Monocyte Chemoattractant Protein-1 Transfection Induces Angiogenesis and Tumorigenesis of Gastric Carcinoma in Nude Mice via Macrophage Recruitment

Tsuyoshi Kuroda,1 Yasuhiko Kitadai,1 Shinji Tanaka,3 Xiaoqin Yang,4 Naofumi Mukaida,4 Masaharu Yoshihara,2 and Kazuaki Chayama1

Abstract

Purpose: Monocyte chemoattractant protein-1 (MCP-1) is a chemokine that has various roles in tumor development and progression. We previously reported that expression of MCP-1 is associated with macrophage infiltration and tumor vessel density in human gastric carcinomas. The present study was undertaken to obtain direct evidence that MCP-1 participates in recruitment of macrophages and induction of angiogenesis.

Experimental Design: We did transfection experiments to analyze the role of MCP-1 in tumorigenicity and angiogenesis in gastric carcinoma in nude mice. The human MCP-1 gene cloned into the BCMGS-Neo expression vector was transfected into the human gastric carcinoma TMK-1 cell line. We examined tumor volumes with the ectopic s.c. xenograft model and tumorigenicity with the orthotopic gastric xenograft model. We determined intratumor microvessel counts and tumor-infiltrating macrophage counts by immunohistochemical staining.

Results: There was no difference in in vitro proliferation between MCP-1-transfected TMK-1 cells and mock-transfected (control) cells; however, MCP-1 transfectants induced tumor growth in ectopic xenografts and increased tumorigenicity and induced lymph node metastases and ascites in orthotopic xenografts. In both ectopic and orthotopic xenograft models, strong infiltration of macrophages was observed within and around the tumors after implantation of MCP-1 transfectants whereas fewer macrophages were seen after inoculation of control cells. The microvessel density was significantly higher in tumors produced by MCP-1 transfectants than in control tumors.

Conclusions: MCP-1 produced by gastric carcinoma cells may regulate angiogenesis via macrophage recruitment. MCP-1 may be a potential target for antiangiogenic therapy for gastric carcinoma.

Solid tumors contain not only malignant cells but also many stromal cells. Interactions between tumor and stromal cells may produce a unique microenvironment that can profoundly influence the process of tumor progression (1, 2). Tumor-associated macrophages are one of the major components of stroma and have dual roles in neoplasms (3). Tumor-associated macrophages can produce a number of angiogenic factors, cytokines, and proteases, all of which influence neoplastic progression (4, 5). Most studies have indicated that infiltration of tumor-associated macrophages has a negative effect on patient survival and that there is a significant correlation between infiltration of tumor-associated macrophages and tumor vessel density in various tumor types including melanoma (6, 7), glioma (8), and breast (9–11), lung (12), prostate (13), and cervical carcinomas (14). It has also been reported that tumor-associated macrophages can be tumor-cidal and can elicit destructive reactions following activation by interleukin (IL)-2, IL-12, and IFN-γ (15, 16).

Due to the large number of chemokines and growth factors produced by human tumors and the broad spectrum of biological functions, the precise roles of specific cytokines in tumor development and progression remain unclear. Macrophage infiltration into tumors is regulated by a number of cytokines and chemokines, in particular monocyte chemo-attractant protein (MCP)-1 (17, 18). MCP-1 is a member of the CC chemokine family (19). It influences monocytes (20, 21), natural killer cells (22), basophils (23), and T lymphocytes (24), all of which express chemokine receptors (25), predominantly CC-chemokine receptor 2 (CCR2; ref. 26). Expression of MCP-1 has been reported in a wide range of tumor types including melanoma (27), glioma (28, 29), sarcoma (30), leukemia (31), hemangioma (32), and breast (33, 34), cervical...
(14), ovarian (35), and esophageal carcinomas (36). The role of MCP-1 in tumor development and progression is also controversial. Expression of exogenous MCP-1 by murine colon adenocarcinoma cells increases the occurrence of lung metastases by augmenting neovascularization (37). In contrast, expression of MCP-1 by Chinese hamster ovary cells completely suppressed their ability to form tumors in nude mice (38).

