Expression of Granulocyte-Macrophage Colony-Stimulating Factor Receptors in Human Prostate Cancer

By Coralia I. Rivas, Juan Carlos Vera, Fernando Delgado-López, Mark L. Heaney, Victor H. Guaiquil, Rong H. Zhang, Howard I. Scher, Ilona I. Concha, Francisco Nualart, Carlos Cordon-Cardo, and David W. Golde

We studied the expression and function of the granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor in the human prostate carcinoma cell line LNCaP and looked for its presence in normal and neoplastic human prostatic tissue. The GM-CSF receptor is composed of two subunits, α and β. While the isolated α subunit binds GM-CSF at low affinity, the isolated β subunit does not bind GM-CSF by itself, but complexes with the α subunit to form a high-affinity receptor. Quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) showed expression of mRNAs encoding the α and β subunits of the GM-CSF receptor in LNCaP cells, and the presence of the α and β proteins was confirmed by immunolocalization with anti-α and anti-β antibodies. Receptor binding studies using radiolabeled GM-CSF showed that LNCaP cells have about 150 high-affinity sites with a kd of 40 pmol/L and approximately 750 low-affinity sites with a kd of 2 nmol/L. GM-CSF signaled, in a time- and dose-dependent manner, for protein tyrosine phosphorylation and induced the proliferation of the LNCaP cells. Immunolocalization studies showed low level expression of GM-CSF α and β subunits in normal prostate tissue, with substantial expression in benign prostatic hyperplasia and prominent expression in neoplastic prostate tissue. Maximal expression of both subunits was observed in prostatic carcinomas metastatic to lymph node and bone. Tumor cells that stained positively with anti-α subunit antibodies were also reactive with anti-β subunit antibodies, indicating that they express high-affinity GM-CSF receptors. Our data show that the LNCaP cells express functional GM-CSF receptors and that prostatic carcinomas have prominent GM-CSF receptor expression. These findings imply that both hyperplastic and neoplastic prostatic tissues may be responsive to GM-CSF.

© 1998 by The American Society of Hematology.

MATERIALS AND METHODS

Cell culture. The human prostatic cell line LNCaP35 was cultured in RPMI-1640 supplemented with 6% heat-inactivated fetal bovine serum, 1% L-glutamine, and antibiotics. The human leukemia cell line HL-6036 was maintained in Iscove’s modified Dulbecco’s medium (IMDM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, and antibiotics.

Proliferation assay. Cell proliferation was assessed by [3H]-thymidine and [125I]-deoxyuridine incorporation. Briefly, 40,000 cells per well were plated in sextuplicate in 6-well plates (Costar, Cambridge, MA) and bacterial recombinant human GM-CSF (0.01 to 100 nmol/L) (a gift from Amgen, Thousand Oaks, CA) was added at the beginning of the culture. Every day for 6 days of culture, 2 µCi of [3H]-thymidine (DuPont NEN, Boston, MA) or 0.45 µCi of [125I]-dUridine (DuPont NEN) was added to each well. The cells were harvested after 20 hours using a cell harvester (Skatron, Sterling, VA) or extracted with 1 N NaOH. The radioactivity incorporated into DNA was quantitated by liquid scintillation or γ spectrometry.

From the Program in Molecular Pharmacology and Therapeutics, Departments of Medicine and Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY; Instituto de Bioquímica, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile; and Departamento de Histología y Embriología, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.

Submitted June 30, 1997; accepted October 2, 1997.

Supported in part by Grants No. R01 CA03888, R01 HL62107, CA-047650, and P30 CA08748 from the National Institutes of Health, Bethesda, MD; by Memorial Sloan-Kettering Institutional funds; by the Schultz Foundation, Verona, NJ; by the PepsiCo Foundation, Purchase, NY; and by the David H. Koch Charitable Foundation, Wichita, KS.

Address reprint requests to David W. Golde, MD, Program in Molecular Pharmacology and Therapeutics, Box 451, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1998 by The American Society of Hematology.

