Monitoring the Response of Orthotopic Bladder Tumors to Granulocyte Macrophage Colony-Stimulating Factor Therapy Using the Prostate-Specific Antigen Gene as a Reporter

Qinghui Wu, Kesavan Esuvaranathan, and Ratha Mahendran
Department of Surgery, National University of Singapore, Singapore

ABSTRACT

Purpose: Although orthotopic animal models of cancer best reflect the disease in humans, a major drawback of these models is the inability to monitor tumor growth accurately. Our aims were to produce a bladder tumor cell line (MB49) that secreted human prostate-specific antigen (PSA), analyze the feasibility and accuracy of PSA as a biomarker for monitoring orthotopic bladder tumor growth, and evaluate the effectiveness of granulocyte macrophage colony-stimulating factor (GM-CSF) gene therapy using this model.

Experimental Design: PSA secretion was assessed after both s.c. and orthotopic implantation of MB49-PSA cells in C57BL/6 mice. PSA levels in mouse serum and urine samples were monitored at 2- to 3-day intervals by ELISA. Using the orthotopic model, mice with confirmed tumors were given liposome-mediated GM-CSF gene therapy twice a week for 3 weeks intravesically and PSA levels monitored.

Results: The MB49-PSA cells behaved similarly as the parental cell line and produced high levels of PSA both in vitro and in vivo. In the s.c. model, the level of PSA produced correlated with tumor volume (r = 0.96). In the orthotopic model, PSA could be detected in serum and urine on the fourth day after implantation. PSA levels over the treatment period indicated that tumor growth was inhibited by GM-CSF gene therapy. Up to 50% of the treated mice were cured. Cytokine array analysis revealed that GM-CSF gene therapy induced the production of other cytokines and chemokines.

Conclusions: MB49 cells modified to secrete PSA are a reliable method to evaluate therapeutic modalities for bladder cancer.

INTRODUCTION

In orthotopic animal models of bladder cancer, the tumor grows in its native site, and this resembles the natural development of the human disease. Although extensively used as a model for cancer therapy, it has major limitations. These include the reported variability in the implantation rate from 50% to 90% (1, 2) and the fact that it is hard to differentiate between an animal in which implantation has failed and one that is cured by therapy. Most noticeable changes in tumor-bearing mice, such as weight loss and palpable tumors, are signs of very late stage and large tumors. More importantly, with an orthotopic model it is difficult to continuously monitor tumor growth and assess response to treatment with time.

Both high-frequency ultrasound (3) and magnetic resonance imaging (4) have been evaluated for the monitoring of orthotopic bladder tumors. These techniques are time consuming, expensive, and operator dependent requiring specially trained staff, and it is still technically difficult to identify a small tumor. In addition, accurate quantification of real tumor burden is extremely difficult, because the tumor mass may contain a necrotic center or infiltrating immune cells, which would cause overestimation of tumor volume. Both luciferase and green fluorescent protein (GFP)-transfected cancer cells (5–8) have been produced. Although the modified tumor cells can be easily visualized, these techniques do not readily enable the quantitative measurement of tumors. Normally, surgical procedures are necessary to expose the bladder for fluorescence/luminescence detection. Urine cytology for shed GFP-modified tumor cells is even less successful at the detection of tumors than surgical exposure of the bladder combined with imaging techniques (8).

We modified the MB49 bladder tumor cell line to produce a secreted marker protein, the human prostate-specific antigen (PSA). PSA is a 34-kDa glycoprotein first characterized by Wang et al. (9). It is a serine protease that is secreted exclusively by human prostate epithelium with a serum half-life of 2 to 3 days (10) and can easily be detected by ELISA. Furthermore, animal models of prostate cancer using LNCaP human prostate tumor cells implanted in nude mice have shown a correlation between PSA secretion and tumor volume (11). Because mice do not produce PSA, any PSA detected would be released from the modified tumor cells. A secreted protein negates the need for surgical exposure of bladders for monitoring of the tumor growth; instead, serum or urinary levels of the marker protein may be monitored. The purpose of this study was to determine the feasibility of using PSA as a reporter for monitoring bladder tumor...

MATERIALS AND METHODS

Tumor Cell Line. The murine transitional cell carcinoma cell line MB49 was maintained in RPMI 1640 supplemented with 10% fetal bovine serum (HyClone, Logan, UT), 2 mmol/L L-glutamine, 50 units/mL penicillin, and 0.05 mg/mL streptomycin (Sigma Chemical Company, St. Louis, MO) at 37°C and 5% CO2.