We previously reported that expression of MCP-1 by gastric carcinoma cells is associated significantly with macrophage infiltration and tumor vessel density in a clinical study (39). To obtain direct evidence that MCP-1 participates in recruitment of macrophages and induction of tumor angiogenesis, we stably transfection vector expressing MCP-1 into human gastric carcinoma cells and examined the effect of MCP-1 on the development and progression of human gastric carcinoma in nude mice.

Materials and Methods

Cell cultures. Two cell lines established from human gastric carcinomas were used. The TMK-1 cell line (a poorly differentiated adenocarcinoma) was kindly provided by Dr. T. Suzuki (Fukushima Medical College, Fukushima, Japan). These cell lines were maintained in RPMI 1640 (Nissui Co., Tokyo, Japan). The MKN-1 cell line (an adenosquamous carcinoma) was provided by Dr. E. Tahara (Hiroshima University, Hiroshima, Japan). The MKN-1 cell line (an adenosquamous carcinoma) was used. The TMK-1 cell line (a poorly differentiated adenocarcinoma) was provided by Dr. T. Suzuki (Fukushima Medical College, Fukushima, Japan). The TMK-1 cell line (a poorly differentiated adenocarcinoma) was kindly provided by Dr. T. Suzuki (Fukushima Medical College, Fukushima, Japan). These cell lines were maintained in RPMI 1640 (Nissui Co., Tokyo, Japan) with 10% fetal bovine serum (FBS; Sigma, St. Louis, MO). Macrophage differentiation of U937 cells was induced by adding 10 ng/mL PMA (Sigma, St. Louis, MO). Macrophage differentiation of U937 cells was induced by adding 10 ng/mL PMA (Sigma, St. Louis, MO). Macrophage differentiation of U937 cells was induced by adding 10 ng/mL PMA (Sigma, St. Louis, MO).

Gene transfection and cloning of transfected cell lines. A 400-bp PstI fragment containing the entire coding region of the human MCAF gene (accession no. NM002982) was subcloned into the expression plasmid BCMGS-Neo to yield BCMGS-Neo-MCAF (41, 42). TMK-1 cells were transfected with BCMGS-Neo-MCAF with Lipofectin (Life Technologies, Gaithersburg, MD) according to the instructions of the manufacturer. After transfection, cells were grown in selective medium (10% FBS-RPMI 1640 containing 400 µg/mL G418). The selective medium was changed every 3 days.

ELISA for monocyte chemoattractant protein-1. Tumor cells were plated at 5 × 10⁴/mL per well in 24-well plates (Nunc, Burlington, ON, Canada). After 24 hours, the supernatants were collected and used to measure MCP-1 protein. An ELISA of human MCP-1 was done essentially as previously described (43). Briefly, microtiter plates were coated overnight with antihuman MCP-1 monoclonal antibody (mAb; ME 61, 1 µg/mL) in 100 µL per well of 0.05 mol/L carbonate buffer (pH 9.6) at 4°C. The plates were then washed thrice with PBS containing 0.05% Tween 20 (buffer A), blocked with a solution of 1% bovine serum albumin in buffer A (buffer B) at 37°C for 1 hour, and washed again with buffer A. The standards and samples diluted in buffer B were then incubated in the wells overnight at 4°C. The wells were then washed thrice and incubated with 100 µL of rabbit antihuman MCP-1 antibody at 37°C for 2 hours. They were then washed at least 10 times and incubated with 100 µL of alkaline phosphatase–conjugated antirabbit immunoglobulin G (Dako, Carpinteria, CA; diluted 1:4,000 with buffer B) at 37°C for 2 hours. The plates were washed and AmpliQ reagent (Dako) was used for the detection of alkaline phosphatase in accordance with the instructions of the manufacturer. Following a 30-minute incubation at 37°C, the reaction was stopped by the addition of 100 µL of 1 mol/L phosphoric acid and the absorbance at 492 nm was determined with an MTP-120 microplate reader (Corona Electric Co., Ibaraki, Japan). All samples were assayed at least thrice.

