Connexin 43 Suppresses Human Glioblastoma Cell Growth by Down-Regulation of Monocyte Chemotactic Protein 1, as Discovered Using Protein Array Technology

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ABSTRACT

Previously, we demonstrated that connexin 43 (cx43) suppressed the growth of human glioblastoma cells. To investigate the molecular mechanisms involved in tumor suppression by cx43, we developed a human cytokine array system, which simultaneously detects the expression of 43 cytokines. By using this new technology, we analyzed the cx43-regulated genes in cx43-transfected cells. The cytokine arrays showed that expression of monocyte chemotactic protein-1 (MCP-1) was profoundly reduced in cx43-transfected cells. RT-PCR, immuno-Western blot, and cDNA microarrays further confirmed this observation. Addition of conditioned medium from control-transfected cells and recombinant MCP-1 to cx43-transfected cells significantly enhanced cx43-transfected cell proliferation and colony formation in soft agar. In contrast, addition of neutralization antibody against MCP-1 significantly inhibited cell proliferation in control-transfected cells. Our results suggested that MCP-1 is involved in the suppression of human glioblastoma cell growth by cx43.

INTRODUCTION

Adjacent cells can directly share ions and small molecules of size <1000 daltons through intercellular channels present in the morphological structure known as gap junctions (1–3). Gap junctions are found in almost all mammalian tissues. cx3 proteins are the major, if not only, component of gap junctions. Thus far, at least 15 family members of cx proteins have been identified. These cx are differentially expressed in a variety of tissues, and this is generally believed to reflect cell-specific regulation of gap junctional coupling and functional demands for gap junctions in different cell types. cx43 is the most widely expressed cx, particularly in the brain and heart (4).

GJC is believed to be involved in the regulation of cell homeostasis, proliferation, and differentiation. Accumulated evidence indicates that cx proteins may function as tumor suppressor genes. Many tumor-promoting agents, oncogenes, and growth factors inhibit GJC (2, 3). GJC is believed to be involved in the regulation of cell homeostasis, proliferation, and differentiation. Accumulated evidence indicates that cx proteins may function as tumor suppressor genes. Many tumor-promoting agents, oncogenes, and growth factors inhibit GJC (2, 3). In contrast, anticancer drugs, such as taxol, vitamin D, and carotenoids up-regulate GJC (1, 2). Expression of gap junction proteins, particularly cx43, is frequently decreased in human cancer cells such as brain (5, 6), lung (7–9), cervical (10, 11), ovarian (12, 13), uterine leiomyomata (14), endometrial (15), mesothelioma (16), and prostate cancer (17, 18) cells. Accumulated evidence indicates that cx proteins may function as tumor suppressor genes. Many tumor-promoting agents, oncogenes, and growth factors inhibit GJC (2, 3). GJC is believed to be involved in the regulation of cell homeostasis, proliferation, and differentiation. Accumulated evidence indicates that cx proteins may function as tumor suppressor genes. Many tumor-promoting agents, oncogenes, and growth factors inhibit GJC (2, 3).

The mechanism(s) responsible for tumor suppression by cx43 are not fully understood and characterized and may involve different mechanisms in different cell types. Expression of cx proteins restored differentiation potential in human mammary carcinoma cells (cx26 and cx43; Ref. 21) and induced myogenic differentiation in rhabdomyosarcoma cells (cx43; Ref. 24). cx43 appears to inhibit proliferation of U2OS cells by increasing the levels of p27 proteins via posttranscriptional regulatory mechanisms (25). Transfection of the cx43 gene also enhanced genetic stability in HeLa cells (26). cx43 may also be involved in the regulation of cell cycle progression (23). Suppression of rat glioma cell growth by cx43 may be attributable to regulation of a number of secreted factors (27–29).

Using human glioblastoma cells as a model system, we demonstrated that cx43 expression was profoundly reduced in high-grade human gliomas, and introduction of cx43 into human glioblastoma cells reversed their transformed growth (5, 6). Furthermore, cx43 enhanced apoptosis of human glioblastoma cells in response to chemotherapeutic agents (30) and under low serum conditions through down-regulation of bcl-2 (31). However, the molecular mechanisms responsible for the control of cell growth by cx43 in human glioblastoma cells remains unclear. To characterize the role of cx43 in growth control, we developed a human protein array system, which can simultaneously detect 43 cytokines, chemokines, and growth factors. The protein array approach revealed that cx43-transfected cells significantly reduced the accumulation of MCP-1 protein. Additional experiments showed that a decrease in MCP-1 expression is involved in the suppression of human glioblastoma cell growth by cx43.

