Serum Prostate Specific Antigen Levels in Mice Bearing Human Prostate LNCaP Tumors Are Determined by Tumor Volume and Endocrine and Growth Factors

Martin E. Gleaves, Jer-Tsong Hsieh, Hsi-Chin Wu, Andrew C. von Eschenbach, and Leland W. K. Chung

Urology Research Laboratory, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

ABSTRACT

The ability of prostate-specific antigen (PSA) to predict tumor volume and stage in patients with prostate cancer would be improved if factors regulating its production and clearance were better defined. A thorough understanding of the pharmacokinetics (regulation of production, metabolism, and excretion) of PSA has been precluded, however, by the absence of an in vivo animal model. The purposes of this study are to develop a murine model for evaluating PSA pharmacokinetics in vivo and to assess factors that influence PSA production in vitro. The human prostate cancer cell line LNCaP, was chosen because it is androgen sensitive and PSA positive. Although LNCaP cells are usually nontumorigenic when inoculated s.c. in athymic mice, coinoculation of 1 x 10^6 LNCaP cells with 1 x 10^6 human bone fibroblasts reliably produces PSA-secreting carcinomas. This LNCaP model provides accurate correlation between tumor volume and serum PSA levels (r = 0.94) and demonstrates that tumor volume and androgens are codeterminants of circulating PSA levels. Following castration, serum PSA levels decrease rapidly up to 8-fold and increase up to 20-fold following androgen supplementation, without detectable castration-induced tumor cell death or concomitant changes in tumor volume. Serum PSA levels increase 0.24 ng/ml/mm^3 of tumor, which is approximately 5-fold less than that estimated for humans. Most likely this reduced PSA index (PSA:tumor volume ratio) results from a 7-fold faster clearance of PSA in athymic mice than in humans; other than this, shorter half-life, PSA elimination in the murine model appears similar to that in human, with both following first-order kinetics characteristic of a two-compartment model. Interestingly, following prolonged growth (>21 days) in castrate hosts, LNCaP tumors are capable of adapting to an androgen-deprived environment whereby LNCaP tumors regain the ability to secrete PSA in amounts similar to the precastrate state.

In LNCaP cells, androgens increase PSA mRNA levels 4-fold in vivo and in vitro. PSA mRNA expression is also altered by various growth factors. Changes in PSA production induced by androgens and growth factors do not always parallel changes in LNCaP cell growth rate induced by these factors, suggesting that PSA production occurs independently of cell growth rate and may be influenced by various interrelated factors, including hormonal and stromal milieu. Observations from this murine model suggest that androgens and tumor volume are independent determinants of serum PSA levels and imply that decreases in circulating PSA following androgen therapy may not always reflect a corresponding reduction in tumor volume.

INTRODUCTION

PSA is a M, 34,000 species- and tissue-specific glycoprotein produced only by human prostatic epithelial cells. Since its isolation by Wang et al. (1) in 1979, clinical experience with PSA has helped to define its utility and limitations in the diagnosis and management of patients with prostate cancer. The role of PSA in following patients with prostate cancer after therapy (2, 3) and in the immunohistochemical confirmation of the prostatic origin of a poorly differentiated adenocarcinoma (4) is undisputed. However, its role in staging and screening of prostate cancer remains controversial (5, 6); clinical investigations indicate that serum PSA levels are roughly proportional to tumor volume and stage (7, 8) but wide variations exist in many patients with either localized disease or advanced metastatic disease (7, 9). Thus, except within broad ranges, serum PSA levels poorly predict total tumor volume in any given patient, presumably due to tumor heterogeneity with development of subpopulations of cells that variably produce the marker. Alternatively, variable PSA levels may be related to changes in tumor cell microenvironment or hormonal, GF, or extracellular matrix milieu. With the exception of tumor volume, little is known regarding factors that influence serum PSA levels; regulators of PSA production at the cellular and molecular level and routes of metabolism and excretion remain poorly defined.

Recent studies suggest that in vitro PSA production by the human prostate cancer cell line, LNCaP, may be regulated by androgens and various GFs (10, 11). Furthermore, serum PSA levels in patients with BPH are reversibly decreased by androgen therapy (12) and increased by androgen supplementation (13). However, controlled investigation of the regulation of PSA production and its pharmacokinetic parameters in vivo has been precluded because there are relatively few animal models available for study. Of the available human prostate cancer cell lines, including PC-3 (14), DU-145 (15), PC-82 (16), LNCaP (17), and HONDA (18), only the LNCaP cell line is androgen responsive, PSA secreting, and immortalized in vitro (17, 19). However, with few exceptions, the LNCaP cell line is generally considered nontumorigenic when inoculated s.c. in athymic mice (20).