Plasmid Vectors. GM-CSF cDNA was amplified by PCR using specific primers and then was inserted into a mammalian expression vector pBudCE4.1 (Invitrogen, Carlsbad, CA) behind the EF-1α promoter. The generated constructs was termed pBud-GMCSF and used for gene therapy (12). To establish the PSA-transfected cell line, a mammalian transfection vector pSecTag2/Hygro/PSA (Invitrogen), which contains a human PSA encoding sequence, and hygromycin resistant gene was used. Plasmid DNA was prepared using Qiagen Endofree Maxi kits (Qiagen GmbH, Hilden, Germany).

Transfection of MB49 Cells with Prostate-Specific Antigen Gene. The liposome-mediated transfection was performed according to our standard transfection protocol (12). In brief, 2 × 10⁶ MB49 cells were transfected using 2.5 μg of plasmid DNA, pSecTag2/Hygro/PSA, complexed with 20 μg of N-[1-(2,3-dioleoyloxy)propyl]-NNN-trimethylammoniummethyl sulfate (Roche Diagnostics, Mannheim, Germany) and 40 μg of methyl-β-cyclodextrin solubilized cholesterol (Sigma Chemical Company). Positive clones were selected by supplementing medium with 1 mg/mL hygromycin B (Invitrogen) followed by limiting dilution. Twenty resistant clones were expanded, and their culture medium was screened by ELISA for PSA production. One clone that secreted 97.2 ng PSA/mL/10⁵ cells was selected and termed MB49-PSA. All of the subsequent experiments were conducted with this cell line. No PSA was detected in the culture supernatant of the parental cell line.

Quantification of PSA Expression by ELISA. A Maxisorp 96-well microtiter plate (Nunc, Roskilde, Denmark) was incubated at 4°C overnight with 1 μg/mL of rabbit antihuman PSA antibody (US Biological, Swampscott, MA) in PBS (pH 7.4). The plate was then blocked with 300 μL of PBS containing 1% bovine serum albumin (Sigma Chemical Company) for 2 hours at room temperature then washed with PBS containing 0.05% Tween 20 (Sigma Chemical Company). Then 50 μL of PSA standards (Chemicon, Temecula, CA) or unknown serum/urine samples were added to the plates, which were incubated for 1 hour at room temperature. The plates were washed five times with PBS containing 0.05% Tween 20. Each well was incubated with a 100 μL of 2 μg/mL mouse anti-PSA monoclonal antibody conjugated with horseradish peroxidase (US Biological) diluted in assay diluent (0.5% BSA and 0.05% Tween 20 in PBS) for 1 hour. After washing five times with buffer, the plate was developed for 30 minutes in the dark using 100 μL Turbo TMB ELISA Substrate (Pierce Chemical Co., Rockford, IL). The reaction was stopped by the addition of 1 N H₂SO₄, and absorbance was measured using an ELISA reader at a wavelength of 450 nm.

In vitro Characterization of MB49-PSA Cells. Two × 10⁵ MB49-PSA and MB49 cells were plated on six-well plates. The cells were counted at 24, 48, and 72 hours after plating using trypan blue exclusion to compare the growth rate with parental cells. Flow cytometry analysis for cell surface markers, MHC class I, and B7.1 were done as described previously (12). Cell culture supernatants were collected to test for PSA production with time.

In vitro GM-CSF Transfection and Measurement of GM-CSF Production by ELISA. Two × 10⁵ MB49 and MB49-PSA cells were plated in six-well tissue culture plates 1 day before transfection. Transfection was performed with pBud-GMCSF (12). After 48 hours, the cell culture media were collected and frozen at −20°C. The cells were counted using trypan blue exclusion and GM-CSF was measured using an ELISA for murine GM-CSF (R&D Systems, Minneapolis, MN).

In vivo Implantation of MB49-PSA Cells. All of the animal studies were conducted according to our institutional guidelines at the National University of Singapore. In all of the experiments, 4 to 6-week-old female C57BL/6 mice were used. In the s.c. model, 5 × 10⁵ MB49 or MB49-PSA cells were injected s.c. into the right flank. Tumor volumes were estimated by caliper measurements weekly. The longest dimension (a) and the perpendicular width (b) were determined, and tumor volume (v) was calculated according to the formula: v = ab²/2 (13). At the same time blood samples were taken and serum PSA levels were determined.

