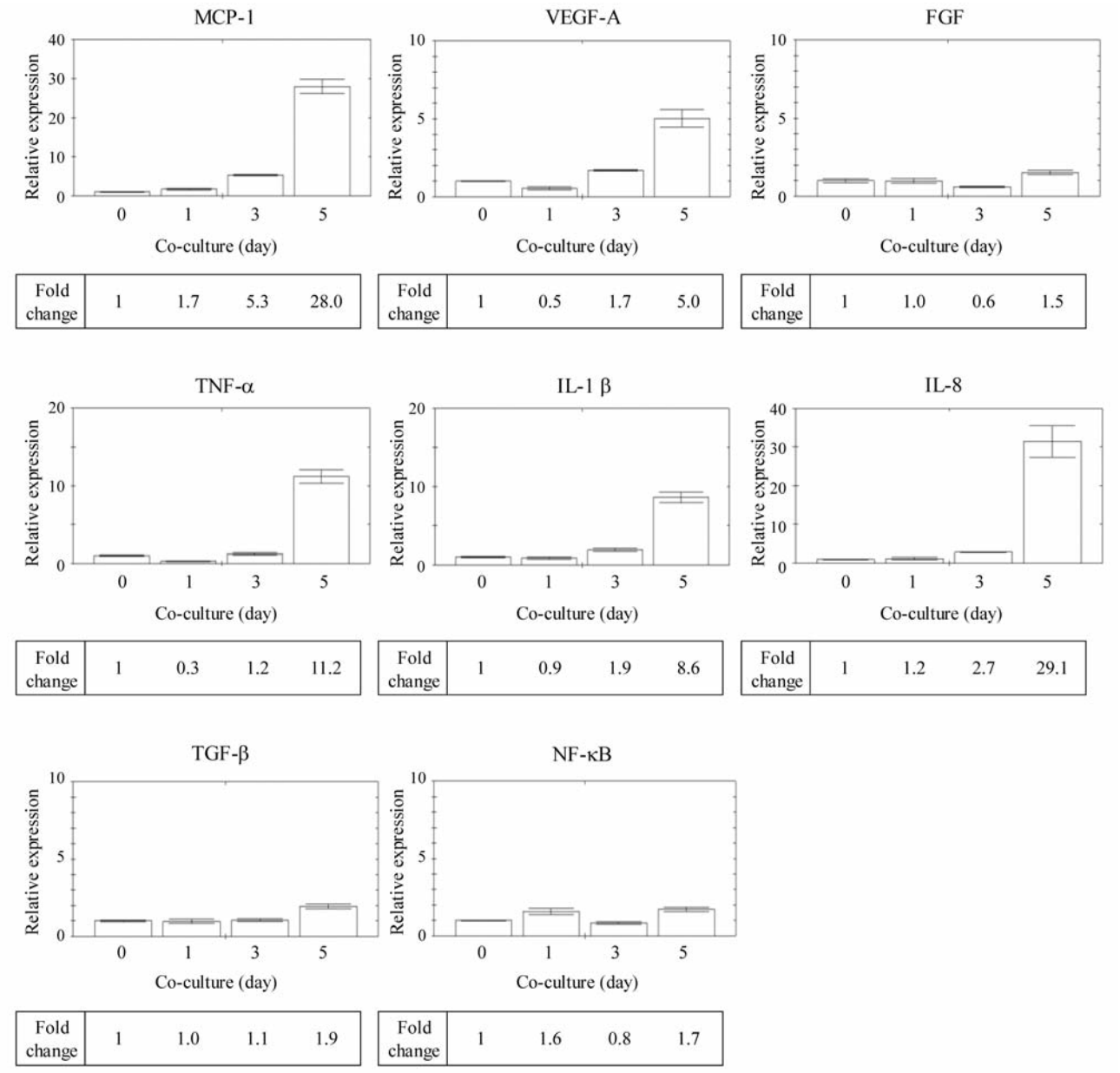

Figure 2. *Differential expression of mRNA in THP-1 cells. After co-culture with DLD-1 cells mRNA expression was analyzed by quantitative real-time PCR. Relative quantifications were achieved by normalization to the value of the housekeeping gene β-actin. Data are expressed as fold-change in mRNA expression compared with co-culture at day 0.*

been reported to produce VEGF in malignant melanoma cells (22). Therefore, activated macrophages may possibly produce angiogenesis-related factors and promote angiogenesis in human colon cancer.

In DLD-1 cells, only *VEGF-A* mRNA expression was increased after co-culture with THP-1 cells. In contrast, *MCP-1, VEGF-A, TNF-α, IL-1β,* and *IL-8* mRNA expression was increased in THP-1 cells after co-culture with DLD-1 cells. These results suggest that THP-1 has a high sensitivity to the changes in its environment. Thus, THP-1 is thought to be a good model to use this co-culture system with colon cancer cells.

MCP-1 mRNA expression in THP-1 cells increased in a time-dependent manner from day 3, and *VEGF-A, TNF-α, IL-1β,* and *IL-8* mRNA expression in THP-1 cells increased at day 5 after co-culture. These results demonstrate that the increase in *MCP-1, VEGF-A, TNF-α, IL-1β,* and *IL-8* mRNA expressions in the THP-1 cells is attributable to the DLD-1

cells. Therefore, it is suggested that MCP-1, VEGF-A, TNF-α, IL-1β, and IL-8 might be associated with human monocyte differentiation into tumor-associated macrophages at human colon cancer sites. Recent studies have revealed that MCP-1 not only acts as a macrophage-recruiting molecule but also promotes the initiation of tumor angiogenesis and early tumor growth in malignant melanoma (23). The increase in mRNA expression of angiogenesis-related factors (VEGF-A, TNF-α, IL-1β, and IL-8) of human monocytes may possibly be attributable to the human monocyte-derived MCP-1 in human colon cancer. Thus, MCP-1 may induce angiogenesis through the production of angiogenesis-related factors by human monocytes/macrophages in human colon cancer and this suggests its utility as a new target for colon cancer therapy.

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