Table 1. Sequences of RT-PCR primers

<table>
<thead>
<tr>
<th>Primer sequences</th>
<th>Ta (°C)</th>
<th>Cycle</th>
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<tr>
<td>MCP-1</td>
<td>60</td>
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<td>57</td>
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</tr>
<tr>
<td>PDE-ECGF</td>
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<td>30</td>
<td>464</td>
</tr>
<tr>
<td>bFGF</td>
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<td>35</td>
<td>234</td>
</tr>
<tr>
<td>IL-8</td>
<td>56</td>
<td>35</td>
<td>247</td>
</tr>
<tr>
<td>GAPDH</td>
<td>51</td>
<td>25</td>
<td>188</td>
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Table 2. Secretion of MCP-1 protein by gastric carcinoma cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>MCP-1 (ng/mL)</th>
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</thead>
<tbody>
<tr>
<td>TMK-1 parent</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TMK-1neo</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TMK-1 MCI-C5</td>
<td>1.1</td>
</tr>
<tr>
<td>MKN-1</td>
<td>1.7</td>
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NOTE: Cells were incubated in RPMI 1640 supplemented with 10% FBS. Culture supernatants were collected after 24 hours and assayed for MCP-1 by ELISA as described in Materials and Methods. These values are each representative of one of a series of three measurements.
Reverse transcription-PCR analysis. Total RNA was extracted from human gastric carcinoma cell lines and tumor tissues with the RNeasy kit (Qiagen, Tokyo, Japan) according to the instructions of the manufacturer. Reverse transcription-PCR (RT-PCR) was done with the obtained RNA. Primers, annealing temperatures, and PCR cycles are listed in Table 1. After the reaction, RT-PCR products were resolved on 5% nondenaturing polyacrylamide gels in Tris-borate-EDTA buffer. PCR without reverse transcriptase showed no specific band. Densitometric analysis was done with NIH Image Analysis software (version 1.63) to quantify the RT-PCR results. The intensities of the PCR products were normalized to that of glyceraldehyde-3-phosphate dehydrogenase.

Immunofluorescence staining for monocyte chemoattractant protein-1. Tumor cells were seeded on a Lab-Tek chamber slide (Nunc, Naperville, IL). After 48 hours, cells were washed with PBS and fixed with 4% paraformaldehyde for 20 minutes at room temperature. After permeabilization with a solution containing 0.5% Triton X-100, the cells were incubated for 1 hour with MCP-1 mAb diluted 1:100 (44, 45). After three washes with PBS, sections were incubated for 45 minutes with FITC-labeled rabbit anti-mouse immunoglobulin G (1:1,000 dilution with PBS-1% bovine serum albumin; Dako) and washed thrice with PBS. Cells were then mounted with Vectashield Mounting Medium (Vector Laboratories, Burlingame, CA). Images were obtained with a Leica DC500 digital camera system (Meyer Instruments, Houston, TX).

In vitro cell growth. Cells (1 × 10^5) were seeded in 24-well plates and cultured in RPMI 1640 containing 0.5% FBS or cells (2 × 10^4) were seeded and cultured in RPMI 1640 containing 10% FBS. The medium was changed every 48 hours. Cell counts were determined from triplicate cultures.

Animal models. Male athymic BALB/c nude mice were obtained from Charles River Japan (Tokyo, Japan). The mice were maintained under specific pathogen-free conditions and used when 4 weeks old. Experiments were done according to the guidelines of the University of Hiroshima.

