Interleukin-6, Secreted by Human Ovarian Carcinoma Cells, Is a Potent Proangiogenic Cytokine

Monique B. Nilsson, Robert R. Langley, and Isaiah J. Fidler

Department of Cancer Biology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas

Abstract

Angiogenesis, a key rate-limiting step in the growth and dissemination of malignant tumors, is regulated by the balance between positive and negative effectors. Recent studies indicate that the pleiotropic cytokine interleukin-6 (IL-6) may contribute to the vascularization of some tumors by disrupting the equilibrium between positive and negative angiogenic regulatory molecules. We determined whether IL-6 participates in the angiogenesis observed during the progression of ovarian carcinoma. We measured IL-6 production by human ovarian cancer cell lines in vitro and in vivo. Not all cell lines secreted IL-6 in vitro; however, when the cell lines were implanted into the peritoneal cavity of female nude mice, every line secreted IL-6. Most human ovarian carcinoma cell lines tested secreted significant levels of the soluble IL-6 receptor (sIL-6R). Endothelial cell lines established from the ovary and mesentery of female nude mice were tested for response to IL-6. Both endothelial cell lines expressed the IL-6R and their stimulation with the exogenous ligand significantly enhanced cell migration and activated the downstream signaling molecule signal transducers and activators of transcription 3. Dual immunohistochemical staining for IL-6R and CD31 revealed IL-6R expression on human endothelial cells within normal ovary and carcinoma specimens. Gelfoam sponges containing 0.4% agarose and IL-6 or basic fibroblast growth factor and implanted into the subcutis of BALB/c mice were vascularized to the same extent. Collectively, the data indicate that ovarian tumor cells secreted IL-6, a highly angiogenic cytokine that supports progression of disease. (Cancer Res 2005; 65(23): 10794-800)

Introduction

The progressive growth and dissemination of solid tumors is dependent on the process of angiogenesis (1, 2), which is regulated by the equilibrium between proangiogenic and antiangiogenic molecules (3). An excess of proangiogenic regulators can lead to the activation of quiescent microvascular endothelial cells causing them to elaborate proteases, migrate toward the stimuli, undergo cell division, and form tube structures (4). The vascular structure these form brings nutrients and oxygen to the developing tumor. Understanding the cellular and molecular mechanisms that regulate angiogenesis in different organs is, therefore, essential for developing effective therapeutic interventions.

Requests for reprints: Isaiah J. Fidler, Department of Cancer Biology, The University of Texas M.D. Anderson Cancer Center, Unit 173, P.O. Box 301429, 1515 Holcombe Boulevard, Houston, TX 77230-1429. Phone: 713-792-8580; Fax: 713-792-8747; E-mail: iidler@mdanderson.org.

Materials and Methods

Mice and tumor cell lines. Female BALB/c and BALB/c athymic nude mice (Ncr-nu) were purchased from the National Cancer Institute-Frederick Cancer Research and Development Center (Frederick, MD). Female H-2Kb-tsA58 mice were purchased from Charles River Laboratories.
Establishment of ovarian, mesenteric, and dermal microvascular endothelial cells. Tissue-specific endothelial cells were established as previously described (21). In brief, tissues (ovary, mesentery, and skin) were harvested from H-2Kb-tsA58 female mice and subjected to mechanical and enzymatic (0.2% type IV collagenease, Sigma, St. Louis, MO) digestion. Tissue digests were resuspended in 10% FBS/DMEM containing 10 units/mL of IFN-γ (PharMingen, San Diego, CA), plated into 75 flasks, and incubated at 33°C in a mixture of 5% carbon dioxide and 95% oxygen. IFN-γ was added to augment the expression of the MHC H-2Kb class I promoter, which regulates the level of large T-antigen protein in H-2Kb-tsA58 mouse-derived cells (22). Cells were expanded and then prepared for flow cytometry by stimulating the primary cultures with 10 ng/mL of recombinant murine tumor necrosis factor-α (R&D Systems, Minneapolis, MN) for 5 hours and then labeling the endothelial cell fraction with 4 μg/mL of phycoerythrin-conjugated rat anti-mouse E-selectin monoclonal antibody (mAb) and 2 μg/mL FITC-conjugated rat anti-mouse VCAM-1 mAb (both from PharMingen). Cell staining was evaluated with a Beckman Epics Elite flow cytometer (Beckman Coulter, Miami, FL) equipped with an air-cooled argon ion laser. Dual positive cells were selected for expansion and then subjected to an additional sort, after which endothelial cell identity was confirmed by a rigorous characterization analysis as previously described (21). IFN-γ was removed from the supporting medium and endothelial cells were expanded by growing in a 33°C incubator. Before analysis, endothelial cells were transferred to a 37°C environment for a period of at least 72 hours at which time the presence of the SV40 large T antigen is no longer detectable by Western blot analysis.

