Expression of Granulocyte-Colony-Stimulating Factor and Its Receptor in Human Ewing Sarcoma Cells and Patient Tumor Specimens

Potential Consequences of Granulocyte-Colony-Stimulating Factor Administration

Jaime Morales-Arias, MD[1]
Paul A. Meyers, MD[2]
Marcela F. Bolontrade, PhD[1]
Nidra Rodriguez, MD[1]
Zhichao Zhou, MD[1]
Krishna Reddy, BS[1]
Alexander J. Chou, MD[2]
Nadezhda V. Koshkina, PhD[1]
Eugenie S. Kleinerman, MD[1]

[1] Division of Pediatrics, The University of Texas M. D. Anderson Cancer Center, Houston, Texas.

[2] Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, New York, New York.

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Address for reprints: Jaime Morales-Arias, MD, Division of Pediatrics, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Unit 87, Houston, TX 77030; Fax: (713) 792-0608; E-mail: jmorales@mdanderson.org

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BACKGROUND. Ewing sarcoma (ES) is a highly vascular malignancy. It has been demonstrated that both angiogenesis and vasculogenesis contribute to the growth of ES tumors. Granulocyte-colony-stimulating factor (G-CSF), a cytokine known to stimulate bone marrow (BM) stem cell production and angiogenesis, is routinely administered to ES patients after chemotherapy. Whether ES cells and patient tumor samples express G-CSF and its receptor (G-CSFR) and whether treatment with this factor enhances tumor growth was examined.

METHODS. Human ES cell lines were analyzed for expression of G-CSF and G-CSFR in vitro and in vivo. Sixty-eight paraffin-embedded and 15 frozen tumor specimens from patients with ES were also evaluated for the presence of G-CSF and G-CSFR. The in vivo effect of G-CSF on angiogenesis and BM cell migration was determined. Using a TC/7-1 human ES mouse model, the effect of G-CSF administration on ES tumors was investigated.

RESULTS. G-CSF and G-CSFR protein and RNA expression was identified in all ES cell lines and patient samples analyzed. In addition, G-CSF was found to stimulate angiogenesis and BM cell migration in vivo. Tumor growth was found to be significantly increased in mice treated with G-CSF. The average tumor volume for the group treated with G-CSF was 1218 mm^3 compared with 577 mm^3 for the control group ($P = .006$).

CONCLUSIONS. The findings that ES cells and patient tumors expressed both G-CSF and its receptor in vitro and in vivo and that the administration of G-CSF promoted tumor growth in vivo suggest that the potential consequences of G-CSF administration should be investigated further. ***Cancer*** **2007;110:1568–77.**

KEYWORDS: Ewing sarcoma, granulocyte-colony-stimulating factor (G-CSF), granulocyte-colony-stimulating factor receptor (G-CSFR), angiogenesis, vasculogenesis.

Ewing sarcoma (ES) is the second most common primary osseous malignancy of childhood and adolescence. Its annual incidence in the U.S. is 2.1 cases per million children.[1] Despite numerous therapeutic trials, to our knowledge there has been no change in the 2-year metastases-free survival over the past 15 years.[2–5] A better understanding of the mechanisms that regulate the growth and progression of ES may help in the development of novel therapeutic approaches for this type of cancer.

Ewing tumors are highly vascular. We have demonstrated that in addition to angiogenesis, vasculogenesis, the process by which endothelial cell precursors are recruited and organized to form a

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vasculature, contributes to the growth of Ewing tumors.[6,7] We also have shown that bone marrow (BM)-derived cells participate in the expansion of the vascular network that supports tumor growth and that vascular endothelial growth factor (VEGF) is overexpressed in ES cells and patient samples and contributes to this process.[6–9] Given that many biologic processes are controlled by more than 1 factor, we hypothesize that other cytokines may participate in tumor vasculogenesis.

Granulocyte-colony-stimulating factor (G-CSF) is a hematologic cytokine that stimulates the proliferation and differentiation of BM precursor cells, mostly of the neutrophil lineage.[10] It is commonly administered to patients after chemotherapy to increase their neutrophil counts and protect them from infection.[11–15] G-CSF exerts its functions by binding and activating its high-affinity receptor, G-CSFR, a member of the hematopoietin receptor superfamily.[16] In addition to its role in the hematopoietic system, G-CSF stimulates the proliferation and migration of human endothelial cells.[17,18] Furthermore, G-CSF has been shown to have angiogenic activity in vivo using rabbit cornea as a model.[17,18]

In normal cells the synthesis of G-CSF is tightly regulated. By contrast, certain malignant cells constitutively secrete high amounts of G-CSF.[19–22] Colorectal cancer, squamous cell carcinoma, melanoma, ovarian carcinoma, meningioma, and glioma cells have been shown to constitutively express either G-CSF or its receptor.[23–28] In the current study, we demonstrate for what we believe to be the first time that Ewing tumor cell lines and patient samples express high levels of both G-CSF and G-CSFR. Using the TC/7-1 nude mouse model, we also further demonstrate that the administration of G-CSF enhanced tumor growth.

Because angiogenesis, and more recently vasculogenesis, have been demonstrated to promote tumor growth in ES,[6–9] we hypothesize that the systemic administration of G-CSF may enhance the release of progenitor cells from the BM that subsequently increase tumor vascular expansion and tumor growth. Indeed, we show that G-CSF chemoattracts BM cells and stimulates the development of vessels.