Ectopic (subcutaneous) xenograft model. To produce subcutaneous tumors, cells growing in culture were harvested by brief treatment with

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Fig. 1. Expression of MCP-1 by TMK-1 cells transfected with a vector encoding MCP-1. A, RT-PCR analysis of MCP-1 mRNA. GAPDH was used as an internal control. B, immunofluorescence staining of MCP-1 in transfected TMK-1 cells. C, expression of CCR2 mRNA by transfected TMK-1 cells as assessed by RT-PCR. Phorbol 12-myristate 13-acetate - treated U937 (PMA-U937) cells were used as a positive control. D, expression of mRNAs for angiogenic factors (VEGF, IL-8, bFGF, and PD-ECGF) by transfected TMK-1 cells as assessed by RT-PCR.

Fig. 2. In vitro growth of TMK-1 cells transfected with a vector encoding MCP-1. A, cells (1 × 10^5) were seeded in 24-well plates and cultured in RPMI 1640 containing 0.5% FBS. B, cells (2 × 10^4) were seeded and cultured in medium containing 10% FBS. Cell number was determined in triplicate cultures. Bars, SE.
tumors, cells (5 × 10^5) were implanted s.c. Mean tumor volumes were determined as described in Materials and Methods. The tumor volumes among mice that received MCP1-transfected TMK-1 cells were significantly larger than those among mice that received neo control TMK-1 cells. Bars, SE. *, P < 0.05.

0.25% trypsin and 0.02% EDTA. Mice were randomly assigned to one of two groups (five mice per group). A single-cell suspension of 5 × 10^5 cells with a viability of >95% was implanted s.c. into the backs of nude mice. Formation and size of the tumors were monitored daily until day 25. Tumor volume (V) was calculated as V = 1/2ab^2, where a is the longest diameter and b is the shortest diameter of the tumor. After 8 weeks, mice were killed and tumors were resected for study. The tumors were fixed in 10% buffered formalin or formalin-free IHC Zinc fixative (PharMingen, San Diego, CA) for immunohistochemistry or snap frozen for RT-PCR.

Orthotopic (gastric mucosa) xenograft model. To produce gastric tumors, cells (5 × 10^5) were implanted into the gastric walls of nude mice as previously described (46). After 8 weeks, mice were killed and tumors were resected for study.

Generation of antimouse CC-chemokine receptor 2 polyclonal antibody. Glutathione S-transferase protein fused with the amino-terminal portion of mouse CCR2 was expressed and purified according to the method described by Lu et al. (47). Sera were obtained from rabbits after repeated immunization with the resulting glutathione S-transferase protein.

Immunohistochemistry. Serial 4-μm sections were cut from each study block. Immunohistochemistry for MCP-1, CCR2, CD31 (platelet/endothelial cell adhesion molecule 1), and macrophage scavenger receptor was done with the LSAB2 System (Dako). MCP-1 was detected with mouse anti-human mAb (1:100; refs. 44, 45). CCR2 was detected with the rabbit anti-mouse polyclonal antibody (1:100). CD31 (platelet/endothelial cell adhesion molecule 1) was detected with a rat anti-mouse mAb (1:50; PharMingen) and macrophage scavenger receptor was detected with a rat anti-mouse mAb (1:200; Serotec Ltd., Oxford, United Kingdom). After deparaffinization and rehydration, tissue sections were pretreated by microwave for 5 minutes (for MCP-1, CCR2, and macrophage scavenger receptor). Subsequent steps were done according to the instructions of the manufacturer. Negative controls were reacted with nonspecific immunoglobulin G as the primary antibody.

Microvessel and macrophage counts. Vessel counts were assessed by light microscopy in immunohistochemistry-stained areas of tumor containing the highest numbers of capillaries and small venules at the invasive edge (48). Highly vascularized areas were first identified by staining tumor sections at low power (40× and 100×). The vessel count was determined for three such areas at 400× (40× objective and 10× ocular) and the mean of the three counts was calculated. A vessel lumen was not necessary for a structure to be defined as a vessel (48).

Macrophage counts were made in the five areas with the greatest density of macrophages by the same methods.

