Glucocorticoid-induced Cell Death Requires Autoinduction of Glucocorticoid Receptor Expression in Human Leukemic T Cells

Jyoti Ramdas, Wei Liu, and Jeffrey M. Harmon

Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814-4799

ABSTRACT

In contrast to the negative autoregulation of glucocorticoid receptor (GR) expression seen in most cells and tissues, GR expression is positively autoregulated in human leukemic T cells and in other cells sensitive to glucocorticoid-induced cell death. To determine whether positive autoregulation is a necessary component of glucocorticoid-induced cell death, a wild-type GR gene under the control of a tetracycline-regulated promoter was stably transfected into glucocorticoid-resistant cells lacking endogenous functional receptor. Transfectants grown in the presence of tetracycline contained about 15,000 receptors/cell, a value approximately equal to basal level GR expression in glucocorticoid-sensitive 6TG1.1 cells before steroid treatment. Under these conditions, dexamethasone had a minimal effect on cell growth, elicited little internucleosomal DNA fragmentation, and induced no cell cycle perturbation. In the absence of tetracycline, GR mRNA and protein expression increased 2–3-fold, and cells expressed 48,000 receptors, a level nearly equivalent to that present in 6TG1.1 cells after 18 h of autoinduction. Under these conditions, dexamethasone markedly inhibited cell growth, caused G1 arrest, and induced significant internucleosomal DNA fragmentation. These studies therefore suggest that basal level GR expression is inadequate to mediate glucocorticoid-induced apoptosis in glucocorticoid-sensitive T cells and that positive autoregulation is a necessary component of this process.

INTRODUCTION

The GR is a ubiquitously expressed ligand-dependent transcription factor that regulates the activities of a large number of genes. In general, studies that have examined the relationship between GR concentration and biological response have found a direct correlation between GR concentration and the magnitude of the response (1). In particular, the sensitivity of many lymphoid cell lines to glucocorticoid-induced growth arrest and apoptosis is directly related to intracellular receptor concentration (2–4). Bourgeois and Newby (3) showed that mouse WEHI-7 cells, which contain two functional copies of the GR gene, are more sensitive to steroid-induced cell death than cells that contain a single copy. Gehring et al. (2) showed a direct correlation between receptor binding activity and steroid-induced growth inhibition of different clones of S49 cells. More recently, Chapman et al. (4) demonstrated a correlation between steroid sensitivity and the level of GR expression in different clones of receptorless S49 cells stably transfected with the mouse GR gene. However, the minimum concentration of GR necessary to mediate an apoptotic response has not been established. In addition, although there is a strong correlation between GR concentration and steroid sensitivity when clonal cell lines are compared, studies that have attempted to correlate GR concentration with therapeutic response in the treatment of leukemias and lymphomas have proven contradictory (5–11). Thus, additional factors must contribute to determining the sensitivity of lymphoid cells to steroid-induced lymphocytolysis.

We have previously shown that in contrast to the negative autoregulation of GR expression seen in most cells and tissues, the hGR is positively autoregulated in glucocorticoid-sensitive human leukemic 6TG1.1 cells (12). In response to glucocorticoid treatment, hGR mRNA and protein increase several-fold during the first 24 h of steroid treatment. These increases appear to be the result of increased transcription of the hGR gene and precede the earliest evidence of glucocorticoid-induced growth arrest and cell death (13). Positive autoregulation of GR expression has also been confirmed in other clonal cell lines derived from human leukemic CEM cells (14, 15) as well as glucocorticoid-sensitive mouse S49 cells (16). In addition, of two human myeloma cell lines established from the same patient, the hGR was positively autoregulated in the cell line that was glucocorticoid sensitive, but not in the one that was glucocorticoid resistant (17). Thus, positive autoregulation of GR expression may be a necessary component of glucocorticoid-induced growth arrest and cell death.