In the orthotopic model, the tumor was implanted as described previously (12). In brief, electrocautery was used to induce point damage, and 1 × 10⁵ MB49-PSA cells were instilled into the bladder. Serum and urine samples were collected every other day from day 2. On day 16, mice were sacrificed, and the presence of tumors in the bladder was confirmed by histologic examination of paraffin-embedded tissues. Serial 6-μm sections were stained with H&E and examined under a light microscope. Two mice from an independent experiment were sacrificed on day 4 to correlate the presence of bladder tumors with the earliest detectable PSA measurements in serum.

Liposome-Mediated Experimental GM-CSF Gene Therapy. Mice with implanted tumors as confirmed by PSA levels were randomly divided into control and treatment groups and treated with intravesical instillation of either pBudCE4.1 or pBud-GMCSF plasmid DNA complexed with N-[1-(2,3-dioleoyloxy)propyl]-NNN-trimethylammoniummethyl sulfate and methyl-β-cyclodextrin solubilized cholesterol. The transfection mixture was prepared in the same manner as for in vitro transfection. The day of implantation was designated as day 0. Intravesical instillations were started on day 5 (after confirmation of the presence of a tumor by serum PSA measurement). Each agent was administered via a 24 G i.v. catheter twice a week in a volume of 0.1 mL for 3 consecutive weeks. Urine and blood from the tail vein were collected every other day from day 4 onwards. The effect of gene therapy on the growth of the tumors was evaluated by serum PSA level measurements. GM-CSF levels were monitored in urine. On day 28, all of the surviving mice were sacrificed and their bladders and other organs examined.
**In vivo Production of GM-CSF and Other Cytokines After Transfection.** In a second set of experiments comparing GM-CSF gene therapy to control vector-treated mice, mouse spot urine samples were collected three times during the day from each mouse and pooled before analysis to give a better representation of urinary cytokine content. After the sixth instillation bladders were harvested, weighed, and homogenized [every 50 mg of tissue, was suspended in 1 mL of lysis buffer (150 mmol/L NaCl, 10 mmol/L Tris, and 1 mmol/L EDTA) with protease inhibitors]. The bladder homogenates were then centrifuged for 10 minutes at 10,000 rpm, and the supernatants collected. GM-CSF levels were determined using an ELISA (R&D systems). The expression of other cytokines and chemokines were examined using a mouse cytokine antibody array, Mouse Cytokine Array 2.1 (Raybiotech, Norcross, GA), according to the manufacturer’s instructions.

**Statistical Methods.** To determine the correlation between cell number and PSA concentration in the medium and the correlation between s.c. tumor volume and serum PSA levels, Pearson’s correlation coefficient was calculated. For analysis of the tumor cell growth rate, *in vitro* transfection and urine GM-CSF study, two-tailed Student’s *t* test, was performed. For the analysis of survival data, *χ*<sup>2</sup> test was used. *P* < 0.05 were considered statistically significant.

**RESULTS**

**In vitro and In vivo Comparison of MB49 and MB49-PSA Cells.** The *in vitro* morphology of MB49-PSA cells was identical with the parental MB49 cells. Their growth rate (Fig. 1A) was similar. Both cell lines expressed similar amounts of MHC class 1 (70.85 ± 2.75% and 66.35 ± 0.45% in MB49 and MB49-PSA, respectively) and B7.1 (1.95 ± 0.05% and 1.75 ± 0.45% in MB49 and MB49-PSA, respectively) surface receptors. The PSA levels in the culture supernatant increased as the cells proliferated (*r* = 0.95; Fig. 1B). After *in vitro* transfection with pBud-GMCSF, the MB49-PSA cells produced high levels of GM-CSF (26374 ± 255 pg/mL), which was comparable with that achieved by the parental cell line (21786 ± 102 pg/mL). *In vitro* GM-CSF transfection produced a growth-inhibitory effect on MB49-PSA tumor cells (data not shown) similar to that observed in the parental cell line (12). Finally, the *in vivo* growth rates of tumors produced by both cell lines were monitored. Twelve mice were injected s.c., 6 with 5 × 10<sup>5</sup> MB49-PSA cells and the other 6 with parental MB49 cells. Tumor volumes were measured once a week for a period of 4 weeks (Fig. 1C). The differences in tumor volume between the two groups were not significant (*P* > 0.05). Thus, PSA does not affect the tumorigenicity of the MB49 cells. When MB49-PSA cells were implanted orthotopically in mice, they developed gross hematuria 1 week after implantation and usually survived only up to 5 weeks without treatment (data not shown), a result similar to that obtained with parental MB49 implanted animals.