From www.bloodjournal.org by guest on October 31, 2017. For personal use only.
Reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was isolated from LNCaP and HL-60 cells using guanidinium thiocyanate (RNAzol B, Cinna/Biotech Laboratories, Houston, TX). Single-stranded cDNA synthesis and quantitative PCR were performed as previously described. Primers were used: a subunit primer: 5′-AGCCGGACAAACACACAA, position 1009-1026 and 3′-CCATGCCCTACATACCT, position 1360-1379; β subunit primer: 5′-CTACAAAGCCCCAGTGGC, position 859-879 and 3′-ACCCGTAGATGGCCACAGAAC, position 1390-1410. The PCR conditions were 94°C for 1 minute and 65°C for 2 minutes for 35 cycles. For quantification of the β subunit, the GM-CSF receptor β cDNA subcloned into pBluescript (Stratagene, La Jolla, CA) was transcribed in vitro (Megascript, Ambion, Austin, TX) and the RNA was digested with DNase and quantitated spectrophotometrically and by the addition of [32P] uridine triphosphate (UTP) (DuPont NEN). RT-PCR of serial dilutions was performed in the presence of 1 µg of RNA derived from HeLa cells, a cell line that does not express the β subunit.

Binding assays. For binding assays, 7 × 10⁶ cells were suspended in RPMI-1640 containing 0.2% bovine serum albumin (BSA) and increasing concentrations of radiolabeled human GM-CSF (DuPont NEN) with or without excess unlabeled human GM-CSF. After incubation for 20 hours at 4°C, the cells were centrifuged for 5 minutes at 4°C through a cushion of fetal bovine serum and the cell pellets were washed with cold phosphate-buffered saline (PBS) pH 7.4. Bound GM-CSF was quantitated by γ spectrometry.

Immunoblotting. Cells were serum starved in RPMI-1640 containing 0.2% BSA for 18 hours, washed, resuspended at 1 × 10⁷ cells/mL and incubated in the absence or in the presence of increasing concentrations of GM-CSF (0.01 nmol/L to 1 µmol/L) for different periods of time (2 seconds to 20 minutes) at 37°C. The cells were washed with cold PBS and the cell pellets were resuspended in 100 µL of lysis buffer and disrupted by sonication. The soluble proteins were resolved by SDS-PAGE (100 µg of cell lysate per lane) in a 10% polyacrylamide gel and transferred to immobilon (Millipore, Bedford, MA). Proteins phosphorylated on tyrosine residues were detected using an antiphosphotyrosine antibody (UBI, Lake Placid, NY). Phosphorylated mitogen-activated protein (MAP) kinase was localized using an antiphosphoMAP kinase antibody (New England Biolabs, Beverly, MA). Anti-JAK2 antibody was purchased from UBI. Antibody blots were developed by chemiluminescence (New England Biolabs).

Immunocytochemistry. For immunoperoxidase localization, LNCaP cells cultured in 6-chamber microscopic slides were fixed in buffered paraformaldehyde-acetone, treated with 0.3% H₂O₂ for 5 minutes and incubated for 30 minutes at room temperature in 4% BSA-PBS pH 7.8, followed by incubation overnight at 4°C in 1% BSA-PBS pH 7.8 and anti-α or anti-β GM-CSF receptor subunit antibodies (1:500) (Alpha Diagnostics, San Antonio, TX). Cells were washed and incubated with antirabbit IgG-horseradish peroxidase (1:100) (Amersham, Arlington Heights, IL) for 2.5 hours at room temperature. Immunostaining was performed using 0.05% dianminobenzidine and 0.03% H₂O₂. As controls, cells were incubated with antibodies preabsorbed with the respective peptide used to generate the antibodies. Cells were counterstained with hematoxylin.