MATERIALS AND METHODS

Cell Lines

TC71 are human ES cells. TC/7-1 is a TC71 cell line stably transfected with the siRNA-VEGF vector.[9] PM3 and PM4 cells were derived from TC71 cells by recycling selection. PM3 has a high bone metastatic potency and PM4 has a high pulmonary metastatic potency. A4573 human ES cells were a generous gift from Dr. Soldatenkov (Georgetown University Medical Center, Washington, DC). All cell lines were cultured in Eagle modified essential medium with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 1 mM sodium pyruvate, 1× nonessential amino acid, and 2× MEM vitamin solution (Life Technologies, Grand Island, NY). SK-ES human ES cells were obtained from the American TypeCulture Collection (ATCC, Manassas, Va) and cultured in McCoy 5A medium with 10% FBS. Normal human osteoblast cells were purchased from Clonetics (San Diego, Calif) and were maintained in the special medium provided by Clonetics. All cell lines were screened with a Mycoplasma Plus PCR Primer Set (Stratagene, La Jolla, Calif) and found to be clear.

Reverse Transcriptase–Polymerase Chain Reaction

Total RNA was extracted from the different cell lines. For RNA extraction from frozen human tissue samples the RNeasy Lipid Tissue Mini Kit (Qiagen, Valencia, Calif) was used and the manufacturer's preparation instructions were followed. The cDNA was synthesized using a Reverse Transcription System (Promega, Madison, Wis). Reverse transcriptase (RT) products were amplified by polymerase chain reaction (PCR) using specific primers for G-CSF (sense, 5′-AGACAGGGAA GAGCAGA ACGG-3′; antisense, 5′GCCA GAGTGAGGGGTGCAA-3′) and G-CSFR (sense, 5′-AACAGCTCAGAGACC TGTGGCCT-3′; antisense, 5′CCAAGGGGC TGGCCTGGA-3′). The initial denaturation was performed at 94°C for 5 minutes. The products were then subjected to denaturation at 94°C for 1 minute, annealing at 58°C for 1 minute, extension at 72°C for 1 minute for 30 cycles, and a final elongation at 72°C for 10 minutes. The PCR products were subjected to electrophoresis on 1.5% agarose gel with ethidium bromide and visualized under ultraviolet light. Water without RNA was used as a negative control sample (data not shown). The 18S primers and competimers (Ambion, Austin, Tex) were used as the internal controls. Quantitative evaluation of the RT-PCR results was performed using a Del Doc 2000 Del Documentation System (Bio-Rad, Hercules, Calif). The relative fold expression of G-CSF and G-CSFR was determined by comparing each band with that of normal osteoblasts and adjusted by 18S internal controls. Primer selectivity for G-CSF and G-CSFR was confirmed by blasting primers against the human genome sequence. We found no homology with other genes with this same amplicon size within the whole human genome using PubMed blasting software.

In Vivo Angiogenesis and Chemotaxis Assays

G-CSF was resuspended in 300 μL aliquots of Growth Factor Reduced Matrigel (BD Biosciences, Pharmingen, San Diego, Calif) at concentrations of 30, 100, 200, and 1000 ng/mL. A matrigel/G-CSF aliquot (or matrigel alone) was subcutaneously (sc) implanted into nude mice. To evaluate angiogenesis, a group of animals were euthanized at 14 days and their matrigel implants were harvested. Frozen sections were prepared and analyzed via immunohistochemistry for CD31 and visualized with immunofluorescence. To assess chemotactic activity, BM cells were harvested from the femurs of nude mice and labeled with CellTracker CM-Dil fluorescent dye (Invitrogen, Carlsbad, Calif). These cells were intravenously injected into the nude mice bearing the matrigel/G-CSF implants at Days 21 and 24 (1.5 and 4 million cells/mouse, respectively). Three days after the second injection of BM cells, animals were euthanized and implants were removed and analyzed using fluorescent microscopy for the presence of CM-Dil+ cells.

Immunohistochemical Analysis

Tumor tissue sections were taken from nude mice bearing intra-tibia TC71 ES. The sections were analyzed by routine pathology using hematoxylin and eosin staining. Paraffin-embedded sections were dewaxed and incubated with Pepsin at 37°C for antigen retrieval. Tissue sections were incubated in 3% hydrogen peroxide in PBS for 12 minutes to block endogenous peroxidase and then incubated with 5% normal horse serum plus 1% normal goat serum in PBS for 20 minutes to block nonspecific protein. The G-CSF and G-CSFR expression was detected by incubating the tissue sections with goat antihuman polyclonal G-CSF antibody (Santa Cruz Biotechnology, Santa Cruz, Calif) or mouse antihuman G-CSFR antibody (BD Biosciences, Pharmingen) as the primary antibody and antigoat or antimouse IgG with HRP (Jackson ImmunoResearch Laboratory, West Grove, PA) as the secondary antibody. The use of goat serum was omitted for the G-CSF stains and no antigen retrieval was used during the G-CSFR staining. The developing product was visualized using chromogen diaminobenzidine (DAB) and Gill hematoxylin was used as a counterstain. Sixty-eight paraffin-embedded human ES patient samples were stained for G-CSF and G-CSFR after the same procedure. Tumors were classified according to immunohistochemical (IHC) staining intensity as weakly positive (1+), moderately positive (2+), and strongly positive (3+). For staining of the various cell lines, cells were seeded in chamber slides and fixed with acetone. For G-CSF, mouse antihuman monoclonal antibody (Oncogene Science, Cambridge, Mass) was used as primary antibody and goat antimouse Alexa 594 (Invitrogen, Molecular Probes, Carlsbad, Calif) was used as secondary antibody. Results were determined using fluorescent microscopy. For G-CSFR, mouse antihuman monoclonal antibody (BD Biosciences, Pharmingen) was used as primary antibody and antimouse IgG-HRP (BD Biosciences, Pharmingen) was used as secondary antibody. Chromogen DAB was then applied to visualize the results. For the matrigel/G-CSF angiogenesis experiment, frozen sections were obtained and fixed in acetone. Fish gelatin was used as protein block. Expression levels of the CD31 gene were detected in blood vessels formed in the matrigel using rat antimouse CD31 (BD Biosciences, Pharmingen) as the primary antibody and goat antirat Alexa 488 (Invitrogen, Molecular Probes) as the secondary antibody. Samples were then analyzed with fluorescent microscopy.

