Establishment and Characterization of a New Lung Cancer Cell Line (MI-4) Producing High Levels of Granulocyte Colony Stimulating Factor

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We established a human lung cancer cell line, MI-4 from the pleural effusion of a 69-year-old male with advanced large cell undifferentiated carcinoma of the lung complicated by leukocytosis. The culture supernatant of MI-4 contained high levels of granulocyte colony stimulating factor (G-CSF). The intracellular localization of the G-CSF was identified by immunocytochemistry. Reverse transcription-polymerase chain reaction (RT-PCR) revealed G-CSF mRNA expression in this cell line. The cell line was successfully transplanted into nude mice. The transplanted nude mice also showed leukocytosis with a high serum G-CSF level. Southern blot analysis did not show amplification or rearrangement of the G-CSF gene in MI-4 cells. Spectral karyotyping (SKY) and fluorescence in situ hybridization (FISH) analyses revealed that this cell line has an additional chromosome 17 attached to a segment of chromosome 10 besides two intact chromosomes 17, and that each of these three chromosomes 17 has a G-CSF gene on chromosome 17q. Inflammatory cytokines, tumor necrosis factor (TNF)-αααα and interleukin (IL)-1ββββ, significantly enhanced G-CSF expression at both the protein and mRNA levels in MI-4. However, these cytokines did not stimulate the growth of MI-4 cells, regardless of abundant G-CSF production. TNF-αααα rather suppressed it, in a dose-dependent manner. Exogenous recombinant human G-CSF and anti-G-CSF antibody did not promote or inhibit the growth of MI-4 cells at any concentration examined. In addition, RT-PCR analysis did not show G-CSF receptor mRNA expression. These results suggest that this cell line does not have an autocrine growth loop for G-CSF. This cell line should be very useful for understanding the biological activity of G-CSF in G-CSF-overproducing lung cancer.

Key words: Lung cancer — G-CSF — Cell line — MI-4

Some cancer patients manifest leukocytosis without overt inflammation. The production of granulocyte colony-stimulating factor (G-CSF) by the tumor itself is thought to be responsible for this paraneoplastic syndrome.1–3 At least 80 cases of G-CSF-producing tumors have been reported up to the present. Although many types of G-CSF-producing tumors such as carcinoma of the thyroid,4–5 kidney,6 oral cavity,7 bladder,8 gallbladder,9 stomach,10 hepatoma11 and melanoma12 have been reported, carcinoma of the lung13–17 is predominant. Large cell carcinoma is the overwhelmingly dominant histologic type in lung cancer.18, 19 However, there have been few reports confirming the establishment of a G-CSF-producing cell line; to our knowledge, 10 G-CSF-producing lung cancer cell lines have been reported. Four of them are derived from large cell carcinoma.14–16 Recently, we studied a patient with large cell lung carcinoma who exhibited leukocytosis, and we established a new cell line in vitro from this patient. This cell line produces a high level of G-CSF in the medium. In this paper, we describe the establishment and characterization of this new lung cancer cell line.

MATERIALS AND METHODS

Patient The patient was a 69-year-old male with large cell carcinoma of the lung. He underwent left upper lobectomy. Histologically, the surgical specimen of the lung tumor showed large cell undifferentiated carcinoma. However, the tumor metastasized to Virchow’s node after 4 years. Chemotherapy and radiotherapy afforded only temporary control and the tumor grew rapidly. As the disease progressed, his peripheral blood white blood cell (WBC) count increased to 92 000/mm3 with 95% mature neutrophils, without evidence of infection. The serum level of G-CSF was 141 pg/ml (normal <30 pg/ml). The patient gradually deteriorated with cachexia, and died 9 months after recurrence. At autopsy, metastatic tumors were found in the bilateral lungs, anterior mediastinum, right adrenal gland and retroperitoneal lymphnodes, besides Virchow’s node. Furthermore, he had a large quantity of malignant pleural effusion and ascites.