Tumor cell expression of interleukin-6 and soluble interleukin-6 receptor. To evaluate the in vitro expression of IL-6 by human ovarian cancer cell lines, 2 × 10^5 cells were seeded into individual wells of a six-well plate. Following a 24-hour incubation, cells were washed with PBS, and 1 mL of 10% FBS/DMEM was added to each well. After 48 hours, tumor cell–conditioned medium was collected, centrifuged to pellet any detached cells, and tested for the presence of human IL-6 by ELISA (R&D Systems). To evaluate the expression of soluble IL-6R by human ovarian cancer cell lines, 1 × 10^6 cells were seeded into individual wells of a 24-well plate and incubated overnight. Cells were then washed with PBS and 0.3 mL of 10% FBS/DMEM was added to each well. After 72 hours, tumor cell–conditioned medium was collected, centrifuged to pellet any detached cells, and tested for the presence of the soluble IL-6R by ELISA (R&D Systems).

To evaluate tumor cell production of IL-6 in vivo, 1 × 10^5 Hey-A8 and 1 × 10^5 SKOV3.ip1 cells were injected into the peritoneal cavity of female nude mice. After 28 days, the mice were killed, and the tumors were harvested and then evaluated using immunohistochemical, ELISA, or Western blot analysis. Tumor sections intended for immunohistochemical evaluation were embedded in optimal cutting temperature medium (OCT; Miles, Inc., Elkhart, IN) and 8 μm sections were mounted on positively charged slides (Fisher Scientific, Houston, TX). Sections were fixed by immersing slides in three acetone preparations for a period of 5 minutes each. Endogenous peroxidase was quenched by incubating sections in 0.3% hydrogen peroxide in PBS for 20 minutes followed by washing in PBS. Samples were blocked in 5% horse serum and 1% goat serum for 20 minutes at room temperature and incubated for 18 hours at 4°C with a mAb directed against human IL-6 (BioSource International, Inc., Camarillo, CA). Samples were washed in PBS and incubated with a peroxidase-conjugated goat anti-mouse antibody (Jackson Research Laboratories, Bar Harbor, ME); a positive reaction was visualized by incubating sections for 15 minutes with 3,3′-diaminobenzidine. Controls consisted of sections incubated with only secondary antibody. Sections were counterstained with Gill's hematoxylin and mounted using Universal Mount (Research Genetics, Huntsville, AL). Additional tumors, as well as normal mouse ovary and peritoneal tissue, were incubated in protein lysis buffer [20 mmol/L Tris-HCl (pH 8.0), 137 mmol/L sodium chloride, 10% glycerol, 2 mmol/L EDTA, 1 mmol/L phenylmethylsulfonyl fluoride, 1% aprotinin, 20 μmol/L leupeptin, and 0.15 units/mL aprotinin] on ice for 2 hours with frequent agitation. Lysates were cleared by centrifugation and protein content was quantified spectrophotometrically. Tumor protein (80 μg) resolved in 10% SDS-PAGE under reducing conditions was transferred to polyvinylidene difluoride membranes. Membranes were blocked with 5% (w/v) nonfat dried milk in 0.1% Tween 20 (Sigma) in PBS for 1 hour and then incubated overnight at 4°C with an anti-human IL-6 antibody (R&D Systems). Mouse anti-human IL-6 antibodies used did not cross-react with mouse IL-6 as confirmed by Western blot.

Immunodetection was done using the corresponding secondary horseradish peroxidase (HRP)-conjugated antibody. HRP activity was detected using enhanced chemiluminescence (ECL, Amersham Pharmacia, Piscataway, NJ). Some of the supernatants were also evaluated by ELISA (R&D Systems).