Cytostasis Assay

TC/7-1 cells were seeded onto 96-well cell culture plates (5000 cells/well) and allowed to adhere overnight. Cells were then treated with concentrations of G-CSF ranging from 10 ng/mL to 1000 ng/mL. Antiproliferative activity was determined by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay at 24 and 48 hours as described previously.[29] Each measurement was performed in triplicate.

Animal Studies

Four-week to 5-week-old specific pathogen-free athymic (T-cell deficient) nude mice were purchased from Charles River Breeding Laboratories (Kingston, Mass). The mice were housed in an animal facility approved by the Association for Assessment and Accreditation of Laboratory Animal Care and in accordance with the current regulations and standards of the U.S. Department of Agriculture and Department of Health and Human Services and the National Institutes of Health. The mice were housed for 1 to 2 weeks before beginning any of the experiments. Either phosphate-buffered saline (PBS) or human recombinant G-CSF (Neupogen, Amgen, Thousand Oaks, Calif) at a dose of 250 μg/kg/day diluted in 0.1% bovine serum albumin in PBS was administered sc every day starting 5 days before tumor inoculation until 14 days after. G-CSF at a dose of 250 μg/kg/day had previously resulted in an increase in the white blood cell count from a mean of 8000/mm^3 on Day 1 of therapy to 14,000/mm^3 by Day 3 and 16,5000/mm^3 by Day 5 (data not shown).

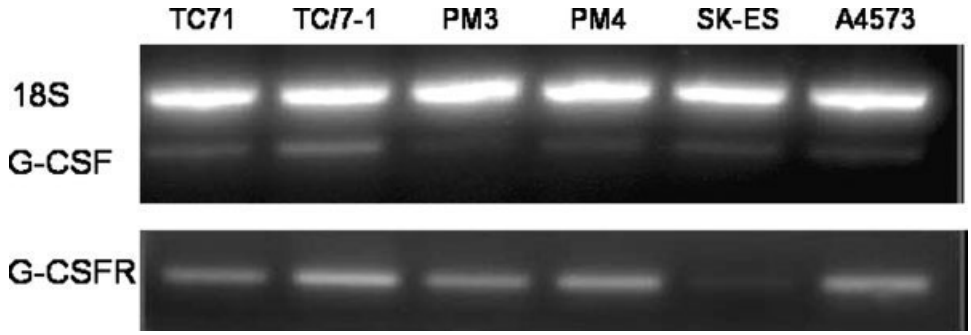

FIGURE 1. Expression of granulocyte-colony-stimulating factor (G-CSF) and its receptor (G-CSFR) RNA in human Ewing sarcoma (ES) cell lines. Reverse transcriptase–polymerase chain reaction for the detection of G-CSF and G-CSFR RNA was performed in 4 primary (TC71, TC/7-1, SK-ES, and A4573) and 2 metastatic (PM3 and PM4) human ES cell lines. Results were normalized using an 18S loading control.