Cell culture Tumor cells were obtained from the pleural effusion and cultured for the establishment of a cell line.

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The cells were cultured in RPMI-1640 medium containing 10% fetal calf serum (FCS) at 37°C in an incubator, under a 100% humidified 5% CO2 atmosphere. Subcultures were carried out twice weekly by removing the cells from 75 cm² tissue flasks with 0.025% trypsin in 0.02% EDTA and splitting them 1:3. After 30 passages, a cell line, named MI-4, was established. The cells were cultured under RPMI-1640 medium containing 5% FCS for the following in vitro experiments.

**Morphological study** The morphological study of MI-4 cells was done under a phase-contrast microscope. Immunocytochemical staining of MI-4 cells was performed using the following monoclonal antibodies: AE1/AE3 (Becton Dickinson, Mountain View, CA), Cytokeratin 8 (Enzo Biochem, New York, NY), Cytokeratin 18 and Cytokeratin 19 (ScyTek, Logan, UT) for keratin, MUC-1 for KL-6 (gift from Dr. Nobuoki Kono), EMA (Dakopatts, Glostrup, Denmark) for epithelial cell membrane antigen, CEA (Bi(science, Emmenbrücke, Switzerland) for carcinomembranous antigen, PE10 (Dakopatts) for surfactant apoprotein A, G-CSF (Genzyme-TECHNE Research Products, Minneapolis, MN) for granulocyte stimulating factor and a polyclonal antibody: Hup-1(Dakopatts) for human urine protein-1.

**Transplantation of cells to nude mice** The MI-4 cells were inoculated subcutaneously to the dorsal side of 5 BALB/c nude mice at 4 weeks of age at the concentration of 10⁷ cells in 0.2 ml of RPMI-1640 medium. Immediately before death, the peripheral leukocytes count was determined and the serum G-CSF concentration of mice was measured by enzyme-linked immunosorbent assay (ELISA) (Genzyme-TECHNE Research Products). Histological sections were prepared from tumors and pertinent organs such as the liver, spleen, lungs, kidneys and bone marrow. The sections were stained with hematoxylin and eosin.

**DNA extraction and Southern blot analysis** The MI-4 cells were treated with protease K and extracted with phenol and chloroform. DNA of peripheral blood mononuclear cells (PBMCs) provided by a healthy donor was extracted as a control DNA. Purified DNA (10 µg) was digested with EcoRI, HindIII, RsaI, PvuII and BglII (Toyobo, Tokyo), electrophoresed through 1% agarose gels, and transferred onto nylon membranes (Bio-Rad, Richmond, CA). The nylon membranes were hybridized to ³²P-labeled human G-CSF cDNA probe, pBRV2 (kindly provided by Chugai Pharmaceutical Co., Tokyo). Hybridization was performed for 16 h at 65°C in 7.5% sodium dodecyl sulfate (SDS)/0.25 M Na₂HPO₄. Blots were washed twice for 10 min in 5% SDS/0.02 M Na₂HPO₄ at 65°C. Bands were visualized by autoradiography.