Detection of interleukin-6 receptor on endothelial cells. Murine endothelial cells isolated from the ovary, mesentery, and skin were seeded into individual chambers of a two-chambered slide at a density of 1 × 10^5 per chamber and incubated for 48 hours. Cells were fixed in acetone for 15 minutes, washed with PBS, and incubated in blocking solution for 20 minutes. The slides were incubated with a rabbit anti-mouse IL-6R antibody (Santa Cruz, Santa Cruz, CA) overnight at 4°C, rinsed thrice with PBS, and then incubated with an Alexa 594–conjugated goat anti-rabbit antibody (Molecular Probes, Eugene, OR). Control cells were incubated with only secondary antibodies. Immunofluorescence microscopy was done using a Zeiss Axiosplan fluorescence microscope (Carl Zeiss, Inc., Thornwood, NY) and images were captured using an air-cooled, charge-coupled device Hamamatsu C3810 camera (Hamamatsu Photonics K.K., Bridgewater, NJ) and Optimas software (Media Cybernetics, Silver Spring, MD). The expression of IL-6R by murine microvascular endothelial cells was also evaluated by Western blot analysis as described above. Protein (50 μg) was separated by SDS-PAGE and blotted onto nitrocellulose. To detect IL-6R, membranes were incubated overnight at 4°C with a 1:500 dilution of rabbit anti-mouse IL-6R antibodies (Santa Cruz) in blocking solution. BJAB cell lysates (Santa Cruz) served as a positive control. A 1:100 dilution of IL-6R blocking peptides (Santa Cruz) were used simultaneously with IL-6R antibodies to confirm the identity of the IL-6R band on the Western blot.

To evaluate expression of IL-6R on human endothelial cells, clinical specimens of ovarian carcinoma were embedded in OCT (Miles, Inc.) and 8 μm sections were mounted on positively charged slides (Fisher Scientific). Sections were fixed by immersing slides in three acetone preparations for a period of 5 minutes each. Sections were rinsed in PBS and incubated in blocking solution for 20 minutes. Slides were incubated for 18 hours at 4°C in a 1:20 dilution of mouse anti-human CD31 antibody (DAKO Corporation, Carpinteria, CA), rinsed thrice with PBS, and then incubated with mouse Alex a 488 secondary antibody (1:500, Molecular Probes). Slides were washed thrice with PBS and then incubated in a 1:25 dilution of rabbit anti-human IL-6 (Santa Cruz) overnight at 4°C. After being rinsed with PBS, slides were treated with a biotinylated antirabbit IgG antibody (Biocare Medical, Walnut Creek, CA) for 30 minutes followed by a 30-minute incubation with a 1:1,000 dilution of Alexa 594–conjugated streptavidin (Molecular Probes). Primary antibodies were omitted in antibody control sections. Immunofluorescence microscopy was done as described above. Endothelial cells were identified by red fluorescence and IL-6R was identified by green fluorescence.
fluctuate. Colocalization of endothelial cells and IL-6R (endothelial cells red + IL-6R green = yellow) was obtained by superimposing two images.

**Functional analysis of microvascular endothelial cell interleukin-6 receptor.** To evaluate the functional status of IL-6R on microvascular endothelial cells, ovarian and mesenteric endothelial cells were stimulated with 100 ng/mL recombinant IL-6 (Biosource International) for 5, 15, and 30 minutes. Cells were lysed and 40 μg of protein was separated by SDSPAGE. Rabbit anti-phospho-signal transducers and activators of transcription 3 (STAT3; Tyr705) antibodies (Cell Signaling, Beverly, MA), mouse anti-STAT3 antibodies (Cell Signaling), rabbit anti-phospho-mitogen-activated protein kinase (MAPK; Cell Signaling) antibodies, and rabbit anti-MAPK antibodies (Cell Signaling) along with appropriate secondary antibodies were used to detect STAT3, phosphorylated STAT3, mitogen-activated protein kinase (MAPK; Cell Signaling) antibodies, and phosphorylated MAPK.

**Cell migration.** To examine whether IL-6 induces endothelial cell migration, 24-well polycarbonate Transwell migration inserts (3.0 μm pore size; Fisher Scientific) were preincubated with serum-free DMEM containing various concentrations (50, 100, or 200 ng/mL) of IL-6 or basic fibroblast growth factor (bFGF) was added to the lower compartment. Tissue-specific endothelial cells (3.5 × 10^4) in 1% FBS/DMEM were then added to each of the upper chambers and incubated at 37°C for 20 hours. Cells in the upper compartment were removed mechanically by scraping. Cells that migrated to the underside of the membrane were stained and counted under a low-power objective (×40). All assays were done in triplicate. Experiments were repeated thrice.