MATERIALS AND METHODS

Materials. All pair antibodies were purchased either from BD PharMingen (San Diego, CA) or from R&D (Minneapolis, MN). Cytokines were obtained from Propetech (Rocky Hill, NJ), BD PharMingen, and R&D. HRP-conjugated streptavidin was purchased from BD PharMingen. Cy3-conjugated streptavidin was the product of Rockland (Gilbertsville, PA).

Preparation of Array Membranes. The preparation of array membranes was essentially described in our previous reports (32, 33). Briefly, a computer-generated template was used to guide spot solutions onto membranes. 0.20 μl of capture antibodies (200 μg/ml) were manually loaded onto membranes by a 2-μl Pipetman in duplicate. HRP-conjugated antibody was spotted onto membranes as the positive control and for identification of array orientation.

Array Assay of Human Cytokine Expression. Membranes immobilized with capture antibodies were blocked with 5% BSA/TBS [0.01 M Tris HCl (pH 7.6), 0.15 M NaCl] for 1 h. Membranes were then incubated with 1 ml of a single or a combination of different cytokines (100 ng/ml) or 1 ml of conditioned medium or 1 ml of 10-fold diluted patient’s sera prepared in 5% BSA/TBS for 2 h at room temperature. After extensive wash with TBS/0.1% Tween 20 (three times, 5 min each) and TBS (two times, 5 min each) to remove unbound cytokines, membranes were then incubated individually or collectively with biotin-conjugated anticytokine antibodies. Membranes were washed and then were incubated with HRP-conjugated streptavidin (2.5 pg/ml) for 1 h at room temperature. Unbound materials were washed out with TBS/0.1% Tween 20 and TBS. Finally, the signals were detected by the Enhanced Chemiluminescence (ECL) system (Amersham Pharmacia Biotech, Aylesbury, United Kingdom).
Human Cytokine Chip Technology. Three hundred picoliters of capture antibodies (500 μg/ml) were printed onto Hydrogel chips (Packard Bioscience, Meriden, CT) using the Biochip Arrayer (Packard Bioscience). After blocking, the chips were incubated with 50 μl of different samples at room temperature for 2 h. The chips were then washed to remove unbound components. Biotin-labeled detection antibody mixture was added (50 μl/chip) and incubated at room temperature for 1 h. After wash, Cy3-labeled streptavidin was added, and the chips were incubated at room temperature for 1 h. The excess amount of Cy3 streptavidin was removed, and the signals were scanned by laser scanner (Affymetrix, Santa Clara, CA). A series of diluted Cy3 streptavidin, Cy5 streptavidin, and biotin IgG were included as a positive control. BSA was used as negative control.

Immunono-Western Blot Analysis. Immuno-Western blot was carried out as described (34, 35). Essentially, conditioned medium was incubated with anti-MCP-1 at 4°C for 2 h. The antigen-antibody complex was precipitated by Staphylococcus aureus. The precipitated complex was analyzed by SDS-PAGE. After transferring the protein to membranes, the presence of MCP-1 was detected by anti-MCP antibody coupled with ECL system.

Reverse Transcription-PCR. RT-PCR was performed according to our previous publication (6). Total RNA was isolated from cultured cells by the guanidine isothiocyanate RNAzol B method (Cinna/Biotec Laboratories, Houston, TX). Five μg of total RNA were used for cDNA synthesis using random hexamer primer (Boehringer Mannheim, Mannheim, Germany). PCR amplification was carried out by using all of the reverse-transcribed RNA. The PCR reaction mixture contained 50 mM KCl, 2.5 mM MgCl2, 10 mM Tris (pH 8.0), 10 mM deoxynucleotide triphosphate, 10 μM of each primer, and 0.5 unit of Taq polymerase (Boehringer Mannheim) in the final volume of 50 μl. The PCR profile was 94°C for 40 s, 52°C for 50 s, and 72°C for 60 s for 25 cycles, followed by 75°C for 5 min. After PCR, the input RNA was removed by RNase digestion. The amplified DNA was then precipitated and separated on 1.8% agarose gel containing ethidium bromide. The sense primer was 5'-CAA ACT GAA GCT ACT CTC GCC-3'. The antisense primer was 5'-GCA AAG ACC CTC AAA ACA TCC CAG G-3'. The expected amplified fragment for human MCP-1 was 327 bp. As an internal control, β-actin primers were used as described previously (11) to detect 245 bp of β-actin product.