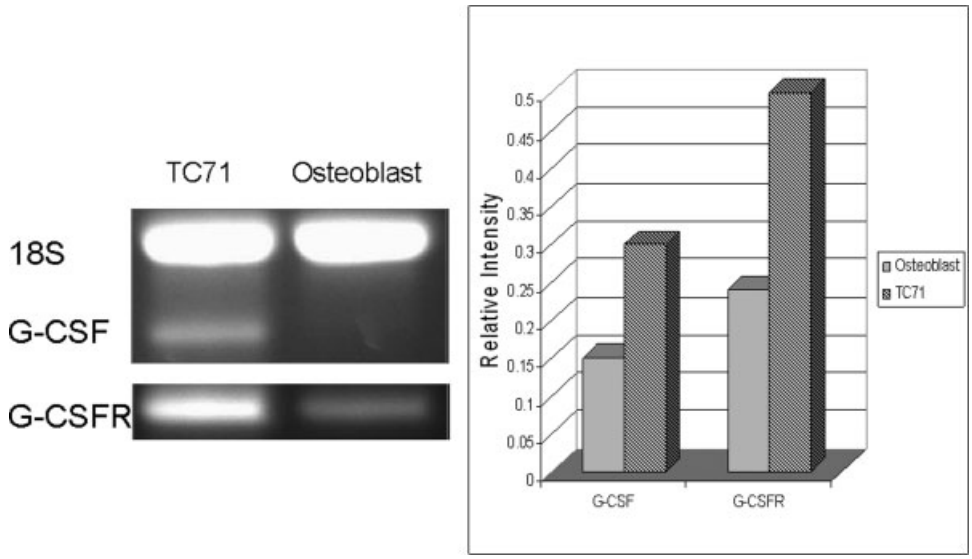

FIGURE 2. Comparison of the expression of granulocyte-colony-stimulating factor (G-CSF) and its receptor (G-CSFR) in Ewing sarcoma and human osteoblast cells. G-CSF and G-CSFR RNA expression of TC71 cells was compared with that of normal human osteoblast cells using reverse transcriptase–polymerase chain reaction (RT-PCR) and the results were analyzed using densitometry. Values were normalized with an 18S loading control. Gel resolution of the RT-PCR product is shown on the left and graphed densitometric analysis is shown on the right.

TC/7-1 ES cells were harvested in mid-log-growth phase. The nude mice were injected sc with 3×10^6 cells in 0.1 mL Hanks balanced salt solution (4°C). The tumors were measured every 3 to 5 days with calipers and their dimensions were recorded. Tumor volumes were expressed in mm^3 and calculated using the formula $½ \times ab^2$, in which a is the longest dimension and b is the shortest dimension. All mice were euthanized 32 days after tumor inoculation. This animal experiment was performed twice, producing similar results each time.

Human Samples

Sixty tumor specimens from ES patients were obtained in paraffin-embedded tissue arrays from Memorial Sloan-Kettering Cancer Center (New York, NY) and 8 additional paraffin-embedded ES patient samples were obtained from the tissue archives at the University of Texas M. D. Anderson Cancer Center (Houston, Tex). Frozen tumor sections from 15 patients with ES were acquired from the Cooperative Human Tissue Network (Columbus, Ohio). The use of these samples was approved by the appropriate Institutional Review Board.

Statistical Analysis

A 2-tailed Student t-test was used to statistically evaluate the difference in tumor volumes between treated and control groups. A P value of <0.05 was considered statistically significant.

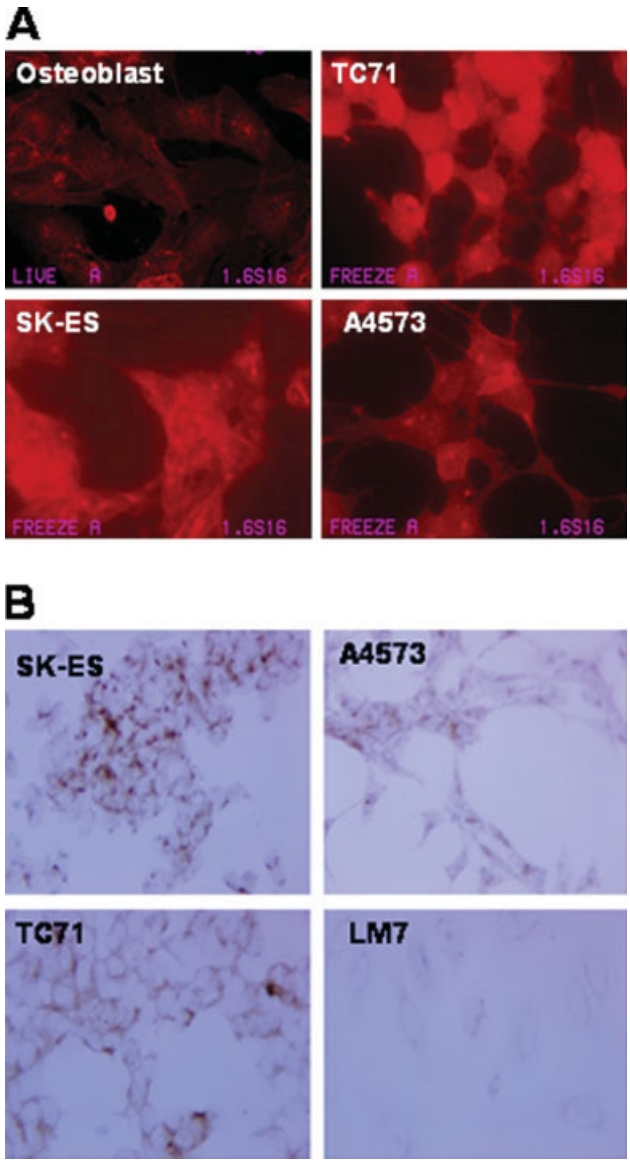

FIGURE 3. Expression of granulocyte-colony-stimulating factor (G-CSF) and its receptor (G-CSFR) protein in human Ewing sarcoma (ES) cell lines. (A) Fluorescent immunohistochemical (IHC) staining for G-CSF protein was performed in the human ES cell lines TC71, SK-ES, and A4573 and compared with a normal human osteoblast cell line. (B) IHC staining for G-CSFR protein in the same ES cell lines was performed using diaminobenzidine. ES cells were compared with a human osteosarcoma cell line (LM7) that did not demonstrate expression.