Statistical analysis. All results are reported as mean ± SE. Differences in tumor growth were evaluated statistically by repeated measures ANOVA. The relative rates of liver and lymph node metastases and tumorigenicity within groups were compared by χ² test or unpaired Student’s t test where appropriate. Differences were considered statistically significant when P < 0.05.

Results

Transfection of the MCP-1 gene into the TMK-1 human gastric carcinoma cell line. We used the TMK-1 cell line, which is derived from poorly differentiated adenocarcinoma, for transfection experiments because it expresses very low levels of MCP-1 (39). The MKN-1 cell line, which overexpresses MCP-1, was used as a positive control (39). After transfection with BCMGS-Neo-MCAF or BCMGS-Neo, we selected a stable clone (MCP1-C5) that overexpressed MCP-1 and a control clone (neo) for subsequent assays. ELISA revealed that MCP1-C5 cells secreted MCP-1 protein into the culture medium at levels similar to those of MKN-1 cells (Table 2). Overexpression of MCP-1 mRNA and protein by MCP1-C5 cells was confirmed by RT-PCR and immunofluorescence, respectively (Fig. 1A and B). The level of MCP-1 expression of control vector–transfected cells was similar to that of the parental TMK-1 cells (Table 2; Fig. 1A). We examined expression of CCR2 (MCP-1 receptor) in TMK-1 cells by RT-PCR. CCR2 mRNA was not expressed by TMK-1 cells and transfection with vector expressing MCP-1 did not induce expression of CCR2. Human dermal microvascular endothelial cells and U937 cells treated with phosphor 12-myristate 13-acetate expressed CCR2 mRNA (Fig. 1C). Moreover, we examined expression of mRNAs for angiogenic factors [vascular endothelial growth factor (VEGF), IL-8, basic fibroblast growth factor (bFGF), and platelet-derived endothelial cell growth factor (PD-ECGF)] in TMK-1 cells by RT-PCR. Transfection with vector expressing MCP-1 did not cause any change in the expression of mRNAs for these angiogenic factors (Fig. 1D).

In vitro growth of monocyte chemoattractant protein-1–transfected gastric carcinoma cells. We next analyzed whether MCP-1 expression stimulates growth of gastric carcinoma cells in vitro. Under culture conditions of 0.5% and 10% FBS, cell growth was not affected by transfection with the MCP-1 gene (Fig. 2).

Table 3. Effect of MCP-1 expression on tumorigenicity and metastasis in an orthotopic xenograft model

<table>
<thead>
<tr>
<th></th>
<th>Parent (%)</th>
<th>Neo (%)</th>
<th>MCP1-C5 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary tumor/total</td>
<td>0/10 (0)</td>
<td>1/14 (71)</td>
<td>4/12 (33.3)</td>
</tr>
<tr>
<td>Lymph node metastasis/total</td>
<td>0/10 (0)</td>
<td>0/14 (0)</td>
<td>3/12 (25)</td>
</tr>
<tr>
<td>Hemorrhagic ascites/total</td>
<td>0/10 (0)</td>
<td>0/14 (0)</td>
<td>3/12 (25)</td>
</tr>
<tr>
<td>Liver metastasis/total</td>
<td>0/10 (0)</td>
<td>0/14 (0)</td>
<td>0/12 (0)</td>
</tr>
</tbody>
</table>

NOTE: TMK-1 parental cells (parent), MCP1-transfected cells (MCP1-C5), or control cells (neo) were implanted into the orthotopic site (gastric mucosa) of nude mice. The mice were killed on day 56 after implantation.
Effect of monocyte chemoattractant protein-1 expression on tumor growth of subcutaneous xenografts. We investigated the effect of MCP-1 on growth of gastric carcinoma cells in vivo in a xenograft tumor model in which mice received subcutaneous implants of TMK-1 neo or MCP1-C5 cells (5 × 10^5 cells). Palpable tumors were first detected in all mice by 8 days after cell implantation. Tumor volume in mice that received MCP-1 gene–transfected TMK-1 cells was significantly larger than that in mice that received neo control TMK-1 cells (P < 0.05; Fig. 3).