**In vivo angiogenesis assay.** Sterile gelfoam sponges (Pharmacia, Peapack, NJ) were cut into 5.5×5×7 mm sections and hydrated in PBS at 4°C for 24 hours. Excess PBS was removed from inserts by blotting onto sterile filter paper. A solution of 0.4% agarose (100 μL) containing either PBS, bFGF (1 μg/mL), IL-6 (1 μg/mL), or denatured IL-6 (1 μg/mL) was then added to each sponge. The sponges were incubated at room temperature for 1 hour and then implanted s.c. into BALB/c mice as previously described (23). Two weeks later, sponges were harvested, embedded in OCT solution (Miles), and frozen in liquid nitrogen. Ten-micrometer sections were transferred to positively charged slides.

Slides were fixed in cold acetone (5 minutes), acetone/chloroform (1:1, v/v, 5 minutes), and acetone (5 minutes); washed with PBS; and incubated for 20 minutes in blocking solution containing 5% normal horse serum and 1% normal goat serum in PBS. Samples were incubated for 18 hours at 4°C in a 1:1000 (v/v) dilution of rat monoclonal anti-mouse CD31 antibody (PharMingen) or anti-mouse VEGF receptor-1 (VEGFR-1) antibody (Chemicon, Temecula, CA). Slides were washed thrice with PBS and then incubated with Alexa 594–conjugated secondary antibody (Molecular Probes) for 1 hour at room temperature. The microvascular density of each insert was determined under immunofluorescence microscopy; we counted the number of structures labeling positive for CD31 in five random 0.159 mm² fields at a magnification of ×100 using Scion software (Scion Corporation, Frederick, MD).

**Statistical analysis.** Statistical analysis of results for cell migration and microvascular density was done by Student's t test (two-tailed).

**Results**

**Expression of interleukin-6 and soluble interleukin-6 receptor by ovarian tumor cells.** ELISA showed various levels of IL-6 expression among ovarian tumor cell lines growing in cell culture (Fig. 1A). The SKOV3.ip1, OVCAR3, and EG lines secreted high levels of IL-6 expression but the Hey-A8 cells produced minimal amounts. sIL-6R expression also varied among the cultured cell lines: SKOV3.ip1, Hey-A8, and OVCAR3-3 cells, but not EG cells, produced detectable levels (Fig. 1B). To determine whether the expression of IL-6 may be regulated by the tissue microenvironment, we implanted Hey-A8 and SKOV3.ip1 cells orthotopically into the peritoneal cavity of female mice. Unlike the case in vitro, both SKOV3.ip1 and Hey-A8 cells expressed IL-6 as determined by immunohistochemistry (Fig. 2A, Western blot (Fig. 2B), and ELISA (Fig. 2C). IL-6 was not detected in lysates prepared from normal mouse ovary and peritoneum, suggesting that the expression of IL-6 by tumor cells may be in response to factors present in the peritoneal microenvironment, such as hypoxia or cytokines produced by host cells. IL-6 has been shown to regulate VEGF expression in some cell lines (11). However, Hey-A8 cells expressed minimal levels of IL-6 and VEGF in vitro, and stimulation of HEY-A8 cells with exogenous IL-6 did not increase VEGF expression. Furthermore, SKOV3.ip1 cells produce high levels of VEGF in vitro and in vivo. Incubation of SKOV3.ip1 cells with IL-6-neutralizing antibodies did not decrease VEGF production (data not shown), suggesting that in these two cell lines, VEGF production is independent of IL-6.