Archived, formalin fixed, and paraffin embedded human prostate tissues were obtained from the Department of Pathology at Memorial Sloan-Kettering Cancer Center. For immunostaining, tissue sections were rehydrated, treated with 3% hydrogen peroxide for 15 minutes at room temperature, and blocked with 4% BSA-PBS pH 7.8, followed by incubation in a humid chamber overnight at 4°C with anti-α or anti-β subunit GM-CSF receptor antibodies (1:500) in 1% BSA-PBS pH 7.8. After extensive washing, sections were incubated for 2.5 hours at room temperature with antirabbit IgG-horseradish peroxidase (1:100, Amersham). The peroxidase activity was developed with 0.05% dianaminobenzidine and 0.03% H₂O₂. Tissues were counterstained with hematoxylin.

RESULTS
LNCaP cells express GM-CSF receptors. A band of approximately 370 nucleotides, the expected size of the amplification product for the membrane-bound form of the α subunit mRNA, was amplified by RT-PCR from LNCaP cells RNA (Fig 1A). Primers specific for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used as an internal standard for the efficacy of the RT-PCR procedure. A similar approach using primers complementary to the β subunit of the GM-CSF receptor showed the expected amplification product of approximately 570 nucleotides (Fig 1A). The size of the amplified

Fig 1. Expression of GM-CSF receptors in LNCaP cells. (A) RT-PCR analysis. Total RNA was isolated from LNCaP (lane 1) or HL-60 (lane 2) cells and subjected to RT-PCR using radiolabeled primers specific for the α (left panel) or the β (right panel) subunits of the GM-CSF receptor. PCR products were size-fractionated on 5% acrylamide gels and autoradiographed. Shown are the PCR products corresponding to the α (370 bp) and the β (570 bp) subunits of the GM-CSF receptor obtained in the presence (+) or in the absence (-) of reverse transcriptase (RT). (B) Binding analysis. Cells were incubated with radiolabeled GM-CSF at concentrations that ranged from 5 pmol/L to 10 nmol/L. GM-CSF binding was dose dependent and saturable approximately at 10 nmol/L. (C) Scatchard analysis of data from (B) showing the presence of two classes of binding sites in the LNCaP cells. (D through G) Immunostaining with antihuman GM-CSF receptor antibodies. Cells were cultured, fixed, and incubated with anti-α (D and F) or anti-β (E and G) antibodies in the absence (D and E) or the presence (F and G) of the peptides used to elicit them (original magnification × 160).
bands corresponded exactly to the size of the respective bands amplified from RNA obtained from HL-60 cells, which express abundant mRNA for the α and β subunits of the GM-CSF receptor (Fig 1A). No amplification products were observed in parallel reactions in which the reverse transcriptase was omitted (Fig 1A), confirming the absence of contaminating DNA in the RNA preparations. The content of α and β subunit mRNAs in LNCaP cells was quantitated by interpolation on a standard curve generated by performing RT-PCR with known amounts of either α or β subunit RNA in parallel with LNCaP cell-derived RNA. LNCaP cells expressed 0.02 to 0.33 fg of α subunit mRNA per 10^5 cells (0.24 to 4 copies per 10^3 cells), a value that is 60 to 200 times lower than the α subunit mRNA levels expressed in HL-60 cells (Fig 1A). LNCaP cells expressed 12 to 83 fg of β subunit mRNA per 10^5 cells (80 to 550 copies per 10^3 cells), which is 30 to 200 times lower than the β subunit mRNA levels expressed in HL-60 cells (Fig 1A). LNCaP cells also expressed low levels of mRNA encoding the soluble isofrom of the α subunit (data not shown).

Radiolabeled GM-CSF bound to LNCaP cells in a dose-dependent and saturable manner (Fig 1B). Scatchard analysis of the binding data (Fig 1C) showed that the LNCaP cells expressed approximately 150 high-affinity binding sites for GM-CSF with a kd of 40 pmol/L, and 750 low-affinity binding sites. The presence of the α and β subunits of the GM-CSF receptor in the LNCaP cells was confirmed by immunolocalization with antibodies specific for each subunit. The LNCaP cells were immunoreactive with both antibodies, with intense immunostaining in both the cytoplasm and the plasma membrane (Fig 1D and F). The LNCaP cells consistently showed enhanced immunoreactivity with the anti-α antibody compared with the anti-β antibody. No immunoreactivity was observed when the primary antibodies were preabsorbed with the peptides used to generate them (Fig 1E and G).