**Chromosome analysis** Chromosome analyses of the MI-4 cells were performed using the Giemsa banding technique. In addition, spectral karyotyping (SKY) analysis was performed to identify precisely the chromosomal rearrangements and the marker chromosomes, as previously described.²³ We also performed fluorescence in situ hybridization (FISH) to detect G-CSF gene on chromosome 17. However, the size of the human G-CSF cDNA probe, pBRV2 was too small to exhibit clear signals in usual FISH analysis. The gene encoding for G-CSF has been mapped to the region 17q11.2–21 by in situ hybridization.²⁴ Therefore, we used a digoxigenin-labeled centromere-specific probe for chromosome 17 (Oncor, Inc., Gaithersburg, MD) and PML SpectrumOrange/RARA SpectrumGreen dual color translocation probe (Vysis, Inc., Downers Grove, IL) to search for the G-CSF gene. The retinoic acid receptor α (RARA) gene is localized at 17q21, which contains one of the breakpoints in the 15;17 chromosome translocation specific for acute promyelocytic leukemia (APL). In situ hybridization has demonstrated that the G-CSF coding region is proximal to the breakpoint on chromosome 17 in APL and is located between the centromere and the RARA gene.²⁵ The centromere of chromosome 17 and the RARA gene were detected as red and green FISH signals respectively. **Detection of G-CSF, GM-CSF, M-CSF, IL-β and TNF-α** G-CSF content in the culture medium or serum was determined using an ELISA kit (Genzyme-TECHNE Research Products) according to the supplier’s instructions. The sensitivity of this kit is 0.4 pg/ml. Contents of granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), IL-β and TNF-α in the culture medium were determined using an ELISA kit (R & D Systems, Minneapolis, MN). The sensitivities of the GM-CSF, M-CSF, IL-β and TNF-α kits are 3, 9, 1 and 4.4 pg/ml, respectively.

**Quantitative reverse transcription-polymerase chain reaction (RT-PCR)** Total RNA was extracted from the cultured cells by the guanidine-thiocyanate method using ISOGEN solution (Nippon Gene Co., Tokyo). OKa-C-1 cells and U937 cells were used as positive controls for detection of G-CSF and G-CSF receptor mRNA expression, respectively. BALL-2 cells were used as a negative control for G-CSF and G-CSF receptor mRNA expression. One microgram of the RNA was converted to cDNA with Moloney murine leukemia virus reverse transcriptase in 20 µl of reaction mixture. For quantification, 1 µl aliquots of cDNA samples were subjected to PCR in 50 µl of reaction solution containing 0.5 µM of each specific primer, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM dNTP, and 1 unit of Taq polymerase. Primers used for G-CSF were 5'-TAGAGCAAGTGAGGAAGATCCAGG-3' for sense and 5'-AGTTCTCTCCATCTGCTGCGCCAAGT-3' for antisense, giving a 328 bp fragment. Primers used for G-CSF receptor were 5'-TGGACTGCAGCTGGTTTCAG-3' for sense and 5'-GGTCTGACAGTTGCCCCG-3' for antisense, giving a 668 bp fragment. Primers
used for β-actin were 5′-ACCTCAACACCCAGCC-ATG-3′ for sense and 5′-GGCCATCTCTTGCTCGAA-GTC-3′, giving a 309 bp fragment. Reaction was performed for 30 cycles for G-CSF, 35 cycles for G-CSF receptor and 20 cycles for β-actin in a DNA thermal cycler (TaKaRa, Ohtsu). The PCR steps for G-CSF and β-actin included denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and polymerization at 72°C for 1 min. The PCR steps for G-CSF receptor included denaturation at 95°C for 55 s, annealing at 72°C for 70 s, polymerization at 65°C for 95 s, and one cycle of 435 s at 72°C. Amplification cycle numbers were optimized for each sequence using the serial dilution method to achieve a dose-dependent amplification. One microliter of PCR product was electrophoresed on 1% NuSieve GTG agarose gel and stained with ethidium bromide. The intensity of the bands was evaluated using a UV-light box imaging system (Atto, Tokyo).

Effects of TNF-α and IL-1β on G-CSF production and propagation of MI-4 cells

For the evaluation of growth sensitivity to TNF-α and IL-1β, cell proliferation assay was performed using the “CellTiter 96” AQeous One Solution Cell Proliferation Assay kit (Promega Corp., Madison, WI). Briefly, MI-4 cells were plated in 96-well plates and exposed for 48 h to the media containing 1% FCS and 0.1 to 10 ng/ml of TNF-α and IL-1β. Then a solution of tetrazolium salt MTS was added directly and the mixture was incubated for 4 h at 37°C. The absorbance of the solution was measured at 490 nm in a spectrophotometer. To examine the effects of TNF-α and IL-1β on G-CSF production in MI-4 cells, cells were exposed to 0.1 to 100 ng/ml of these cytokines for 48 h, or to examine G-CSF mRNA expression, for 24 h. Then G-CSF in cultured medium and mRNA expression in harvested cells were analyzed by quantitative ELISA and RT-PCR respectively. The values of G-CSF in the conditioned medium were normalized with respect to the number of cells using values obtained in the cell proliferation assay. The experiment was done in triplicate, and the mean and standard deviation were calculated.