To investigate the role of positive autoregulation of hGR expression in steroid-induced growth arrest and cell death, we have introduced a functional copy of the hGR gene under the control of a tetracycline-regulated promoter into glucocorticoid-resistant ICR27TK.3 cells. ICR27TK.3 cells are ΔGR/GR753F and were derived from the glucocorticoid-sensitive cell line 6TG1.1, whose genotype is GR+/GR753F (18). The mutant hGR present in these cells is activation deficient; although it can repress activator protein 1 activity, it cannot induce transcription from the mouse mammary tumor virus promoter or mediate steroid-induced growth arrest or cell death (19, 20). Using a stably transfected cell line that expresses a functional hGR gene whose expression is regulated by the concentration of tetracycline in the growth medium, we show that steroid treatment of transfected cells that express a concentration of GR equivalent to that present in 6TG1.1 cells before steroid treatment elicits only a small decrease in cell growth and induces little cell death. In contrast, steroid treatment of transfected cells that express a concentration of GR greater than that present in 6TG1.1 cells after steroid treatment results in increased growth arrest and substantial apoptosis, suggesting that basal levels of hGR expression are inadequate to mediate an apoptotic response and that positive autoregulation of hGR expression may provide a mechanism to amplify the hormonal signal in cells programmed to die in response to steroid treatment.

MATERIALS AND METHODS

Plasmids. Plasmid pZeoSV was obtained from Invitrogen (Carlsbad, CA). pZeo was constructed by removing the 622-bp BamHI/ClaI fragment of pZeoSV containing the SV40 promoter and polyA addition signal sequence and replacing it with a linker sequence containing XhoI and PvuII sites. Plasmids pUHD10-3 and pUHD15-1 neo were generous gifts of Drs. Hermann Bujard (Zentrum fur Molekulare Biologie der Universitat Heidelberg, Heidelberg, Germany) and Steven Reed (Scripps Research Institute, La Jolla, CA) (21, 22).

Received 11/2/98; accepted 1/21/99.

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.

1 Supported by USPHS Grant CA22226 awarded by the National Cancer Institute to J. M. H. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences.

2 Present address: Laboratory of Cellular Biology, National Institute on Deafness and Other Communication Disorders, NHI, Rockville, MD 20850.

3 To whom requests for reprints should be addressed, at Department of Pharmacology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814-4799. Phone: (301) 295-3248; Fax: (301) 295-3220; E-mail: jharmon@usuhs.mil.

4 The abbreviations used are: GR, glucocorticoid receptor; hGR, human GR; HA, influenza hemagglutinin; EMSA, electrophoretic mobility shift assay; RT-PCR, reverse transcription-PCR.

5 The abbreviations used are: GR, glucocorticoid receptor; hGR, human GR; HA, influenza hemagglutinin; EMSA, electrophoretic mobility shift assay; RT-PCR, reverse transcription-PCR.

6 Received 11/2/98; accepted 1/21/99.

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.

1 Supported by USPHS Grant CA22226 awarded by the National Cancer Institute to J. M. H. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences.

2 Present address: Laboratory of Cellular Biology, National Institute on Deafness and Other Communication Disorders, NHI, Rockville, MD 20850.

3 To whom requests for reprints should be addressed, at Department of Pharmacology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814-4799. Phone: (301) 295-3248; Fax: (301) 295-3220; E-mail: jharmon@usuhs.mil.

4 The abbreviations used are: GR, glucocorticoid receptor; hGR, human GR; HA, influenza hemagglutinin; EMSA, electrophoretic mobility shift assay; RT-PCR, reverse transcription-PCR.
buffer (pH 8.3) containing 5 mM MgCl₂, 50 mM KCl, 1 mM of each deoxyribonucleotide triphosphate, 2.5 μM random hexamers, 1 unit of RNase inhibitor, 2.5 units of murine leukemia virus reverse transcriptase, and 1 μg of total RNA. The reaction was incubated at room temperature for 10 min, at 42°C for 15 min, at 99°C for 5 min, and at 5°C for 5 min in a Perkin-Elmer Thermal Cycler model 480. Amplification was performed by adding MgCl₂ to a final concentration of 2 mM and adding sufficient buffer to maintain a concentration of 50 mM KCl. Primers A (5′-TCGAAGATAAGCCTCACATAGC-3′), B (5′-TCCAGGAACTCTGCTGACATA-3′), and C (5′-ATGAGACGCT-GTTTGTTCACGCC-3′) were added to a final concentration of 0.15 μM, and AmpliTaq DNA Polymerase (2.5 units; Perkin-Elmer Corp.) was added to initiate the reaction. After denaturation at 95°C for 3 min and 30 s, amplification was accomplished by 35 cycles of incubation at 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min, followed by a final extension at 72°C for 7 min. PCR products were purified using the QIAquick-spin column (Qiagen, Valencia, CA), and 200 ng of each product were electrophoresed at 6 V/cm in a 1.5% agarose gel in 1× Tris-borate EDTA containing 0.5 μg/ml ethidium bromide. To quantify the amount of PCR product, DNA was visualized with a UV transilluminator and photographed using Polaroid type 55 film. Negatives were scanned with a LKB Ultrascan laser densitometer.