GM-CSF signaling in LNCaP cells. Proliferation assays, measuring the incorporation of [3H]-thymidine or [125I]-deoxyuridine in DNA, showed that GM-CSF stimulated proliferation of the LNCaP cells in a dose- and time-dependent manner (Fig 2B and C); only [3H]-thymidine incorporation is shown). An increase in [3H]-thymidine incorporation of 20% to 40% was observed after 3 or 4 days of culture in the presence of 0.3 or 100 nmol/L GM-CSF (Fig 2B). The effect of GM-CSF on [3H]-thymidine incorporation was evident during the exponential phase of cell growth and decreased at later stages (Fig 2A and B). Dose-response studies indicated a biphasic effect of GM-CSF on [3H]-thymidine incorporation (Fig 2C). GM-CSF induced a measurable increase in [3H]-thymidine at a concentration of 0.03 nmol/L, an effect that reached saturation at 1 nmol/L GM-CSF, with no further increase observed at concentrations of GM-CSF from 1 to 30 nmol/L. However, 100 nmol/L GM-CSF induced an additional increase in [3H]-thymidine incorporation, which was also evident in the time-course experiments (Fig 2B and C).

We next analyzed whether GM-CSF induced protein tyrosine phosphorylation in the LNCaP cells. A transient increase in tyrosine phosphorylation was observed in several proteins when LNCaP cells were treated with GM-CSF (Fig 3A). Proteins with apparent molecular weights of 160, 130, 75 to 80, 68 to 70, 55, 47, and 40 kD (black arrowheads, Fig 3A) showed maximal phosphorylation during the first 30 seconds of treatment with 1 nmol/L GM-CSF and remained phosphorylated for 20 minutes, with phosphorylation returning to basal levels after 1 to 2 hours (Fig 3A). Phosphorylation of these proteins was induced at concentrations of GM-CSF ranging from 0.03 nmol/L to 1 μmol/L (Fig 3A). Interestingly, we observed the rapid dephosphorylation of proteins with an apparent molecular weight of 45 and 110 kD after incubating the cells with 1 nmol/L GM-CSF (white arrowheads, Fig 3A). These proteins were dephosphorylated after treating the cells with GM-CSF at concentrations from 0.03 nmol/L to 1 μmol/L and remained dephosphorylated for at least 18 hours (Fig 3A).

We did not detect tyrosine phosphorylation of MAP kinase in LNCaP cells treated with GM-CSF using antiphosphotyrosine antibodies (Fig 3A). The p42 MAP kinase was identified by reprobing the membrane with an anti-MAP kinase antibody and was found to be present at a constant level at the expected position in the blots (Fig 3B). Phosphorylation of MAP kinase, however, was evident when using a monoclonal antibody.

Fig 2. GM-CSF signals for proliferation in LNCaP cells. (A) Growth curve. LNCaP cells were maintained in continuous culture with no stimulation and the cell number was determined by counting the cells every day for 6 days and the cell viability was assessed by exclusion of trypan blue. (B) Time course. Cells were incubated with 0.3 (⚫) or 100 nmol/L (⚪) GM-CSF for 1 to 6 days and parallel cultures were pulse-labeled with [3H]-thymidine for 20 hours every day. (C) Dose response. Cells were incubated with increased amounts of GM-CSF (0.01 to 100 nmol/L) for 3 (⚫) or 4 days (⚪) and pulse-labeled with [3H]-thymidine for the last 20 hours.
Phosphorylation of MAP kinase occurred only at concentrations of GM-CSF of 3 nmol/L or higher and reached a maximal level at 100 nmol/L GM-CSF. Maximal phosphorylation was observed at 30 seconds, with phosphorylation returning to basal levels after 20 minutes (Fig 3B). No phosphorylation of JAK2 was evident in cells treated with GM-CSF.