Effect of exogenous G-CSF and anti-G-CSF antibody on propagation of MI-4 cells

We examined whether administration of a specific anti-human G-CSF monoclonal antibody (Genzyme-TECHNE Research Products) and exogenous recombinant G-CSF (kindly provided by Chugai Pharmaceutical Co., Tokyo) would inhibit and promote the growth of tumor cells. The proliferating activity of the cultured cells was measured by the cell proliferation assay described above. Anti-G-CSF antibody (500 ng/ml) was diluted 512-, 256-, 128-, 64-, 32-, and 16-fold with phosphate-buffered saline (PBS) and added to the culture to make 10%. Serial concentrations (0.1–100 ng/ml) of recombinant G-CSF were also added to the culture. NFS-60 cells (ATCC CRL-1838), which proliferate in response to G-CSF in a dose-dependent manner, were used as a control. The experiment was done in triplicate, and the mean and standard deviation were calculated.

RESULTS

Morphological features of the cultured cells (MI-4)

MI-4 cells adhered weakly to the culture dish and were easily detached (Fig. 1). The doubling time was 96 h. The adherent cells were morphologically polygonal and epithelial-like. The cells contain granules in the cytoplasm.

Immunocytochemical analysis

Immunocytochemical staining showed that MI-4 cells were positive to AE1/AE3, Cytokeratin 8, Cytokeratin 18, Cytokeratin 19, EMA, G-CSF and MUC-1, but negative to Hup-1, CEA, and PE10 (Table I).

![Fig. 1. Morphological features of cultured MI-4 cells by phase-contrast microscopy (×200).](image)

Table I. Immunohistochemical Staining of MI-4 Cell Line

| Antibody     | Specificity                          | MI-4 |
|--------------|--------------------------------------|------|
| AE1/AE3      | Epithelial cells                     | ++   |
| Cytokeratin 8| Epithelial cells                     | +    |
| Cytokeratin 18| Epithelial cells                  | +    |
| Cytokeratin 19| Epithelial cells                  | +    |
| Epithelial membrane antigen| Epithelial cells, plasma cells and some lymphoma cells | +    |
| MUC-1        | KL-6                                 | +    |
| Hup-1        | Human urinary protein-1             | -    |
| CEA3         | Carcinoembryonic antigen            | -    |
| PE10         | Surfactant apoprotein A             | -    |
| G-CSF        | G-CSF-producing cells               | +    |
Chromosome analysis Although the patient’s original tumor cells were not examined, MI-4 cells at the 40th passage had a modal chromosome number of 69 with complex structural abnormalities (Fig. 2A). G-banding analysis showed only an intact pair of chromosome 17 and sixteen marker chromosomes. SKY analysis demonstrated another

![Chromosome analysis of MI-4 cell](image_url)