**Analysis of hGR Protein.** The binding of [3H]dexamethasone to intact cells was performed as described previously (24). For immunoblotting, total cell protein was extracted and solubilized as described previously (19). Protein (50 μg) was electrophoresed in an 8% SDS-polyacrylamide gel and transferred to a 0.45 μM Protran nitrocellulose membrane (Schleicher and Schuell, Keene, NH), and the filter was incubated overnight at 4°C in TTBS buffer [20 mM Tris-HCl (pH 7.6) containing 137 mM NaCl and 0.1% Tween-20] containing 5% nonfat milk. The filter was washed twice at room temperature for 15 min in 100 mM of TTBS, incubated for 2 h at room temperature in TTBS containing 1:1000 dilution of anti-hGR antibody 710 (18) or anti-HA epitope antibody pZeotetGRHA was constructed by replacing the 1-kb SalI/ClaI fragment of the corresponding fragment of pShGrHR17A1 and inserting the 3456-bp XhoIV/PvuII fragment of the resulting plasmid into XhoIV/PvuII-digested pZeo.

**Cell Culture and Transfection.** The glucocorticoid-sensitive T-cell line 6TG1.1 and its glucocorticoid-resistant derivative, ICR27I2.3, have been described previously (24). The glucocorticoid-sensitive T-cell line was constructed in which the concentration of the receptor could be regulated in a hormone-independent manner. Accordingly, the tetO-tetR system was used to express HA-tagged hGR in glucocorticoid-resistant ICR27TK3.2 cells. The resistance of these cells to steroid-induced cell death is the consequence of a deletion of one hGR gene and a mutation in the other (L753F) that renders it activation-deficient and unable to mediate glucocorticoid-induced growth arrest or cell death.
Plasmid pUHD15-1 neo, which expresses the chimeric tTA transactivator protein, was transfected into ICR27TK.3 cells, and neomycin-resistant clones were screened for tTA expression by EMSA using the tetO sequence as a probe and by assaying β-galactosidase activity in cells that were transiently transfected with the tetracycline-responsive reporter plasmid pHUC16-3. Cells that expressed the highest level of tTA (as measured by EMSA) and in which β-galactosidase expression from a transiently transfected tTA-regulated, reporter plasmid was repressed by growth in medium containing tetracycline (data not shown) were then transfected with plasmid pZeotetGRHa17 that expresses the HA-tagged GR downstream of a heptameric repeat of the tetO sequence and a minimal eukaryotic promoter (pCMV(−1)) and also contains a gene conferring resistance to zeocin. Cells resistant to both Geneticin and zeocin were assayed for dexamethasone-induced growth inhibition in the absence or presence of tetracycline. The majority of drug-resistant clones showed greater steroid-induced growth inhibition in the absence of tetracycline. Several of these clones were assayed for hGR expression in the absence or presence of tetracycline, and clone CEMtetGRHA was chosen for further study.

The ability of tetracycline to regulate hGR mRNA expression in CEMtetGRHA cells was determined by RT-PCR (Fig. 2). Forward primers A and C, which are specific for the transfected HA-tagged hGR gene and the endogenous mutant GR753F gene, respectively, and reverse primer B, which hybridizes to both genes, were used to amplify mRNA transcribed from the endogenous mutant and transfected HA-tagged hGR genes. The 147-bp product from the endogenous mutant GR753F gene is present in samples prepared from both parental ICR27TK.3 and transfected CEMtetGRHA cells and serves as an internal control for the efficiency of the amplification. In contrast, the 204-bp product from the transfected gene is present only in CEMtetGRHA cells (Fig. 2B). More importantly, growth of CEMtetGRHA cells in 1 μg/ml tetracycline for 4 days resulted in a 50% reduction in the amount of mRNA from the transfected gene, demonstrating tetracycline-regulated expression of hGR mRNA in CEMtetGRHA cells.