**Increased expression of GM-CSF receptors in human prostate tumors.** We examined whether the α and β subunits of the GM-CSF receptor were expressed in normal prostate, benign prostatic hyperplasia, and prostatic carcinomas, including primary tumors and metastatic lesions to lymph node and bone (Fig 4 and Table 1). Very weak to undetectable immunostaining was observed in the luminal epithelial cells of the acini of the normal prostate, although weak to moderate reactivity was evident in basal cells (Fig 4). Ducts exhibited a more intense immunoreactivity than acini for both antibodies. Moderate immunostaining was also found in the epithelial cells of most of the samples of benign prostatic hyperplasia analyzed with both antibodies, although moderate to strong reactivity was observed in two specimens (Fig 4). In addition, basal cell hyperplasia, a histologically different form of hyperplasia, was seen in one sample and displayed strong reactivity with both antibodies. All primary tumors analyzed, which exhibited Gleason scores from 5 to 9, were positive for both antibodies, with homogeneous and heterogeneous staining patterns of moderate to strong intensity (Fig 4 and Table 1). The metastatic tumors displayed homogeneity, as well as heterogeneity in immunostaining profiles, with moderate to strong reactivity to both antibodies and had increased staining compared with the primary tumors (Fig 4). One sample from a bone metastasis was negative for both antibodies. Tumor cells that were immunopositive for the anti-α antibody were also positive for the anti-β antibody, as immunohistochemistry was conducted in consecutive sections and numerous lesions showed homogeneous staining. In addition, most of the cases studied showed a more intense anti-α immunoreactivity than the anti-β immunoreaction.

**DISCUSSION**

GM-CSF is a hematopoietic growth factor and a host defense regulator used clinically to stimulate hematopoietic cell proliferation after chemotherapy, as well as autologous or allogeneic bone marrow transplantation.40-42 The physiologic role of GM-CSF receptors in nonhematopoietic tissue is unknown. Even more problematic are the implications and consequences of GM-CSF receptor expression on malignant, nonhematopoietic tissue. Distinct types of neoplastic cells have been shown to have functional GM-CSF receptors.25,30,31 Whether or not therapeutically administered hematopoietic growth factors can stimulate nonhematopoietic cell growth, including solid tumor cells, has remained a controversial issue.

We report here a detailed study addressing the issue of GM-CSF receptor expression in human prostate cancer. The presence of GM-CSF receptors in the LNCaP cells was defined by quantitative RT-PCR, ligand binding, immunolocalization, and functional assays. Quantitative RT-PCR showed that the LNCaP cells expressed mRNAs for the α and β subunits of the GM-CSF receptor. The immunolocalization experiments confirmed the presence of the α and β proteins in the LNCaP cells.
and the ligand-binding studies showed that LNCaP expressed both high- and low-affinity GM-CSF receptors. The number of high-affinity receptors present in the LNCaP cells was similar to the number present in cells of hematopoietic origin in which GM-CSF induces proliferation and differentiation and in nonhematopoietic cells such as mouse fibroblasts expressing the human high-affinity receptors, which respond to GM-CSF with cell proliferation and protein phosphorylation. The identification of approximately 750 low-affinity GM-CSF receptors in the LNCaP cells, compared with about 150 high-affinity sites, indicates the presence of an excess of α as compared with β subunit in these cells. Consistent with these findings, the immunolocalization experiments showed greater immunoreactivity with the anti-α subunit antibodies than with the anti-β subunit antibodies in the LNCaP cells. The RT-PCR experiments, however, indicated that LNCaP cells express a higher number of mRNA molecules per cell encoding the β than the α subunit of the GM-CSF receptor. These data suggest that, in the LNCaP cells, the expression of the α and β subunit proteins is regulated at the level of translation or protein stability.