Fig. 2. Chromosome analysis of MI-4 cell. A typical karyotype of MI-4 cells (69, add(X)(q11), add(1)(q11), +add(2)(p21), add(3)(q11), add(3)(q21), −4, +5, −6, −6, +add(7)(q11), add(9)(p11), add(11)(p11), add(11)(p11), +add(12)(p11), add(12)(p11), add(13)(p11), add(14)(q32), +add(16)(p11), +add(16)(p11), +add(16)(p11), +add(16)(p11), −18, +add(19)(q13), +20, +add(20)(q13), −21, +22, +22, +del(22)(q13), +16mar) (A). SKY analysis of metaphase chromosome. The G-banded karyotype is on the left and the classified SKY images on the right. The colors of chromosomes of origin in the classified image are listed. There are two intact chromosomes 17 and another with a segment of chromosome 16 (B). A marker chromosome with a segment of chromosome 17 (C). FISH analysis of metaphase chromosomes. The α satellite DNA of chromosome 17 was detected as a red signal (D). The RARA gene was detected as a green signal (E). An illustration of RARA gene, G-CSF gene and α satellite DNA mapped on chromosome 17 (F).
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chromosome 17 with a segment of chromosome 16 (Fig. 2B) and a marker chromosome with a small segment of chromosome 17 (Fig. 2C). FISH analysis showed three red signals for the centromere of chromosome 17 (Fig. 2D) and three green signals for the \( \text{RARA} \) gene in metaphase cells (Fig. 2E). Each locus of the \( \text{RARA} \) gene, \( \text{G-CSF} \) gene and centromere on chromosome 17 is illustrated (Fig. 2F). These results indicate that the three chromosomes 17, except the marker chromosome, might have \( \text{G-CSF} \) genes.

**Southern blot analysis of the \( \text{G-CSF} \) gene** In order to define if the constitutive expression of \( \text{G-CSF} \) by the tumor cells is due to the changes in genomic structures, we carried out Southern blot analysis. DNA (10 \( \mu \)g) isolated from MI-4 tumor cells and normal PBMCs was digested with \( \text{EcoRI} \), \( \text{HindIII} \), \( \text{RsaI} \), \( \text{PvuII} \) or \( \text{BglII} \) and subsequently electrophoresed on 1% agarose gel. They were transferred to nylon membrane, then hybridized to \( \text{G-CSF} \) cDNA probe. The size of bands detected with MI-4 DNA were identical to that in the case of PBMC DNA, regardless of digestion with the different restriction enzymes (Fig. 3). This result is compatible with the reported restriction maps of the \( \text{G-CSF} \) gene. Moreover, there was little difference in the signal intensities between MI-4 and PBMC DNA. Thus, the \( \text{G-CSF} \) gene of MI-4 cells showed no rearranged configuration or amplification.

**Transplantation to nude mice** Approximately 10\(^7\) MI-4 cells were inoculated subcutaneously into five mice. A tuberous tumor was formed in 1 to 2 weeks at the site of inoculation in all 5 nude mice and grew gradually. The animals died of the tumor in about 2 months, with massive metastasis. Immediately before death, the mean WBC count of tumor-bearing mice was 350 000/mm\(^3\) with 96% mature neutrophils and the mean serum \( \text{G-CSF} \) level was 850 pg/ml. A histological study of the transplanted tumor tissue showed a morphology similar to that of the original tumor tissue (Fig. 4A). The cells contain granules in the cytoplasm with obscure cytoplasmic outline. Nuclei were lobulated with multiple, enlarged and irregular nucleoli. The lungs of tumor-bearing mouse showed marked capillary leukostasis and alveolar septae packed with mature neutrophils in all cases (Fig. 4B).

![Fig. 3. Southern blot analysis of human G-CSF in MI-4 cells and PBMCs from a healthy volunteer. DNA was hybridized with the G-CSF probe. Lanes 1, 3, 5, 7 and 9, DNA from normal PBMCs; lanes 2, 4, 6, 8 and 10, DNA from MI-4 cells; 10 \( \mu \)g of DNA was digested with \( \text{EcoRI} \) (lanes 1, 2), \( \text{HindIII} \) (lanes 3, 4), \( \text{RsaI} \) (lanes 5, 6), \( \text{PvuII} \) (lanes 7, 8) or \( \text{BglII} \) (lanes 9, 10).](image)