Effect of monocyte chemoattractant protein-1 expression on orthotopic xenografts. Because the organ microenvironment influences tumor growth and metastasis (49), we next implanted the TMK-1 parental, TMK-1 neo, and MCP1-C5 cells into an orthotopic site (gastric mucosa) in nude mice. As shown in Table 3, transfection of TMK-1 cells with vector expressing MCP-1 increased tumorigenicity. By 8 weeks after inoculation with 5 × 10^5 cells, MCP1-C5 cells formed tumors 14 to 18 mm in diameter with bloody ascites in 4 of 12 nude mice whereas neo control cells formed tumors 4 mm in diameter in only 1 of 14 nude mice. Parental cells did not form tumors. The number of mice implanted with MCP1-C5 cells that developed lymph node metastases was higher than that of mice implanted with control cells or parental cells but the differences were not statistically significant. Liver metastasis was not observed in any mouse.

Expression of mRNAs for angiogenic factors in mouse tumor tissues. We next examined expression of mRNAs for angiogenic factors (VEGF, IL-8, bFGF, and PD-ECGF) in mouse tumor tissues using semiquantitative RT-PCR. Representative images of RT-PCR of VEGF-A are shown in Fig. 7. VEGF-A mRNA was expressed at higher levels in MCP-1-expressing tumors than in control tumors in both ectopic and orthotopic xenograft models of gastric carcinoma. The expression levels of mRNAs for IL-8, bFGF, and PD-ECGF were not different between the MCP-1-expressing tumor and control tumor (data not shown).

Discussion

In the present study, we did transfection experiments to analyze the role of MCP-1 on the growth and progression of gastric carcinoma. Transfection with vector expressing MCP-1 did not affect in vitro cell proliferation of TMK-1 cells, which do not express CCR2. However, elevated expression of MCP-1 stimulated tumor growth in vivo in a s.c. xenograft model and increased the tumorigenic potential of these cells in an orthotopic xenograft model in nude mice. In addition, MCP-1 transfection induced lymph node metastasis and bloody ascites in the orthotopic xenografts. Consistent with our findings, Nakashima et al. (37) reported that transfection with vector expressing MCP-1 into a murine colon adenocarcinoma cell line increased lung metastases by augmenting neovascularization. In contrast, Nokihara et al. (50) reported that xenogenous expression of the MCP-1 gene in human lung adenocarcinoma cells suppressed metastasis in severe combined immunodeficient mice when cells were inoculated i.v. although it did not affect tumorigenicity or growth rate when cells were inoculated into the s.c. space. Although the reason for this discrepancy is unclear, it is possible that differences in the level of MCP-1 expression after transfection and the resulting infiltration of macrophages might have affected the results. The level of MCP-1 in the transfectants used by Nokihara et al. (50) was ~100 times higher than that in the transfectants that we and Nakashima et al. (37) used. Indeed, Nesbit et al. (51) showed that the level of MCP-1 secretion influences the rate of tumor growth, with high levels promoting tumor rejection and low levels supporting tumor growth. In addition, the inconsistencies may be due to differences in the routes of tumor inoculation and the genetic...
backgrounds of the experimental mice. We injected tumor cells into gastric mucosa (orthotopic site) of nude mice whereas Nokihara et al. (50) inoculated cells into severe combined immunodeficient mice i.v.