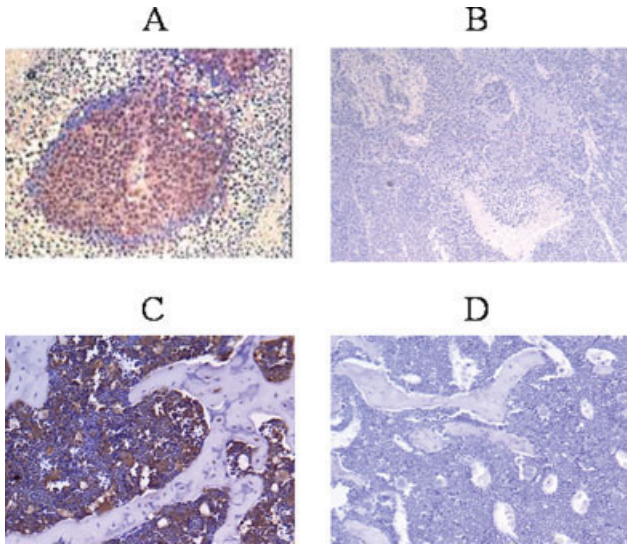

FIGURE 4. In vivo expression of granulocyte-colony-stimulating factor (G-CSF) and its receptor (G-CSFR) in TC71 Ewing sarcoma (ES) tumors. (A) Immunohistochemical (IHC) staining for G-CSF protein performed in a TC71 ES tumor removed from a nude mouse. (B) Negative control with the omission of primary G-CSF antibody. (C) IHC staining for G-CSFR protein in similar tissue. (D) Negative control with omission of the primary G-CSFR antibody.

RESULTS

G-CSF and G-CSFR Expression in Human ES Cell Lines

G-CSF and G-CSFR expression was determined in 4 primary (TC71, TC/7-1, SK-ES, and A4573) and 2 metastatic (PM3 and PM4) human ES cell lines using RT-PCR. Our findings indicated that both G-CSF and its receptor were expressed in all of these cell lines (Fig. 1). We compared the expression of G-CSF and G-CSFR between the TC71 tumor cells and a normal human osteoblast cell line. As shown in Figure 2, there was approximately a 2-fold higher degree of expression for both G-CSF and G-CSFR in the TC71 cells. We next evaluated the expression of G-CSF and G-CSFR proteins in TC71, SK-ES, and A4573 cells using IHC analysis. Expression of both G-CSF and G-CSFR protein was demonstrated (Fig. 3A and B).

To confirm the in vitro findings described above, we determined the G-CSF and G-CSFR expression in TC71 tumors that were induced after intratibial injection into nude mice. IHC revealed positive staining in all specimens analyzed (Fig. 4A and C). These data indicate that both proteins were produced in vivo.

G-CSF and G-CSFR Expression in Patient Samples

Samples from 68 patients with ES tumors (both primary and metastatic) were analyzed for G-CSF and G-CSFR expression using IHC. All samples demonstrated positive staining for both G-CSF (Fig. 5) (Table 1) and its receptor (Table 1). We also analyzed RNA that had been isolated from fresh specimens from 15 other ES patients. As seen with the ES cell lines, both G-CSF and G-CSFR were expressed at varying levels (Fig. 6). The highest expression of G-CSF was seen in tumors from the larynx and paraspi-