![Fig. 4. Histology of nude mouse tumor produced by transplantation of MI-4 cells (A). The neoplastic cells showed round lobulated nuclei with multiple, enlarged and irregular nucleoli and cytoplasm with an obscure cytoplasmic outline. Hematoxylin and eosin staining (×200). Lung of nude mouse transplanted with MI-4 cells showing capillary leukostasis and alveolar septae packed with mature neutrophils (B). Hematoxylin and eosin staining (×100).](image)
Detection of human G-CSF and G-CSF receptor mRNA in MI-4 cells  
RT-PCR analysis showed the presence of G-CSF transcript (Fig. 5A), but not G-CSF receptor (Fig. 5B) in this cell line.

Detection of G-CSF, GM-CSF, M-CSF, IL-β and TNF-α in the medium  
MI-4 cells were plated in 96-well plates and cultured in the media containing 5% FCS for 48 h. Then the medium was collected for ELISA. All cytokines in the fresh medium containing 5% FCS were present, if at all, at levels below the sensitivity of the ELISA kits. Contents of G-CSF, GM-CSF and M-CSF in the medium were 2230±40, 140±19 and 160±23 pg/ml respectively while TNF-α and IL-1β were undetectable by the ELISA kits.

Effect of TNF-α and IL-1β  
IL-1β and TNF-α stimulated G-CSF production by MI-4 cells in a dose-dependent manner (Fig. 6A). Maximal G-CSF production by IL-1β and TNF-α was observed at concentrations of 10 ng/ml and 100 ng/ml respectively. Maximal G-CSF levels induced by these cytokines were about ten times the control. IL-1β and TNF-α (0.1 ng/ml) enhanced production and mRNA expression of G-CSF (Fig. 6B). However, these cytokines did not promote the growth of MI-4 cells at any concentration (Fig. 6C). TNF-α rather suppressed cell growth in a dose-dependent manner.

Effect of anti-G-CSF antibody and recombinant G-CSF  
Exogenous recombinant G-CSF (Fig. 7A) and anti-G-CSF antibody (Fig. 7B) did not promote and inhibit the growth of MI-4 cells compared with NFS-60 cells at any concentration, respectively.
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DISCUSSION

In this study, we established a new cell line (MI-4) which produces G-CSF from the pleural effusion of a patient with large cell undifferentiated carcinoma of the lung with marked leukocytosis, without any evidence of infection. Culture medium of MI-4 cells also contained a high level of G-CSF. Immunocytochemistry showed cytoplasmic staining for human G-CSF in MI-4 cells. Moreover, RT-PCR analysis revealed G-CSF mRNA expression in this cell line. Nude mice transplanted with MI-4 cells showed marked leukocytosis with a high serum level of G-CSF. We thought that this cell line would be a useful tool for understanding the cellular and molecular basis for G-CSF production in lung cancer.