**PSA Secretion Corresponds to Tumor Growth in a Subcutaneous Tumor Model.** Six mice were injected s.c. with 5 × 10<sup>5</sup> MB49-PSA cells. In the s.c. model, tumor mass was usually detectable only 7 days after a period of treatment (data not shown), a result similar to that obtained with parental MB49 implanted animals. PSA secretion corresponds to tumor growth in a subcutaneous tumor model. Mice were monitored for a period of 4 weeks. Whereas tumor volumes and growth rates varied between mice, PSA levels were found to correspond closely to calculated tumor volume within individual mice. Statistical analysis showed a good correlation (*r* = 0.96) between serum PSA levels and tumor volume (Fig. 2C).

![Fig. 1](image-url)
To check whether PSA is a good indicator of tumor growth in an orthotopic model, we implanted 6 mice orthotopically with MB49-PSA. PSA levels in both urine and serum were monitored every other day from day 2. All 6 of the mice had measurable PSA levels. The experiment was terminated on day 16, and all of the mice bladders harvested for histologic analysis of the presence of tumors. PSA was detectable both in serum and in urine as early as day 4 after implantation and increased progressively (Fig. 3, A and B) indicating the growth of the tumors. By histology, big tumors always correlated with higher PSA levels in serum. On the basis of these results, day 4 serum PSA levels greater than basal serum values (background due to nonspecific binding) from normal mice +3 SD were considered representative of the presence of tumors.

In an effort to determine the minimal size of tumors that could be detected by this system, bladders were harvested from 2 mice in an independent experiment on the fourth day after implantation for histologic examination. Serum PSA levels were also measured in these mice. In both animals, small tumors measuring ~0.2 to 0.3 mm in diameter were successfully identified by histology at identical locations in the bladder (Fig. 3, C and D), and these were sufficient to produce measurable PSA.

When using metabolic cages to collect urine samples it was found that there was little variation in 12-hourly PSA production within a day. However, due to a lack of sufficient metabolic cages for large-scale experiments we measured spot urine samples, which were collected at several time points during the day. We found that single time point urinary PSA levels varied considerably even in the same animal within the same day. This could be due to the different amounts of urine produced, which, in turn, were affected by liquid intake of the mice. Moreover, the collection of urine is a problem when the general condition of the mice deteriorated as the tumors grow larger in size. Therefore, serum PSA levels were considered a preferable indicator of tumor growth in this setting and were used in all of the subsequent work.

Response of MB49-PSA Orthotopic Model to GM-CSF Gene Therapy. To assess the advantages of our monitoring system, we proceeded to treat mice with orthotopic MB49-PSA bladder tumors with GM-CSF gene therapy. A total of six instillations were given twice a week for 3 consecutive weeks. Surviving mice were sacrificed on day 28 for histologic examination. The experiment was repeated and the data combined (n = 6 control group and n = 19 in the treated group). The results showed that the both serum and urine PSA levels increased progressively in the control group. However, the PSA levels started to decrease in some mice after the second week in the treated group (Fig. 4). In the control group 67% (4 of 6) of mice died before the end of the experiment, whereas only 16% (3 of 19) died in the treated group. There was a significant survival advantage when compared with control group (P < 0.05). More than half of the treated mice (10 of 19) were cured as indicated by the serum PSA levels and confirmed by histology. This was also consistent with our previous GM-CSF treatment results with parental MB49 cells.