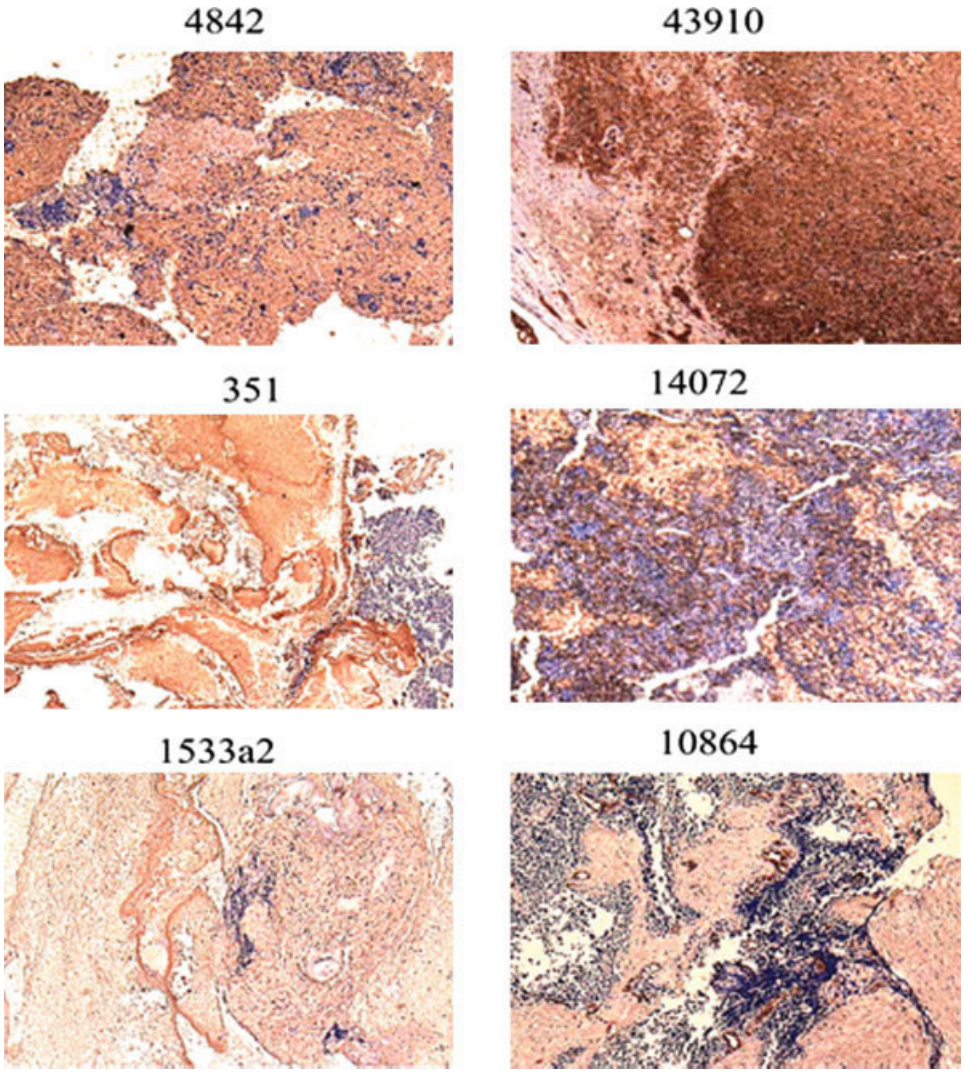

FIGURE 5. Expression of granulocyte-colony-stimulating factor (G-CSF) in samples from Ewing sarcoma (ES) patients. Paraffin-embedded specimens from 68 patients with ES tumors (both primary and metastatic) were analyzed. Immunohistochemical staining for G-CSF protein in 6 representative patient samples using anti-G-CSF antibodies is shown. Omission of the primary antibodies demonstrated no staining (data not shown).

nous region. The highest expression of G-CSFR was seen in tumors arising from the thigh and fibula.

G-CSF Stimulates Angiogenesis and BM Cell Migration In Vivo

To evaluate the effect of G-CSF on endothelial cell growth in vivo, matrigel implants containing different concentrations of G-CSF were injected into nude mice. Increased CD31+ cells integrating vascular networks were observed in the matrigel/G-CSF plugs compared with the control (data not shown). This corroborates other investigator's findings that G-CSF stimulates angiogenesis in vivo.[17,18]

TABLE 1
Percentage of Tumors From Ewing Sarcoma Patients Expressing G-CSF and G-CSFR by Immunohistochemical Grade (N = 68)

	G-CSF expressed (%)		G-CSFR expressed (%)	
Grade	All tumors	Metastatic tumors	All tumors	Metastatic tumors
1+ (weakly positive)	2	0	2	0
2+ (moderately positive)	36	16	12	0
3+ (strongly positive)	62	84	86	100

G-CSF indicates granulocyte-colony-stimulating factor; G-CSFR, granulocyte-colony-stimulating factor receptor.

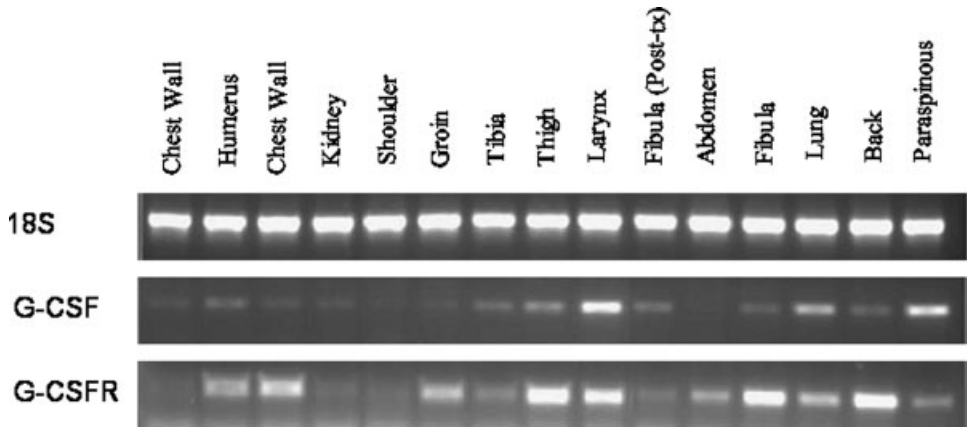

FIGURE 6. Expression of granulocyte-colony-stimulating factor (G-CSF) and its receptor (G-CSFR) RNA in samples from Ewing sarcoma (ES) patients. RNA was extracted from frozen tumor sections from 15 patients with ES and analyzed for the expression of G-CSF and G-CSFR using reverse transcriptase–polymerase chain reaction. Results were normalized using an 18S loading control. Post-tx indicates post-treatment.

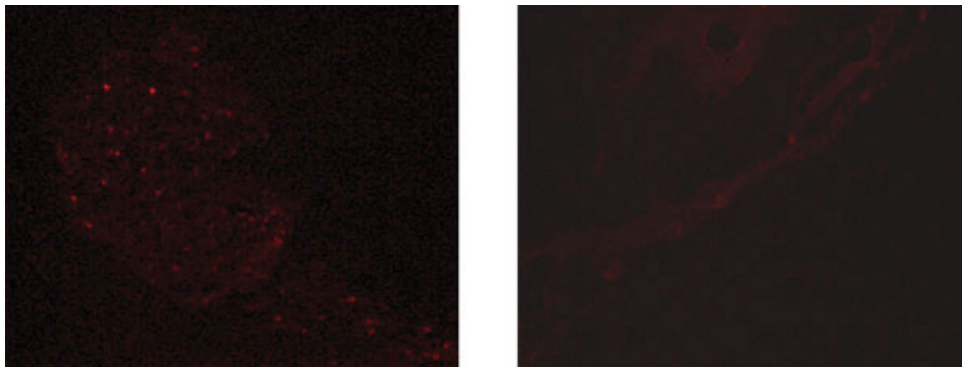

FIGURE 7. In vivo effect of granulocyte-colony-stimulating factor (G-CSF) on bone marrow (BM) cell migration. Fluorescent microscopy analysis for the presence of CM-DiI-positive BM cells after their intravenous administration into nude mice previously implanted with a matrigel/G-CSF plug (left) and with a matrigel plug without G-CSF (right) used as the control.