**Expression of interleukin-6 receptor on tissue-specific microvascular endothelial cells.** Both murine ovary and mesenteric-derived microvascular endothelial cells expressed the receptor for IL-6 as determined by immunohistochemical evaluation (Fig. 3A) and Western blot analysis (Fig. 3B). Dermal-derived microvascular endothelial cells also expressed the IL-6R. Lysates from a mouse B-cell lymphoma cell line (BJAB) served as a positive control. Interleukin-6 receptor is expressed on endothelial cells within normal ovary and human ovarian carcinoma specimens. To determine whether the observed IL-6R expression by murine endothelial cells is consistent with the phenotype of the vasculature within human ovarian carcinomas, we evaluated 25 human ovarian cancer specimens and three normal ovaries. Figure 3C illustrates an example of the immunohistochemical analysis. IL-6R was expressed on both tumor cells and tumor-associated endothelial cells (19 of 25 samples). IL-6R expression was also detected on endothelial cells from normal ovaries (2 of 3 samples).
Interleukin-6 initiates activation of signal transduction pathways in microvascular endothelial cells. IL-6 exerts its biological effects by binding the non-signal-transducing IL-6R, thus activating the signal-transducing receptor gp130. The formation of an IL-6, IL-6R, and gp130 hexamer results in the phosphorylation of downstream signaling molecules, such as signal transducers and activators of transcription 3 (STAT3), which then dimerizes, translocates to the nucleus, and functions as a transcription factor (24). To show that the IL-6R detected on endothelial cells is functional, endothelial cells were stimulated with 100 ng/mL of IL-6. The phosphorylation of downstream signaling molecules were then determined. Immunoblot analysis showed that IL-6 induced STAT3 phosphorylation in ovarian and mesentery endothelial cells (Fig. 4A and B). Phospho-STAT3 was detected as early as 5 minutes and for up to 30 minutes following the addition of IL-6 to the endothelial cells. Neutralizing antibodies against IL-6 or IL-6R

Figure 2. Human ovarian carcinoma cell lines express IL-6 in vivo. Although Hey-A8 cells secrete low levels of IL-6 in tissue culture conditions, both SKOV3.ip1 and Hey-A8 cells growing in the peritoneal cavity of female nude mice express IL-6 as determined by immunohistochemistry (A), Western blot analysis (B), and ELISA (C). Columns, mean; bars, SD.

Figure 3. IL-6R is expressed on endothelial cells (EC). Organ-specific murine endothelial cells (ovary, mesentery, and skin) express IL-6R as detected by immunohistochemistry (A) and Western blot analysis (B). C, fluorescent double-labeled immunohistochemistry of clinical human ovarian cancer specimens show IL-6R expression on tumor vasculature. Representative ×100 images show immunohistochemistry for CD31 (red), IL-6R (green), and the colocalization (yellow) of IL-6R on endothelial cells.
blocked IL-6-induced STAT3 phosphorylation (data not shown). In addition to signaling via STAT3, IL-6/IL-6R/gp130 interactions have been shown to induce MAPK phosphorylation (25). Our Western blot analysis also indicated that stimulation of endothelial cells with IL-6 (100 ng/mL) induced transient phosphorylation of extracellular signal-regulated kinase 1/2 (p42/22, MAPK; Fig. 4C and D). These data confirm that a functional IL-6R is expressed on endothelial cells.

Interleukin-6 induces migration of ovarian and mesentery microvascular endothelial cells. To examine the chemotactic effects of IL-6 on ovarian, mesentery, and dermal endothelial cells, we stimulated cells with exogenous protein and measured their migration in a Boyden chamber assay. As shown in Fig. 5, IL-6 significantly enhanced endothelial cell migration, reaching values essentially equivalent to that induced with bFGF. Denatured IL-6 had no effect on the migration of any cell line tested (data not shown).
Interleukin-6 induces angiogenesis in vivo. To investigate the proangiogenic activity of IL-6 under in vivo conditions, we used the gelfoam sponge assay (23) in which surgical hemostatic gelfoam sponges containing 0.4% agarose and PBS, IL-6, heat-denatured IL-6, or bFGF were implanted into BALB/c mice. Because our results showed that the effects of IL-6 on endothelial cells were not limited to the endothelial cells of the ovary and mesentery but also involved skin endothelial cells, and because the trauma of implanting sponges in the peritoneal cavity causes nonspecific angiogenesis, we implanted the sponges s.c. After 2 weeks, the gelfoam sponges were harvested. Vessel density was determined by staining with antibodies against CD31 and VEGFR-1. As shown in Fig. 6, the gelfoam sponges containing IL-6 had significantly ($P < 0.0001$) more CD31$^+$ and VEGFR-1$^+$ vessels than sponges containing PBS or denatured IL-6. The endothelial cells within the sponges were negative for VEGF-A (data not shown).