As the causes of G-CSF over-production in tumors, trans-acting regulatory mechanisms, amplification and rearrangements of the G-CSF gene and co-amplification of oncogenes such as c-myc and c-ki-ras have been suggested.\textsuperscript{27-30} In MI-4 cells, Southern blot analysis did not show any amplification or rearrangement of the G-CSF gene. We also studied whether chromosomal aberrations specific for chromosome 17 could be a cause of G-CSF over-production in the MI-4 line. G-banding analysis showed only two intact chromosomes 17, whereas SKY analysis revealed an additional chromosome 17 with a segment of chromosome 16 and one marker chromosome containing a part of chromosome 17. To examine whether these chromosomes 17 have G-CSF genes, we performed FISH analysis using G-CSF cDNA probe. However, the size of the G-CSF probe we used was too small to clearly demonstrate the FISH signal. Therefore, we used DNA probes for the centromere of chromosome 17 and the RARA gene instead of the G-CSF gene. The G-CSF gene should be localized between the centromere and the RARA gene on chromosome 17q. The results of FISH analyses suggested that the G-CSF gene might not be disturbed in the three chromosomes 17. The G-CSF gene was not detected in any marker chromosome. Previously we have established a squamous cell lung cancer cell line OKa-C-1, which abundantly produces G-CSF.\textsuperscript{16} In OKa-C-1 cells, Southern blot analysis did not show any amplification or rearrangement of the G-CSF gene. SKY analyses showed another chromosome 17 with a segment of chromosome 10 besides three intact ones and eight marker chromosomes without any segment of chromosome 17 (data not shown). The FISH analysis suggested the existence of the G-CSF gene on the four chromosomes 17 in OKa-C-1 (data not shown). Thus, it is unlikely that overexpression of G-CSF would be caused by amplification and rearrangement of the G-CSF gene or specific translocation of chromosome 17 in G-CSF-producing lung cancer. The increased number of chromosomes having the G-CSF gene was observed in MI-4 and OKa-C-1 cell lines. However, numerical changes of chromosomes have been frequently revealed in lung cancer cell lines, regardless of cytokine production.\textsuperscript{31, 32} Therefore, it is not likely that the increased number of chromosomes would directly correlate with G-CSF production in these cell lines. Further approaches will be needed to define the mechanisms of G-CSF production by lung tumor cells.

It has been suggested that in a certain kind of hematopoietic malignant cell, such as acute myeloid leukemia cells with G-CSF receptor, an autocrine mechanism for aberrant secretion of G-CSF may exist and may lead to abnormal cell growth.\textsuperscript{33} Several reports have also demonstrated the presence of an autocrine growth loop for G-CSF in non-hematopoietic tumor cells.\textsuperscript{10, 34, 35} However, in

![Fig. 7. Proliferation of MI-4 cells in the presence of various concentrations of exogenous recombinant G-CSF (A) and anti-G-CSF antibody (B). MI-4 cells and control murine NFS-60 cells were grown for 48 h in serum-free medium in the presence of increasing concentrations of G-CSF (0.1–10^4 U/ml) and anti-G-CSF antibody (dilution by 16- to 512-fold). The data represent the mean±SD of three experiments.](image)
MI-4 cells, we could not detect the expression of G-CSF receptor mRNA by RT-PCR. In addition, exogenous recombinant human G-CSF and anti-G-CSF monoclonal antibody had no influence on the growth of MI-4 cells. It has been reported that IL-1β and TNF-α stimulate G-CSF production from various cell types, for example, astrocytoma cell line, medulloblastoma cell line, and bone marrow stromal cells. We have previously demonstrated that IL-1β and TNF-α significantly stimulate G-CSF production by the tumor cell line OKa-C-1. In this study, we showed that both cytokines also significantly enhanced G-CSF production by MI-4 cells. MI-4 cells produced neither TNF-α nor IL-1β themselves. However, these cytokines did not promote the growth of MI-4 cells regardless of abundant G-CSF production. TNF-α rather inhibited the growth of both cell lines in a dose-dependent manner. We could not detect the presence of an autocrine growth loop for G-CSF in OKa-C-1 cell line (data not shown). Thus, our molecular biological study could not demonstrate a crucial role for G-CSF in mediating a growth advantage for these lung cancer cell lines. To our knowledge, there has been no report about autocrine growth control by G-CSF in lung cancers. Some studies have reported that several tumor cell types simultaneously secrete GM-CSF and M-CSF, in addition to G-CSF. We also detected that MI-4 cells secreted some GM-CSF and M-CSF, besides a large amount of G-CSF. Although the presence of receptor for GM-CSF or M-CSF was not examined in this study, we can not rule out the possibility that these cytokines could support the growth of MI-4 cells. Further studies will be needed to elucidate the biological activity of G-CSF in lung cancer with G-CSF over-production. It has been reported that the prognosis is significantly worse in non-small cell lung cancer showing G-CSF gene expression. Regulation of G-CSF production by tumor cells might be an approach to improve the prognosis of patients with G-CSF-producing lung cancer.

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