cDNA Microarrays. Assays were carried out according to the manufacturer’s instruction. Briefly, two Atlas human cDNA expression array membranes were purchased from Clontech (Palo Alto, CA). Five μg of mRNA isolated from cx43-transfected cells (U251cx43-216) and control-transfected cells (U251N23) were then treated with DNase I, and first-strand cDNA synthesis was carried out in the presence of [32P]dATP. Equal amounts of cDNA from cx43-transfected and control-transfected cells were then hybridized to two identical Atlas human cDNA expression arrays in separate bags. The expression arrays were washed, and the image was obtained by exposure to X-ray film and PhosphorImager.

[3H]Thymidine Incorporation Assay. This procedure was carried out according to Huang et al. (36, 37). Cells were seeded in 96-well plates. Twenty-four h later, cells were incubated in the presence of cytokine or conditioned medium for 48 h. 1.12 μCi of [3H]thymidine was added to each well, and incubation was continuous for 24 h. The incorporated [3H]thymidine was then determined by a scintillation counter.

CyQUANT Cell Proliferation Assay. The assay was carried out according to the manufacturer’s instruction (Molecular Probe, Eugene, OR). One thousand cells were seeded in 96-well plates. Twenty-four h later, different concentrations of antibody were added to the tissue culture wells. The plates were incubated at 37°C for another 48 h. Cell number was determined by incubation with CyQUANT dye, and the fluorescence was measured using a CCD imaging system (Bio-Rad, Hercules, CA) with filters for 480 nm excitation and 520 nm emission.

Soft Agar Assay. Soft agar assay was performed as described previously (6, 35, 38). Briefly, cx43-transfected cells and control-transfected cells were assayed by seeding 2000 cells in 0.26% agar medium into six-well plates lined previously with 0.65% agar medium. The plates (in duplicate and repeated twice) were cultured for 3–4 weeks for various experiments and then stained with 0.1% crystal violet for overnight before photography and counting. Colony size ≥15,625 μm² was scored as positive.

RESULTS

Development of a Human Cytokine Array System to Simultaneously Detect 43 Human Cytokines. Recently, we developed a human cytokine expression array system that can simultaneously detect the expression levels of 23 cytokines (32, 33). The system is simple, flexible, and high sensitive. More importantly, the system can be performed in a general laboratory setting. Here, we further expanded the number of cytokines detected to 43.

The system has the combined advantages of the specificity of ELISA, sensitivity of ECL, and high-throughput of microspot. In this approach, capture antibodies are spotted onto membranes, and the membranes were then exposed to a sample containing proteins of interest. The corresponding antigen binds to its cognate antibody spotted onto the membrane and then detected by a mixture of detection antibodies.

The specificity of this system to detect 43 human cytokines is demonstrated in Fig. 1. Captured antibodies against individual cytokines and other controls were spotted onto Pharmacia Hybond ECL membranes. The membranes were then incubated with the individual cytokine, followed by corresponding biotin-conjugated antibody. Specific signals were observed in all 43 cytokines with representative arrays (Fig. 1B). When the membrane was incubated with epidermal growth factor, followed by antibody against IL-8, no signal was detected. When the membrane was incubated with detection antibodies alone, no signal appeared (data not shown), demonstrating a high specificity of the system.

This system is a feasible approach to simultaneously detect multiple cytokines as shown in Fig. 1C. The cytokine antibody array membranes were incubated with different combinations of cytokines and corresponding antibodies. As expected, specific signals were detected in all cases.

Recently, several labs and companies have successfully produced protein chip technology. To test whether our system can be easily adapted to a chip platform, we examined the cytokine expression from different biological samples: conditioned medium, patient serum, cell lysate, tissue lysate, and urine using Hydrogel chips. In this case, 43 capture antibodies were printed onto Hydrogel chips to generate human cytokine chips. The cytokine chips were then incubated with 50 μl of different biological samples, followed by a mixture containing 43 biotinylated detection antibodies and Cy3-conjugated streptavidin. As shown in Fig. 2, specific cytokines could be detected in different samples on the cytokine chips, suggesting that our system is readily adapted to a protein chip format, and such protein chips can be used to detect cytokines from different biological sources.