Monitoring Orthotopic Tumor Growth via PSA Levels in Urine/Serum. To check whether PSA is a good indicator of tumor growth in an orthotopic model, we implanted 6 mice orthotopically with MB49-PSA. PSA levels in both urine and serum were monitored every other day from day 2. All 6 of the mice had measurable PSA levels. The experiment was terminated on day 16, and all of the mice bladders harvested for histologic analysis of the presence of tumors. PSA was detectable both in serum and in urine as early as day 4 after implantation and increased progressively (Fig. 3, A and B) indicating
chemokine arrays. Mice treated with GM-CSF \((n = 9)\) that had undetectable levels of PSA, as well as those that produced detectable PSA, were terminated and their bladder tissues used to produce extracts to probe the arrays. In general there was an increase in macrophage inflammatory protein (MIP)-2, macrophage chemoattractant protein (MCP)-1, and tissue inhibitor of metalloproteinases (TIMP)-1, although a number of cured mice also had up-regulation of interleukin (IL)-12 (p40 and p70

---

**Fig. 3** PSA levels in mice with orthotopic bladder tumors were representative of the presence of tumors and could be detected as early as day 4. Six mice were given no treatment after initial tumor implantation and monitored for 2 weeks. (A) serum and (B) urine PSA levels were plotted with time. In an independent experiment, mice were sacrificed 4 days after implantation and their bladders removed, fixed, and sectioned for histologic examination. C and D, histologic sections of tumors on day 4 after tumor implantation and the corresponding serum PSA values. The hematoxylin and eosin staining revealed tiny tumors at the dome of the bladder where damage to the epithelium was caused by cautery. The tumors were superficial and their diameters were <0.3 mm (original magnification, ×100).

---

**Fig. 4** PSA levels in serum of mice with orthotopic tumors treated with GM-CSF or empty vector. The mice were given intravesical instillations of either pBudCE4.1 or pBud-GMCSF twice a week. PSA levels for (A) pBudCE4.1-treated and (B) GM-CSF–treated mice were plotted with time. PSA levels started to decrease in some mice after the second week in the GM-CSF–treated group. By day 28, 10 of 19 mice were found to be cured.
subunits), KC, and Eotaxin as well. The cytokine/chemokine profile of 1 GM-CSF-treated and cured mouse and 1 tumor-bearing control mouse is shown in Fig. 6. Densitometric analysis of the blots showed a 2-fold increase in MIP-2, MCP-1, and TIMP-1 in the GM-CSF-treated group. Using a mouse MCP-1 ELISA, we confirmed that there was a 3-fold increase in production after GM-CSF gene therapy. Both tumor-bearing and cured mice showed increased expression of RANTES and 6-chemokine.

DISCUSSION

We have shown that secreted PSA is an excellent reporter protein that enables the quick confirmation of the presence of tumors at a very early stage of the disease, namely 4 days after implantation. Histologic analysis revealed that at day 4, tumors were ~0.2 to 0.3 mm in diameter. A comparison of serum PSA levels at day 4, as determined by analysis of 33 mice, indicates that PSA levels of 1.298 ± 0.494 ng/mL (mean ± SD) correspond to the presence of tumors. It has been reported previously that using ultrasonography, tumors could be identified ~3 weeks after implantation (3), and usually this was a tumor >1.5 mm in diameter, whereas with magnetic resonance imaging it was initially reported that tumors could only be confirmed 14 days after implantation (4). However, with more recent advances in magnetic resonance imaging, tumors have been discerned 10 days after implantation corresponding to a tumor size of 1 mm (14). Using GFP-modified cells, tumors could be confirmed 7 days after implantation (7). Thus, tumor-secreted PSA seems to be the most sensitive indicator of the presence of tumor. This is advantageous, because it permits the administration of therapy at very early stage of the disease when the tumor is superficial, thus mimicking the clinical situation where most therapy occurs after removal of the tumor burden.

A number of genes have been used as reporters, and the best known are GFP and luciferase. GFP requires molecular oxygen to catalyze post-translational cyclization to produce fluorescence, and its expression is variable within tumor areas making it a less reliable marker for tumor growth (15). Luciferase does not require post-translational modification, but to produce luminescence it requires molecular oxygen and ATP at the time of activation of luciferin (15). In contrast, PSA secretion in serum in the s.c. model showed an excellent correlation with tumor volume. In our hands using the orthotopic model all of the animals with less than basal PSA +3 SD levels of PSA were confirmed by histology (6-μm sections) to not have tumors.

A study by Shih et al. (16) used β-human chorionic gonadotropin (β-hCG) as a reporter gene to assess tumors in living animals. They assessed the efficacy of β-hCG as a reporter by modifying several cell lines to express this gene and found that they had an excellent urinary monitoring system to confirm the presence of tumors, which had been implanted by s.c., i.p., intrasplenic, and i.v. injections in mice. However, they did not do intravesical implantations or serum measurements of β-hCG and, thus, it is difficult to compare and contrast the efficacy of β-hCG and PSA as reporter genes.