Biologic response analyses confirmed the presence of functionally active GM-CSF receptors in the LNCaP cells. A previous study reported a 2.8-fold increase in LNCaP cell proliferation in the presence of suprapharmacologic concentrations of GM-CSF (>1 µmol/L). These concentrations of GM-CSF are at least four orders of magnitude higher than the concentrations we used here (∼100 pmol/L) and are not compatible with the expression of high-affinity GM-CSF receptors in the LNCaP cells. On the other hand, although our data indicated that GM-CSF increased LNCaP cell proliferation at concentrations consistent with the presence of high-affinity receptors in these cells, these concentrations of GM-CSF (∼100 pmol/L) were at least one order of magnitude higher than that necessary in cells such as HL-60 (∼10 pmol/L), which express a similar number of high-affinity GM-CSF receptors. The origin of these discrepancies is not evident from these studies;
The lack of immunoreactivity or weak pattern of staining using anti-
and -subunit expression may modulate signaling through the
GM-CSF receptor in prostate tumors. 4,9,10,13-15,45 We found
that modulate the functional activity of the GM-CSF receptor.

The immunohistochemical analysis of human prostatic tissue
has a role in maintenance of function in the normal prostate, as
are not well understood, our results suggest that GM-CSF may
normal prostate physiology and progression of prostate cancer
metastatic disease.

Table 1. Expression of GM-CSF Receptors in Human Prostatic Tissue

<table>
<thead>
<tr>
<th>Grade (Gleason score)</th>
<th>GMαa</th>
<th>GMβa</th>
<th>GMαb</th>
<th>GMβb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal prostate</td>
<td>4/7†</td>
<td>±/+</td>
<td>4/7</td>
<td>±/+</td>
</tr>
<tr>
<td>Benign hyperplasia</td>
<td>7/7†</td>
<td>+/+</td>
<td>7/7</td>
<td>+/+</td>
</tr>
<tr>
<td>Prostatic carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>4/5</td>
<td>++++</td>
<td>4/5</td>
<td>++++</td>
</tr>
<tr>
<td>Lymph node</td>
<td>4/4</td>
<td>++++</td>
<td>4/4</td>
<td>++++</td>
</tr>
</tbody>
</table>

*No. of positives cases/total no. of cases studied.
† = , very weak; +, weak; +++, moderate; ++++, strong.
#Epithelial cells.

however, the data suggest the existence of cell-specific effects that
modulate the functional activity of the GM-CSF receptor.

Tyrosine phosphorylation of p42 and p44 MAP kinases is an
early step in GM-CSF signal transduction.9,10,15-15,45 We found
that GM-CSF induced time- and dose-dependent tyrosine
phosphorylation of several proteins in the LNCaP cells and
induced tyrosine phosphorylation of the p42 and p44 MAP
kinases. Phosphorylation of proteins other than MAP kinase
was observed at GM-CSF concentrations of 0.1 nmol/L or less,
which is consistent with the presence of high-affinity GM-CSF
receptors in the LNCaP cells. On the other hand, phosphoryla-
tion of MAP kinase was triggered only at GM-CSF concentra-
tions of at least 3 nmol/L. Furthermore, we failed to observe
JAK2 phosphorylation in these cells. Because the LNCaP cells
express about 750 low-affinity sites that likely correspond to
excess of α subunits (in addition to approximately 150 high-
affinity sites), the data raise the intriguing possibility that excess
of α-subunit expression may modulate signaling through the
high-affinity receptor.

The immunohistochemical analysis of human prostatic tissue
using anti-α and -β antibodies confirmed the presence of the α
and the β subunits of the GM-CSF receptor in prostate tumors.
The lack of immunoreactivity or weak pattern of staining
observed in normal epithelial cells, contrasts with the substan-
tial immunoreactivity with anti-α and -β antibodies observed in
cases of benign prostatic hyperplasia. Although primary
tumors showed higher levels of expression compared with
benign prostatic hyperplasia, we observed a further increase in
the immunoreactivity of metastases to lymph node and bone
compared with that of primary tumors. These data are compat-
ible with increased expression of the GM-CSF receptor as the
disease progresses from localized tumors to the development of
metastatic disease.