We previously reported that MCP-1 is associated with macrophage infiltration and tumor vessel density in human gastric and esophageal carcinomas (36, 39). In the present study, we observed that MCP-1-transfected tumors are more angiogenic than control tumors when implanted into nude mice and that MCP-1-induced angiogenesis is accompanied by massive infiltration of macrophages. These results are consistent with those reported by Goede et al. (52). They showed that MCP-1 can induce angiogenesis via macrophage recruitment, suggesting that MCP-1 induces angiogenesis indirectly by recruiting macrophages. Tumor-associated macrophages have been reported to produce various angiogenic factors including VEGF-A, bFGF, tumor necrosis factor-α, PD-ECGF, and IL-8 (53, 54). Liss et al. (55) speculated that, in addition to the infiltrating macrophages, tumor cells themselves may be activated by the macrophages and secrete angiogenic factors, which might contribute to tumor angiogenesis in head and neck squamous cell carcinomas. In the present study, MCP-1 did not influence expression of angiogenic factors by TMK-1 cells in vitro. However, expression of VEGF-A was increased by MCP-1 in vivo. These findings suggest that

Fig. 5. Immunohistochemical staining of macrophage scavenger receptor and CD31.
In both ectopic (A and B) and orthotopic (C and D) xenograft models, strong infiltration of macrophages was observed within and around the MCP-1-transfected tumors (B and D) whereas fewer macrophages were seen in the control tumors (A and C). Additionally, in both ectopic (E and F) and orthotopic (G and H) xenograft models, the vascularity was higher in MCP-1-transfected tumors (F and H) than in control tumors (E and G).
interactions between macrophages and tumor cells through MCP-1 may be an important pathway for tumor-associated angiogenesis via VEGF-A.

Salcedo et al. (56) reported that MCP-1 binds to CCR2 expressed by vascular endothelium and directly promotes angiogenesis. Hong et al. (57) reported that MCP-1 binds to CCR2 on vascular endothelial cells in the arterial wall and induces angiogenesis mediated through pathways involving VEGF-A and activation of the small G protein RhoA. In the present study, we examined immunohistochemically whether MCP-1 receptor (CCR2) was expressed on endothelial cells. CCR2 was not expressed on endothelial cells but was expressed on mononuclear cells, suggesting that MCP-1 expression indirectly influences angiogenesis through macrophage recruitment and activation in our experimental model.

Although MCP-1 expression increased tumorigenicity and angiogenicity, neither control cells nor MCP-1 transfectants produced liver metastases. To produce a distant metastasis, tumor cells must complete a series of sequential and selective steps including growth, vascularization, invasion, adhesion, and extravasation (49). The increase in proliferative and angiogenic activity by MCP-1 expression may not be sufficient to cause liver metastasis of TMK-1 cells. However, MCP-1 transfectants induced lymph node metastases and bloody ascites. Tumor-associated macrophages have been reported to be a major source of VEGF-A, VEGF-C, and VEGF-D. VEGF-A regulates permeability of endothelial cells as well as angiogenesis and VEGF-C and VEGF-D induce lymphangiogenesis and subsequent dissemination of cancer through lymphatic systems in humans (58). It is of particular interest whether macrophages recruited by MCP-1 overexpress lymphangiogenic factors such as VEGF-C and VEGF-D.

In conclusion, we found that MCP-1 produced by gastric carcinoma cells induces angiogenesis via macrophage recruitment. Therefore, MCP-1 may be a potential target for antiangiogenic therapy for gastric carcinomas.

Fig. 6. Immunohistochemical staining of CCR2 and CD31. These are adjacent cross sections of the tumors. CCR2 immunoreactivity was visible only in the cytoplasm of mononuclear cells but not in vascular endothelial cells (A), which were stained with a mAb against CD31 (B).

Fig. 7. Expression of VEGF-A mRNA in mouse tumor tissues. Representative results of RT-PCR analysis of VEGF-A. VEGF-A mRNA was expressed at higher levels in MCP-1-expressing tumors (MCP1-C5) than in control tumors (neo) in both ectopic and orthotopic xenograft models of gastric carcinoma. Ethidium bromide staining values were calculated as described in Materials and Methods. The ratio of expression of VEGF-A to that of GAPDH is indicated. The number below each lane represents the ratio of VEGF-A mRNA level compared with that of neo.

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References


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