PSA secretion permits serial noninvasive monitoring of the tumor burden during treatment, rather than just at the termination of the experiment. This means that the requirement for large numbers of animals to be sacrificed at various time points, so that efficacy during treatment can be monitored, as well as the overall cost of experiments will be reduced. Also, animals where implantation has failed can be easily identified and re-
moved before initiation of therapy. In our experiment animals receiving GM-CSF therapy started to show a decrease in PSA levels after the third to fourth instillations. Future experiments will determine whether four instillations are sufficient for successful therapy.

Due to the association between PSA and prostatic disease, it has been analyzed as a possible antigen to stimulate immune responses to tumors bearing PSA. Dendritic cells transfected with mRNA for PSA have been found to be successful in inducing PSA-specific immunity (17). However, our results have shown that the MB49-PSA cells were as tumorigenic as the parental cells as determined by the kinetics of tumor growth in the s.c. and orthotopic models. Our data indicate that there is no PSA-specific immune response strong enough to affect the growth kinetics of the tumor. This may due to the secretary nature of PSA, which was used as a marker protein. Therefore, we concluded that MB49-PSA is an appropriate model to study immunotherapy for bladder cancer.

Successful transfection of urothelial cells (12) was confirmed by the measurement of urinary GM-CSF levels. To our knowledge, this is the first report that cytokines can be detected in mouse urine after intravesical gene delivery. GM-CSF gene therapy appears to act by inducing the production of other cytokines and chemokines, which is the actual aim of all cytokine gene therapy protocols. The sensitivity of this assay is 100 pg/mL for 6Ckine, RANTES, MIP-2, KC, Eotaxin, and IL-12 p70; 200 pg/mL for TIMP-1 and GM-CSF; and 10 pg/mL for MCP1 and IL-12 p40. Thus, although we could detect GM-CSF in urine by ELISA, it was not detectable by the array, which has a higher minimum threshold. The chemokines RANTES and 6Ckine were expressed by bladder tissue transfected with the pBudCE4.1 and pBudGM-CSF, whereas transfection with the pBudGM-CSF resulted in increased expression of MCP-1, MIP-2, TIMP-1, and IL-12 in some samples.

The literature on the effect of RANTES, 6Ckine, and MIP-1 is controversial. Tumors producing high amounts of RANTES and 6Ckine have been shown to result in desensitization of spleen T cells and, thus, increased tumor growth (18). Increasing MIP-1 expression has also been correlated with high invasive bladder and breast tumors (19, 20). On the other hand transfection with RANTES, MIP-1α, and MIP-1 has been reported to induce antitumor immunity (21–23). The production of RANTES and 6Ckine in vector-treated bladder malignant cells may be the result of CpG sequences present in the plasmid DNA or a characteristic of the tumor cell line. It has been reported previously that feeding of mice with CpG oligodeoxynucleotides induces chemokine production in the gastric mucosa (24). If so, the inflammatory responses induced by plasmid DNA (vector alone) were not sufficient to induce antitumor activity, but GM-CSF was able to do so. This effect of CpG sequences has been shown to also result in decreased expression of genes carried on plasmid DNA (25) and, thus, this could be a contributory factor to the 50% efficacy of GM-CSF therapy. The increased expression of MIP-1α, MCP-1, and IL-12 by GM-CSF has been observed previously in skin transfected with GM-CSF DNA but TIMP1, a known inhibitor of tumor metastasis, was not reported previously to be up-regulated (26). This may be a tumor-specific effect of GM-CSF.

In summary, this reporter gene model will be useful for preclinical evaluation of new potential intravesical chemotherapy and immunotherapeutic agents as shown here.

REFERENCES


Monitoring the Response of Orthotopic Bladder Tumors to Granulocyte Macrophage Colony-Stimulating Factor Therapy Using the *Prostate-Specific Antigen* Gene as a Reporter

Qinghui Wu, Kesavan Esuvaranathan and Ratha Mahendran


Updated version
Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/10/20/6977

Cited articles
This article cites 24 articles, 9 of which you can access for free at: http://clincancerres.aacrjournals.org/content/10/20/6977.full.html#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at: /content/10/20/6977.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.