Discussion

Previous studies have shown that IL-6 can function as a regulator of ovarian tumor cell proliferation and migration (26, 27) and that IL-6 levels are significantly elevated in the serum of women with ovarian cancer and hence associated with poor prognosis (9, 10). We wished to determine whether IL-6 facilitates progression of this disease by stimulating angiogenesis. The results reported here provide new information regarding the role of IL-6 expression in ovarian carcinoma progression. First, ovarian tumor cells secreted significant quantities of IL-6 and sIL-6R. Second, both murine and human tumor–associated microvascular endothelial cells residing in the peritoneum expressed IL-6R. Third, the microvascular endothelial cell IL-6R was functional and stimulation with IL-6-activated STAT3 and MAPK signaling and induced endothelial cell migration. Fourth, IL-6 was a potent inducer of the formation of new blood vessels in vivo.

All but one of the malignant ovarian tumor cell lines under study secreted measurable levels of sIL-6R, which is capable of binding its ligand and mediating signal transduction on cells that do not express membrane-bound IL-6R. This process, called trans-signaling, occurs when IL-6/sIL-6R complex binds the membrane-bound signal transducer gp130, which is ubiquitously expressed (28). Therefore, the soluble form of the IL-6R not only potentiates the effects of secreted IL-6 but also widens the range of cells affected by this cytokine. The observation that sIL-6R is expressed by ovarian tumor cells warrants further investigation to determine the significance to cancer progression.

Previous studies have indicated that endothelial cells lack receptors for IL-6 (16). However, we found that endothelial cells derived from organs relevant to the progression of ovarian carcinoma expressed IL-6R. This observation was consistent with our findings that the IL-6R is expressed on endothelial cells within clinical specimens of human ovarian carcinomas as well as on endothelial cells of the normal human ovary. Additionally, treatment of endothelial cells with IL-6 activated STAT3 and MAPK, signal transduction molecules known to regulate cellular processes, including proliferation and migration. Although we did not detect any significant increase in endothelial cell proliferation or VEGF production as a result of IL-6 stimulation, we did observe

$^1$ Unpublished data.
that IL-6 significantly enhances endothelial cell migration, a key step in the process of angiogenesis. We also observed that endothelial cells derived from the skin express IL-6R. Because IL-6 was capable of inducing a robust angiogenic response in the cutaneous microenvironment, it is possible that IL-6 also contributes to the vascularization of skin tumors. Our finding correlates with published reports that serum levels of IL-6 are elevated in patients with metastatic melanoma (29) and that overexpression of IL-6 in basal cell carcinoma is associated with enhanced angiogenesis and tumor growth (30). Additional evidence for the role of IL-6 in angiogenesis comes from a most recent report that a peptide specifically binding to the IL-6R can inhibit vessel formation and growth of tumors in the subcutis of severe combined immunodeficient mice (31).

The identification of an angiogenic function of IL-6 in ovarian carcinoma may have important implications for therapies designed to target the tumor vasculature. It has been well established that tumor cells are heterogeneous and can produce a wide variety of proangiogenic molecules. Previous studies have highlighted the importance of proangiogenic factors, such as platelet-derived growth factor (32), VEGF/VPF (33), and IL-8 (34) in ovarian carcinoma. Because tumor cells secrete a variety of proangiogenic molecules, therapies targeting IL-6 in addition to other proangiogenic factors will likely be useful in the treatment of women with ovarian carcinoma.

**Acknowledgments**

Received 2/22/2005; revised 8/10/2005; accepted 9/15/2005.

**Grant support:** Cancer Center Support Core grant CA16672 and Specialized Programs of Research Excellence in Ovarian Cancer grant CA93039 from the National Cancer Institute, NCI.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked ‘advertisement’ in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Rachel Tuan for technical assistance, Walter Pagel for critical editorial review, and Lola López for expert assistance with the preparation of the manuscript.

**References**


Interleukin-6, Secreted by Human Ovarian Carcinoma Cells, Is a Potent Proangiogenic Cytokine

Monique B. Nilsson, Robert R. Langley and Isaiah J. Fidler


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/65/23/10794

Cited articles
This article cites 34 articles, 13 of which you can access for free at:
http://cancerres.aacrjournals.org/content/65/23/10794.full#ref-list-1

Citing articles
This article has been cited by 39 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/65/23/10794.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.