To determine the chemotactic activity of G-CSF for BM cells, matrigel plugs with or without G-CSF were implanted into nude mice that were later injected with fluorescently labeled BM cells. There was an increase in the number of labeled BM cells that migrated to the G-CSF-containing matrigel plugs when compared with the control plugs (Fig. 7). This suggests that G-CSF is a chemoattractant for BM cells in vivo.

Effect of G-CSF on Tumor Growth In Vitro and In Vivo

Treatment of TC/7-1 cells with G-CSF in vitro had no effect on cell proliferation (Fig. 8). To test the effect of G-CSF on tumor growth in vivo, nude mice were treated with either G-CSF or PBS for 5 days before the sc injection of VEGF-deficient TC/7-1 cells.[9] Treatment continued for 14 days posttumor injection. Tumor growth was significantly increased in the G-CSF-treated mice compared with the mice treated with PBS. The average tumor volume for the G-CSF-treated mice was 1218 mm^3 compared with 577 mm^3 for the control group ($P = .006$) (Figs. 9 and 10).

DISCUSSION

The data presented demonstrated that different ES cell lines and patient tumor specimens express both G-CSF and G-CSFR. We further demonstrated that G-CSF stimulated angiogenesis and the migration of BM cells in vivo and enhanced tumor growth when administered sc. Taken together, these data suggest that G-CSF may contribute to tumor angiogenesis and ultimately support tumor growth by positively influencing tumor vascular expansion.

The concept that G-CSF alters the tumor microenvironment in a way that enhances growth is supported by a number of other studies. G-CSF has

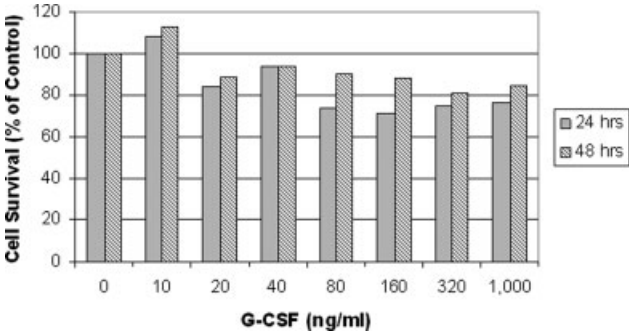

FIGURE 8. In vitro effect of granulocyte-colony-stimulating factor (G-CSF) on TC/7-1 Ewing sarcoma cells. TC/7-1 cells were seeded into culture plates and allowed to adhere overnight. G-CSF at different concentrations was administered and the percentage of cell survival was evaluated at 24 hours and 48 hours using the [4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. TC/7-1 cells incubated without G-CSF were used as the control.

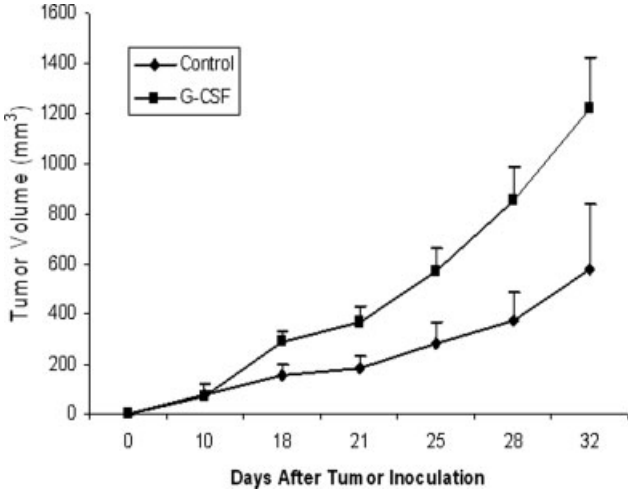

FIGURE 9. In vivo effect of granulocyte-colony-stimulating factor (G-CSF) on Ewing sarcoma tumor volumes. Graphic comparison of average tumor volumes in treated mice and controls ($P = .006$) after the administration of G-CSF at a dose of 250 μg/kg/day subcutaneously beginning 5 days before tumor inoculation and continuing until 14 days after.

been shown to promote endothelial cell growth and the mobilization of circulating stem cells in addition to stimulating the proliferation, differentiation, and mobilization of neutrophil precursors.[17,18] The administration of G-CSF to mice with colon cancer resulted in accelerated growth that was attributed to increased neovascularization and the participation of BM-derived endothelial progenitor cells.[30] G-CSF was also shown to contribute to the angiogenic and more malignant phenotype of skin carcinoma cells in vivo.[31] Moreover, treatment with this cytokine promoted tumor angiogenesis and growth in a Lewis lung carcinoma animal model through increased circulating endothelial progenitor cells.[32] A more recent study showed that the administration of G-CSF significantly increased the bone tumor burden in an intratibial melanoma mouse model through osteoclast activation.[33]