To examine the regulation of hGR protein, extracts of total cell protein prepared from parental ICR27TK.3 and transfected CEMtetGRHA cells in 1 μg/ml tetracycline for 4 days resulted in a 50% reduction in the amount of mRNA from the transfected gene, demonstrating tetracycline-regulated expression of hGR mRNA in CEMtetGRHA cells.

Antibody HA.11 reacted exclusively with HA-tagged GR in CEMtetGRHA cells. In contrast, antibody HA.11 reacted exclusively with HA-tagged GR in CEMtetGRHA cells were analyzed by immunoblotting with anti-hGR antibody 710 or anti-HA antibody HA.11. The results (Fig. 3B) showed that antibody 710 reacts with the 98-kDa hGR protein present in the human B-cell line IM-9 that contains approximately 107 receptors/cell (27) and the glucocorticoid-sensitive T-cell line 6TG1.1, which, in the absence of steroid, expresses both the mutant L753F hGR protein and approximately 15,000–20,000 normal receptors/cell (18). Antibody 710 also reacted with the mutant hGR (L753F) protein present in both parental ICR27TK.3 and transfected CEMtetGRHA cells. The amount of immunoreactivity was essentially equal in extracts prepared from these two cell lines and was approximately half that present in extracts prepared from glucocorticoid-sensitive 6TG1.1 cells, consistent with the fact that the wild-type gene is deleted in these cells and indicating that there is no reaction of antibody 710 with the HA-tagged GR. In contrast, antibody HA.11 reacted exclusively with HA-tagged GR in glucocorticoid-induced T-cell apoptosis.
transfected CEMtetGRHA cells (Fig. 3C); there was no reaction with either mutant L753F or wild-type protein in ICR27TK.3 cells or in 6TG1.1 cells.

The addition of as little as 0.05 μg/ml tetracycline to the growth medium resulted in a 50–75% reduction in the amount of immuno-reactive HA-tagged hGR protein expression (Fig. 4). However, increasing the concentration of tetracycline did not elicit a further reduction in the amount of hGR protein expressed. Thus, regulation of hGR expression from the promoter that contains the heptameric repeat of the \( \text{tetO} \) sequence occurs over a very narrow range of drug concentration. In addition, even at 1 μg/ml tetracycline, it is not possible to fully repress hGR expression.

To measure the intracellular concentration of receptor, \([^{3}H]\)dexamethasone binding assays were performed on intact cells. Under the conditions of this assay, there is virtually no binding of \([^{3}H]\)dexamethasone to mutant L753F receptors (Ref. 28; data not shown). CEMtetGRHA cells grown in the absence of tetracycline expressed approximately 48,000 receptors/cell (Fig. 5). In contrast, cells grown in 1 μg/ml tetracycline expressed only 15,000 binding sites/cell (Fig. 5). This decrease was comparable to the decrease in immunoreactive protein seen by immunoblotting. More importantly, the concentration of active hGR in CEMtetGRHA cells grown in the presence and absence of tetracycline corresponds well with the amount of functional hGR present in glucocorticoid-sensitive 6TG1.1 cells before and 18 h after glucocorticoid treatment. Thus, tetracycline regulation of hGR expression in CEMtetGRHA cells is a suitable system for evaluation of the role of positive hGR autoregulation in glucocorticoid-induced growth arrest and cell death.