Identification of cx43-regulated Cytokines by Human Cytokine Array System. Using this new technology, we screened the potential cx43-regulated cytokines in cx43-transfected and control-transfected cells. As shown in Fig. 3, expression of MCP-1 was significantly reduced in cx43-transfected cells. All other cytokines are similar between cx43-transfected and control-transfected cells. To further confirm the human cytokine array results, we performed immunoprecipitation of conditioned medium from cx43-transfected cells and control-transfected cells with antibody against MCP-1. The immunoprecipitated complex was then separated by SDS-PAGE, and the levels of MCP-1 protein were detected by Western blot using antibody against MCP-1. As shown in Fig. 4, the MCP-1 protein was predominantly expressed in conditioned medium from control-transfected cells (U251N23) but not from cx43-transfected cells (U251cx43-216).

To examine whether the down-regulation of MCP-1 expression was mediated by transcription regulation, semiquantitative RT-PCR was applied to measure MCP-1 mRNA levels. As shown in Fig. 5, MCP-1 is only detected in U251N23 cells. To make sure that this result did
not simply reflect clonal variation, we examined the expression of MCP-1 in other cx43-transfected cells (U251cx43-217) and other control-transfected cells (U251N2). Again, MCP-1 was highly expressed in the control-transfected cells but not in cx43-transfected cells.

Fig. 1. Detection of purified cytokines in array format with high specificity. A, a template of the human cytokines in the array. Pos, positive; Neg, negative; GCSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte/macrophage-colony stimulating factor; GRO, growth-regulated oncogene; MCSF, macrophage colony stimulating factor; MDC, macrophage-derived chemokine; MIP, macrophage inflammatory protein; SCF, stem cell factor; SDF, stromal cell-derived factor; TARC, thymus and activation regulated chemokine; TGF, transforming growth factor; EGF, epidermal growth factor; IGF, insulin-like growth factor; Ang, angiotensin; OSM, oncostatin; Tpo, thrombopoietin; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor. B, detection of individual cytokine with high specificity. Different capture antibodies against different cytokines were immobilized on Hybond ECL membranes and incubated subsequently with individual cytokines or control as indicated in the figure. Corresponding biotin-conjugated anticytokine or control antibodies with HRP-conjugated streptavidin were used to detect specific cytokines bound to the capture antibodies. The signals were visualized by ECL. Cyto, cytokine; Ab, antibody; MCP, monocyte chemotactic protein; Ang, angiotensin; TGF, tumor necrosis factor.

Analysis of cx43-regulated Genes by cDNA Microarrays. To further exploit the molecular mechanisms responsible for the cx43-mediated tumor suppression, we applied the Atlas human cDNA microarray system. Five μg of mRNA prepared from U251cx43-216
Suppression of Human Glioblastoma Cell Growth by cx43

Fig. 3. cx43-transfected cells (U251cx43-216) reduce secretion of MCP-1 into medium as detected by the human cytokine array system. A, a template of the human cytokines in the array. Abbreviations are as in the legend to Fig. 1A. B, human cytokine arrays from cx43-transfected cells and control-transfected cells. One ml of 10-fold diluted conditioned medium and control medium was incubated with human cytokine array membranes. After removing unbound materials, the membranes were incubated with a mixture of biotin-labeled antibodies. Signals were detected with HRP-conjugated streptavidin and ECL. The experiment was repeated at least three times.

Fig. 4. cx43-transfected cells (U251cx43-216) reduce secretion of MCP-1 into medium as detected by immunoprecipitation and Western blot. Cells were seeded at a density of $1 \times 10^5$ per 100-mm dish. After 48 h, conditioned medium was collected. Nonconcentrated medium (1X) or 10-fold concentrated medium (10X) was used in immunoprecipitation assays with anti-MCP-1 antibody. The immunoprecipitated complexes were then separated by SDS-PAGE, and the levels of MCP-1 protein were detected by Western blot.

Fig. 5. MCP-1 mRNA is reduced in cx43-transfected cells compared with controls as measured by RT-PCR. Two separate clones of U251 cx43-transfected cells were tested for reduced MCP-1 mRNA. Five $\mu$g of total RNA were used for cDNA synthesis using a random hexamer primer (Boehringer Mannheim). Specific primers to MCP-1 or $\beta$-actin were used for PCR amplification. The expected amplified fragments for human MCP-1 (327 bp) and $\beta$-actin (245 bp) were separated in 1.5% agarose gel electrophoresis.