Although the action of growth factors and their receptors in
normal prostate physiology and progression of prostate cancer
are not well understood, our results suggest that GM-CSF may
have a role in maintenance of function in the normal prostate, as
well as in prostate cancer progression. The presence of both
subunits of the GM-CSF receptor in the tumor cells indicate that
they express functional high-affinity GM-CSF receptors and
therefore this hematopoietic growth factor may have an effect
on prostate carcinoma cells, which have a proclivity to metasta-
size to bone. The increased expression of GM-CSF receptors in
prostatic hypertrophy and neoplastic prostate epithelium sug-
Suggest a relationship between prostatic epithelial cell growth and
GM-CSF.

REFERENCES

1. Gasson JC: Molecular physiology of granulocyte-macrophage
2. Gasson JC, Kaufman SE, Weisbart RH, Tonomaga M, Golde DW:
High-affinity binding of granulocyte-macrophage colony-stimulating
factor to normal and leukemic human myeloid cells. Proc Natl Acad Sci
USA 83:669, 1986
3. Park LS, Friend D, Gillis S, Urdal DL: Characterization of the cell
surface receptor for human granulocyte/macrophage colony-stimulating
4. Cannistra SA, Koenigsmann M, DiCarlo J, Groshek P, Griffin JD:
Differentiation-associated expression of two functionally distinct classes
of granulocyte-macrophage colony-stimulating factor receptors by
Molecular cloning of a second subunit of the receptor for human
granulocyte-macrophage colony-stimulating factor (GM-CSF):
Reconstitution of a high-affinity GM-CSF receptor. Proc Natl Acad Sci
USA 87:9655, 1990
6. Kitamura T, Sato N, Ari K, Miyajima A: Expression cloning of the
human IL-3 receptor cDNA reveals a shared beta subunit for the
7. Isfort I, Ihle JN: Multiple hematopoietic growth factors signal
8. Kanakura Y, Druker B, Cannistra SA, Furukawa Y, Torimoto Y,
Griffin JD: Signal transduction of the human granulocyte-macrophage
colony-stimulating factor and interleukin-3 receptors involves tyrosine
phosphorylation of a common set of cytoplasmic proteins. Blood
76:706, 1990
JD, Druker BJ: Granulocyte-macrophage colony-stimulating factor,
interleukin-3, and steel factor induce rapid tyrosine phosphorylation of
p42 and p44 MAP kinase. Blood 79:2880, 1992
10. Eder M, Griffin JD, Ernst TJ: The human granulocyte-
macrophage colony-stimulating factor receptor is capable of initiating
signal transduction in NIH3T3 cells. EMBO J 12:1647, 1993
Yazaki H, Hirai H: c-fps/fev protein-tyrosine kinase is implicated in a
signaling pathway triggered by granulocyte-macrophage colony-
stimulating factor and interleukin-3. EMBO J 12:1641, 1993
13. Gomez-Cambronero J, Huang CK, Gomez-Cambronero TM,
Waterman WH, Becker EL, Sha’ali RI: Granulocyte-macrophage
colony-stimulating factor-induced protein tyrosine phosphorylation of
microtubule-associated protein kinase in human neutrophils. Proc Natl
Acad Sci USA 89:7551, 1992
14. Raines MA, Golde DW, Daepour M, Nel AE: Granulocyte-
macrophage colony-stimulating factor activates microtubule-associated
protein 2 kinase in neutrophils via a tyrosine kinase-dependent pathway.
Blood 79:3350, 1992
15. Welham MJ, Duronio V, Sanghera JS, Pelech SL, Schrader JW:
Multiple hematopoietic growth factors stimulate activation of mitogen-
regulation of early response genes and cell proliferation through the
human granulocyte macrophage colony-stimulating factor receptor:
Selective activation of the c-fos promoter by genistein. Mol Biol Cell
4:983, 1993


Expression of Granulocyte-Macrophage Colony-Stimulating Factor Receptors in Human Prostate Cancer

Coralia I. Rivas, Juan Carlos Vera, Fernando Delgado-López, Mark L. Heaney, Victor H. Guaiquil, Rong H. Zhang, Howard I. Scher, Ilona I. Concha, Francisco Nualart, Carlos Cordon-Cardo and David W. Golde