**Effect of Receptor Concentration on Glucocorticoid-induced Growth Arrest and Cell Death.** To ascertain the role of receptor concentration on glucocorticoid-induced growth arrest, CEMtetGRHA cells cultured in the absence or presence of 1 μg/ml tetracycline were grown for 4 days in the absence or presence of 1 μM dexamethasone. Determination of the cell number revealed that cells grown in the presence of tetracycline (15,000 receptors/cell) experienced a partial growth inhibition in response to steroid. This was greater than that seen after the treatment of steroid-resistant ICR27TK.3 cells but was substantially less that seen after steroid treatment of CEMtetGRHA cells grown in the absence of tetracycline (48,000 receptors/cell), which experienced a 75% reduction in growth (Fig. 6). Tetracycline itself had no effect on either hGR expression or cell growth (data not shown). Thus, there is a direct correlation between receptor concentration and the steroid-induced inhibition of cell growth.

The effect of receptor concentration on cell cycle arrest and cell death was investigated by flow cytometric analysis of cells cultured in the presence or absence of tetracycline and then grown for 48 h in the absence or presence of 1 μM dexamethasone. Growth of steroid-resistant ICR27TK.3 cells in 1 μM dexamethasone had no effect on...
cell cycle distribution, and no apoptotic cells were detected, as evidenced by the absence of subdiploid nuclei (Fig. 7). Similarly, steroid treatment of CEMtetGRHA cells grown in the presence of tetracycline (a condition approximating the initial hGR concentration in steroid-sensitive 6TG1.1 cells) resulted in only a small decrease in the proportion of cells in S phase, with no substantial increase in the number of cells with a subdiploid amount of DNA. In contrast, steroid treatment of CEMtetGRHA cells grown in the absence of tetracycline (a condition approximating the concentration of hGR in 6TG1.1 cells after 24 h of steroid treatment) elicited both a decrease in the percentage of S-phase cells and a substantial number of apoptotic cells (Fig. 7). A comparable result was obtained when apoptosis was assessed by examining chromatin fragmentation; there was substantially more internucleosomal DNA fragmentation in steroid-treated cells grown in the absence of tetracycline than in cells cultured in the presence of tetracycline (Fig. 8).

When growth arrest and cell death were examined in steroid-sensitive 6TG1.1 cells in which the hGR is autoinduced (12, 13), the effects of steroid treatment were similar to those seen in CEMtetGRHA cells grown in the absence of tetracycline (Figs. 7 and 8); there was a substantial decrease in the percentage of S-phase cells, a marked increase in internucleosomal DNA fragmentation, and an increase in the percentage of dead cells with less than a G$_1$ content of DNA. Thus, glucocorticoid-induced growth arrest and cell death seem to require hGR concentrations comparable to those seen after receptor autoinduction.

**DISCUSSION**

In most cells and tissues, the GR is negatively autoregulated by both transcriptional and posttranscriptional mechanisms (29, 30). Such negative autoregulation is consistent with the negative feedback loops characteristic of many endocrine systems. However, we have previously shown that hGR mRNA and protein are positively autoregulated in glucocorticoid-sensitive 6TG1.1 cells at the level of transcription (13). Positive autoregulation has been observed in other cells programmed to die in response to steroids (16, 17, 31), suggesting that the increase in GR could provide a mechanism for amplifying the hormonal signal in cells destined to undergo apoptosis. To investigate the role of positive autoregulation in steroid-induced cell death, the hGR gene, under the control of a tetracycline-regulated promoter, was stably transfected into cells that lack functional GR. Glucocorticoid treatment of cells grown in the absence of tetracycline (induced) resulted in the accumulation of cells in the G$_1$ phase of the cell cycle, the inhibition of cell division, and increased chromatin fragmentation. In each case, the response was qualitatively similar to the response seen in 6TG1.1 cells in which expression of the hGR gene is controlled by its own promoter. Because untreated 6TG1.1 cells express approximately the same amount of hGR ligand binding activity (15,000 receptors/cell) as CEMtetGRHA cells grown in the presence of tetracycline (24) and because the 2–3-fold increase in hGR protein and ligand binding activity seen after the removal of tetracycline is comparable to the 3–4-fold increase in hGR protein seen after steroid treatment of autoinducible 6TG1.1 cells (12, 13), we conclude that autoinduction is an essential component of steroid-induced cell death in human leukemic 6TG1.1 cells.