Fig. 6 shows the raw images of cDNA expression arrays.

The quantification of the Atlas human cDNA expression array membrane was performed using a computer program in AWK Script running under the Unix environment to automate the comparison procedure. The intensity of signal in the membranes was calculated by the Image QUANT program (Molecular Dynamics). The quantitative scores were normalized using the scores of $\beta$-actin spotted on the same membrane. To identify genes, which are up-regulated or down-regulated in cx43-transfected cells, the ratios of the sum scores (minus background) between U251cx43-216 and U251N23 were calculated for each spot. Because each cDNA was spotted in duplicate, there are two spots for each cDNA and thus two ratios. Those cDNAs, which are consistent across both ratios, were considered to be genuinely regulated in U251cx43-216 cells. A ratio of $>2$ was taken as a cutoff score to access whether a gene is up- or down-regulated. According to this standard, we found that MCP-1 is specifically down-regulated in cx43-transfected cells ($>5$-fold reduction in cx43-transfected cells).

Down-Regulation of MCP-1 by cx43 Is Involved in Cell Proliferation. The down-regulation of MCP-1 in cx43-transfected cells raises the question of whether reversion of the transformed phenotype in cx43-transfected cells is mediated by the reduction of MCP-1 expression. To test this possibility, we added anti-MCP-1 neutralization antibody into the tissue culture medium to block the MCP-1 activity and examined the cell proliferation by CyQUANT assay. The
addition of anti-MCP-1 antibody significantly inhibited the cell proliferation rate in U251 cells transfected with control vector, U251N23, which expressed a high amount of MCP-1, but not in cx43-transfected cells, which accumulated a very low level of MCP-1 (Fig. 7A). In contrast, U251N23 cell conditioned medium specifically enhanced the cell proliferation rate in cx43-transfected cells as shown in Fig. 7B. Furthermore, addition of MCP-1 specifically stimulated cell proliferation rates in cx43-transfected cells but not in control-transfected cells (Fig. 7C), suggesting the involvement of MCP-1 in cx43-mediated growth control. Combined, this evidence suggests that MCP-1 might be an important factor contributing to cell proliferation control in human glioblastoma cells.

To examine the effect of down-regulation of MCP-1 on transformed growth, cx43-transfected and control-transfected clones were assayed for their anchorage-independent growth in soft agar in the presence of MCP-1. Fig. 8 and Table 1 demonstrate that the addition of MCP-1 increased colony formation of cx43-transfected cells in soft agar.

We finally assayed cx43-transfected clones and control-transfected clones for apoptosis in the presence of MCP-1 or anti-MCP-1 antibody. The addition of MCP-1 or anti-MCP-1 antibody did not have any effect on apoptosis under normal culture conditions, under low serum conditions, or in response to chemotherapeutic drugs. Therefore, enhanced apoptosis under low serum conditions and decreased cell growth in cx43-transfected cells involve at least two separate pathways.

DISCUSSION

Growing evidence suggests that cx43 functions as a tumor suppressor gene. However, the molecular mechanisms involved in tumor suppression are still ill defined. To determine whether secreted factors

![Image](https://example.com/image)

**Table 1** Effect of MCP-1 on colony formation in soft agar

<table>
<thead>
<tr>
<th>MCP-1 (ng/ml)</th>
<th>U251N23</th>
<th>U251N2</th>
<th>U251cx43-216</th>
<th>U251cx43-217</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>36.0 ± 5.65</td>
<td>24.5 ± 0.70</td>
<td>4.0 ± 1.41</td>
<td>7.0 ± 1.41</td>
</tr>
<tr>
<td>1</td>
<td>18.0 ± 2.83</td>
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<tr>
<td>10</td>
<td>37.5 ± 3.53</td>
<td>24.5 ± 2.12</td>
<td>24.5 ± 2.12</td>
<td>24.5 ± 2.12</td>
</tr>
<tr>
<td>100</td>
<td>28.5 ± 4.95</td>
<td>27.5 ± 3.53</td>
<td>27.5 ± 3.53</td>
<td>27.5 ± 3.53</td>
</tr>
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*Colonies containing >100 cells (i.e., ≈200 μm diameter) were scored positive.*
SUPPRESSION OF HUMAN GLIOBLASTOMA CELL GROWTH BY cx43

Fig. 8. MCP-1 stimulates colony formation in soft agar of cx43-transfected cells. cx43-transfected and control-transfected cells were grown in soft agar with medium alone or medium plus different concentrations of MCP-1 for 3 weeks. Colonies formed were visualized by staining with p-iodotetrazolium violet.