Our studies add further support to the hypothesis that G-CSF promotes tumor growth by stimulating angiogenesis and perhaps vasculogenesis as well. G-CSF did not appear to enhance the in vitro growth of our TC/7-1 cells. By contrast, increased in vivo tumor growth was demonstrated after its sc administration. We have previously shown that BM cells participate in the tumor vascular expansion in our TC-71 ES animal model.[6,7] Approximately 10% of the tumor vessels formed during the first week of tumor growth were derived from the migrated BM cells, indicating that vasculogenesis in addition to angiogenesis plays an important role in Ewing tumor vascular expansion. Therefore, enhancing the number of circulating stem cells may aid in this vasculogenesis process.

The ability of G-CSF to stimulate BM-derived cells has been extensively studied with regard to its potential therapeutic use for the treatment of ischemic or injured tissue. G-CSF treatment was shown to induce the homing of large numbers of BM cells to salivary glands damaged by radiation. This subsequently led to repair of the damaged tissue, with improved function and morphology.[34] A clinical trial of myocardial infarction patients showed that administration of G-CSF induced secondary mobilization of $CD34^+$ cells, which resulted in an improvement of the left ventricular ejection fraction. These findings suggest that G-CSF potentiated BM cell migration to the damaged heart, which subsequently led to the restoration of organ function.[35] An additional study demonstrated that G-CSF improved cerebral ischemia by stimulating both BM and neural stem progenitor cells.[36] The studies cited above demonstrated that G-CSF stimulated the number of circulating BM-derived cells, which then migrated to the injured tissues. Tumors have been called "a continuous nonhealing wound." Therefore, this same mechanism may be operational in tumors. Damaged tissue and/or inflammatory cells surrounding the tumor and the tumor cells themselves may produce cytokines that induce BM cells to migrate to the location of the tumor. These migrated BM cells may then participate in local neovascularization contributing to malignant growth and invasion.

VEGF, a well-known angiogenic factor, plays an important role in tumor progression and metastasis

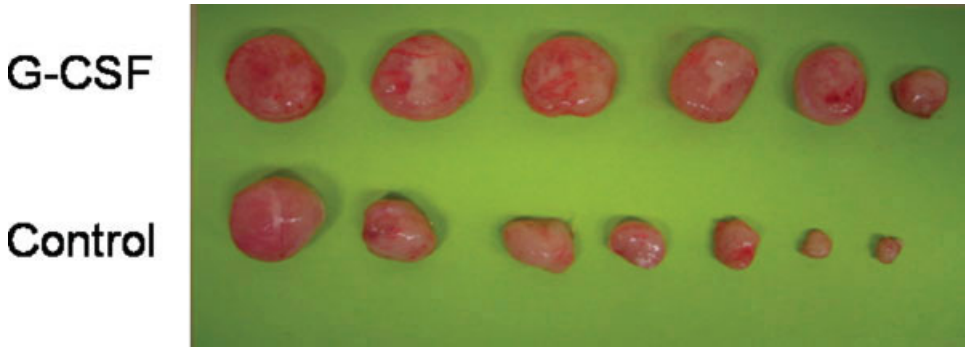

FIGURE 10. Macroscopic effect of granulocyte-colony-stimulating factor (G-CSF) treatment on Ewing sarcoma tumors. Visual comparison of representative tumors removed from the mice treated with G-CSF and from the control mice.

in many different solid cancers, including ES.[8,37] We have previously shown that inhibition of $VEGF_{165}$ production in ES cells using siRNA technology suppressed tumor growth.[9] Here we demonstrated that G-CSF promoted the growth of the $VEGF_{si\text{-}RNA}$ TC/7-1 tumors. In contrast to its usual slow-growing behavior, mice developed aggressive fast-growing tumors after receiving G-CSF therapy. These data suggest that G-CSF may compensate for the lack of VEGF by stimulating the mobilization and migration of BM cells that assist in tumor vascular expansion in the absence of VEGF.

Clinical trials investigating the efficacy of antiangiogenic therapy including VEGF inhibitors are currently under way.[37] Indeed, 1 of the targets for chemotherapy has been shown to be endothelial cells. It has been demonstrated that by altering the schedule of chemotherapy to provide sustained apoptosis of endothelial cells, an antiangiogenic effect is evident.[38] Thus, standard chemotherapy regimens may also inhibit tumor angiogenesis in addition to targeting the tumors directly. Furthermore, radiation therapy has been shown to have an effect on endothelial cells.[39] The results presented in the current study suggest that tumor cells may be able to use alternative mechanisms to stimulate vascular expansion, thereby circumventing the blockage of VEGF or chemotherapy/radiotherapy-induced antiangiogenesis. We suggest that the administration of G-CSF may therefore have a deleterious effect on certain cancer therapies.

In summary, the results of the current study demonstrated that ES cells and patient tumor specimens express both G-CSF and its receptor in vitro and in vivo. The administration of G-CSF promoted tumor growth in vivo. Because G-CSF is routinely administered to patients with solid tumors after chemotherapy, our studies suggest that evaluating the potential consequences of G-CSF therapy in this situation may be warranted.

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