One possible complication to the interpretation of these results is that in replacing the epitope used to generate anti-hGR antibody 710 with a trimer of the HA epitope, the activity of the GR was reduced, thereby requiring increased expression of the HA-tagged receptor to achieve growth arrest and cell death. Two factors suggest that this is not the case: (a) the region of the GR that was replaced is downstream from the strong transactivating function ($\tau_1$) present in the NH$_2$-terminal domain of the hGR (32, 33); and (b) more importantly, transient transfection of the HA-tagged GR gene into COS-7 cells gave the same level of glucocorticoid induction of pMSG-CAT and the same level of glucocorticoid repression of the activator protein 1-responsive reporter plasmid –73 COL-CAT as did wild-type GR (data not shown).

Although positive autoregulation of GR expression is seen in several cell lines sensitive to glucocorticoid-induced cell death, it is not simply an *in vitro* phenomenon restricted to such cells. Positive autoregulation of GR expression is also seen in several regions of the brain, indicating that it is not a sufficient condition for steroid-induced cell death (34). There has been only limited characterization of the organization and regulation of the hGR promoter (35–39). Consequently, the mechanism(s) that underlies the differential autoregulation of hGR expression is unclear. However, differential autoregulation of the closely related androgen and mineralocorticoid receptors has also been observed (40–42), suggesting that differential tissuespecific autoregulation may be a feature common to other members of the nuclear receptor family.

Recently, it has been reported that glucocorticoid-resistant CEM-C1 cells can be rendered sensitive to glucocorticoid-induced...
cell death by overexpression of rat GR (43). However, we have previously shown that CEM-C1 cells expressing approximately the same basal level of GR as glucocorticoid-sensitive 6TG1.1 cells exhibit the same level of positive hGR autoregulation as their glucocorticoid-sensitive counterparts (13). In addition, it was shown that both CEM-C1 cells and murine SAK8 cells could be rendered stably sensitive to glucocorticoid-induced apoptosis by treatment with 5-aza-cytidine (44, 45), presumably as a result of the demethylation of glucocorticoid-regulated genes involved in the lytic response. Thus, it is probable that the relative sensitivity of various cells and cell lines to glucocorticoid-induced growth arrest and cell death reflects a balance between the amount of GR present and the sensitivity of the

Fig. 7. Effect of hGR concentration on dexamethasone-induced cell cycle arrest. Glucocorticoid-resistant ICR27TK.3 cells (A and B), CEMtet-GRHA cells grown in the presence (C and D) or absence (E and F) of 1 μg/ml tetracycline, or glucocorticoid-sensitive 6TG1.1 cells (G and H) were grown in the absence (A, C, E, and G) or presence (B, D, F, and H) of 1 μM dexamethasone for 48 h. Cells were stained with propidium iodide and analyzed by flow cytometry as described in “Materials and Methods.” The percentage of particles with a subdiploid content of DNA is indicated in each panel.
suggest that the synthesis of new hGR protein is also required. Thus, the requirement for new protein synthesis could reflect, in part, the requisite autoinduction of hGR expression. If this is the case, then there may be more than one component whose induction is required for steroid-induced cell death.

We have demonstrated that autoinduction of hGR expression is a necessary component of glucocorticoid-induced cell death in human leukemic CEM cells in culture. Studies examining the relationship between hGR concentration and clinical response in human leukemic cells obtained directly from patients have not found the same strong positive correlation between receptor concentration and response seen for estrogen receptors in breast cancer (5–11). However, these studies examined intracellular hGR concentration in the absence of hormone. Thus, if autoinduction of hGR expression is a necessary component of the therapeutic response, it is possible that the absence of a strong correlation between receptor concentration and clinical response is due to the measurement of basal levels rather than induced levels of hGR expression.

REFERENCES


Glucocorticoid-induced Cell Death Requires Autoinduction of Glucocorticoid Receptor Expression in Human Leukemic T Cells

Jyoti Ramdas, Wei Liu and Jeffrey M. Harmon


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/59/6/1378

Cited articles
This article cites 49 articles, 21 of which you can access for free at:
http://cancerres.aacrjournals.org/content/59/6/1378.full.html#ref-list-1

Citing articles
This article has been cited by 19 HighWire-hosted articles. Access the articles at:
/content/59/6/1378.full.html#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.