Contribute to the tumor suppression by cx43, we developed a human cytokine array system, which allowed us to simultaneously detect 43 cytokines, chemokines, and growth factors. The system described in this paper has several important features. The system can be used to detect multiple human cytokines from a variety of biological sources, such as conditioned medium, serum, cell lysate, tissue lysate, and urine. Our system can be used in both membrane (macroarray) format and protein chip (microarray) format. The macroarray format allows designing arrays in a simple, cheap, and flexible way. Sophisticated equipment is not required in the process, and the system should be able to access a wider research community. The protein chip format allows the development of a high-throughput approach to screen large quantities of samples with very efficient reagents. The system has high specificity and sensitivity to detect physiological levels of these cytokines, chemokines, and growth factors. Considering the important role of cytokines in cell growth, differentiation, survival, immunity, apoptosis, and angiogenesis and the implication of cytokines in the development of many diseases such as cancer, cardiac diseases, and arthritis (39, 40), the human cytokine array systems developed here have significant promise in basic research and clinical application.

By applying this human cytokine array system, we found that MCP-1 was down-regulated in cx43-transfected cells. This conclusion was further confirmed by immunoprecipitation analysis, RT-PCR, cDNA microarray, and enhanced protein arrays (data not shown), suggesting that this human cytokine array system developed here is a reliable means to simultaneously measure the expression of multiple cytokines.

A wealth of evidence suggests that MCP-1 may play an important role in tumor formation. In contrast to the majority of normal cells, many human and murine tumor cells are known to constitutively produce high levels of MCP-1, including human glioblastoma (41), melanoma (42), ovarian cancer (43), breast carcinoma (44), Hodgkin’s disease (45), and lung cancer (44). Clinical studies suggest that high expression of MCP-1 was a significant indicator of early relapse of human breast cancer (46). MCP-1 expression may contribute to the high malignant phenotype of murine mammary adenocarcinoma cells (47). MCP-1 is capable of inducing angiogenesis, which is a very critical event for tumor growth (48). Expression of MCP-1 is tightly associated with chronic inflammation, which may promote tumor development (49, 50). CDNA microarray technology revealed the association between the development of drug resistance in ovarian cancer cells and the accumulation of MCP-1 (51). Furthermore, other chemokines or chemokine receptors such as RANTES, CXCR2, and CXCR4 have been shown to be associated with the tumor development (52). Considering the possible role of MCP-1 in tumor development, our results suggest that down-regulation of MCP-1 in cx43-transfected cells contributed to the reversion of transformed cell growth. The hypothesis was tested by several experiments. Addition of anti-MCP-1 antibody to tissue culture medium of control-transfected and cx43-transfected cells reduced cell proliferation rates in control-transfected cells but not in cx43-transfected cells. In contrast, MCP-1 and conditioned medium from control-transfected cells promoted cx43-transfected cell growth both in monolayer culture and in soft agar. The role of MCP-1 on the human glioblastoma cell growth is probably mediated through an autocrine mechanism because both cx43-transfected cells and control-transfected cells expressed the MCP-1 receptor, CCR2. Consistent with the notion that MCP-1 is a major factor involved in the control of human glioblastoma cell growth, we showed previously that expression of cx43 was decreased in human glioblastoma cells and patient’s tissues (5, 6).

On the other hand, production of MCP-1 in glioblastoma cells is responsible for the infiltration of macrophages and monocytes in these tumors. It is well known that tumor-associated macrophages represent one of the first lines of immunological defense against neoplastic cell growth. Therefore, the tumor growth in vivo must be regulated by the balance between stimulation of tumor cell growth by MCP-1 and the MCP-1-mediated macrophage infiltration.

Our previous observations suggest that cx43 enhances apoptosis under low serum conditions and in response to chemotherapeutic agents (30, 31). In the this study, MCP-1 appears not to be involved in the enhanced apoptosis in cx43-transfected cells. Therefore, suppression of transformed cell growth and enhanced apoptosis by cx43 in human glioblastoma cells involves at least two distinct pathways.

ACKNOWLEDGMENTS

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REFERENCES