Recently we have reliably induced LNCaP tumor growth in vivo by coinoculating LNCaP cells with nontumorigenic human bone fibroblasts (20). The tumors are histologically poorly differentiated carcinomas that secrete PSA. The s.c. location of these tumors permits rapid, accurate, and sequential tumor volume measurements. This model allows for the study of PSA production both in vitro and in vivo and provides a means to define the pharmacokinetic profile (regulation of production, metabolism, and excretion) of PSA and its relationship to tumor volume, serum androgen levels, and tumor cell microenvironment or metastatic site.

In this communication, we evaluated the relationship between tumor volume and PSA and whether this relationship is altered by androgen ablation or supplementation. This LNCaP tumor model provides accurate correlation between tumor volume and serum PSA levels (r = 0.94) and demonstrates that tumor volume and androgen are codeterminants of serum PSA levels. Serum PSA levels decrease up to 8-fold following castration and increase up to 20-fold following androgen supplementation without detectable cell death or concomitant changes in tumor volume. Interestingly, we observed that LNCaP tumors are capable of adapting to an androgen-deprived environment; following a prolonged period of growth (>21 days) in castrated...
hosts, LNCaP tumors regain their ability to secrete PSA in amounts similar to their precastrate state without androgen supplementation. Differential changes in PSA mRNA expression in vitro induced by androgens and various GFs suggest that LNCaP cell growth rate and PSA production are not correlated. These results suggest that serum PSA levels are likely influenced by numerous factors in addition to tumor volume, including tumor cell hormonal and stromal milieu.

MATERIALS AND METHODS

Cell Lines and Establishment of LNCaP Tumors. LNCaP cells, passage 29 of the original line developed by Horoszewicz et al. (17), were kindly supplied by Dr. Gary Miller (University of Colorado, Denver, CO) and grown in RPMI 1640 (Irvine Scientific, Santa Ana, CA) with 5% FBS. A human bone fibroblast cell line, MS, derived from a patient with an osteogenic sarcoma, was established by Dr. A. Y. Wang (The University of Texas M. D. Anderson Cancer Center). MS cells were maintained in T-medium (20) with 5% FBS and passages 35–40 were used.

Six- to 8-week-old male nude mice (BALB/c strain; Charles Rivers Laboratories, Wilmington, MA) were coinoculated s.c. with 1 x 10⁶ LNCaP and 1 x 10⁶ MS bone fibroblasts. Up to 5 x 10⁶ LNCaP and 2 x 10⁶ MS cells are nontumorigenic when inoculated s.c. alone. Cells were suspended in RPMI 1640 with 5% FBS prior to injection and 0.1 ml was inoculated using a 0.27-gauge needle. Tumors were measured twice weekly and their volumes were calculated using the formula L x W x H x 0.5236 (21).

To determine whether PSA is regulated by androgen in vivo, 10 animals bearing LNCaP/MS chimeric tumors were castrated and their serum PSA levels assessed. Tumor volume was followed for 4 weeks (see below). Castration was performed transabdominally under methoxy-fluran anesthesia (Pitman-Moore, Mundelein, IL). Five of the 10 castrated mice were treated with 3 mg/kg/day of TP (Sigma Chemical Co., St. Louis, MO) injected s.c. in 0.1 ml peanut oil for 10 days. TP therapy was started 8 days after castration in 2 mice and 26 days after castration in 3 mice. Another cohort of 3 intact males was treated by s.c. injection daily for 7 days with 0.3 mg/kg 17β-estradiol dipropionate suspended in 0.1 ml peanut oil. Serum PSA and tumor volume were measured over a 4-week observation period.

At sacrifice, sternotomy was performed to obtain serum for PSA analysis. Tumor specimens were subjected to various morphological and biochemical analyses (see below). Selected tumors were cut into 1–2-mm cubes and transplanted in male recipient mice.

Determination of Serum PSA Values. Once tumors became measurable blood samples for sequential PSA measurements were obtained. The dorsal tail vein was incised and a sample was collected using a 21-gauge needle. The absorbance of each well was measured by a Titertek Multiscan TCC/340 (Flow Laboratories, McLean, VA) at 560 nm. The dye was eluted with 100 μl of Sorensen's solution (9 mg of trisodium citrate in 305 ml of distilled H2O, 195 ml of 0.1 N HCl, and 500 ml of 90% ethanol). The absorbance of each well was measured by a Titertek Multiscan TCC/340 (Flow Laboratories, McLean, VA) at 560 nm. Control experiments demonstrated that absorbente is directly proportional to the number of cells in each well.

Northern analysis was used to determine the effects of androgens and GFs on PSA mRNA levels by LNCaP cells. LNCaP cells were plated in 150-mm tissue culture dishes (Falcon) in T-medium, 2% TCM, and 1% charcoal-stripped calf serum. When the cells were 70–80% confluent, the media were changed, cells were washed with PBS, and media were replaced with serum-free T-medium with 2% TCM and various concentrations of androgens or GFs, or 100 ng/ml of 4-hydroxyflutamide (see above). Medium was changed on day 2, and 2 days later cells were harvested and their RNA was isolated and subjected to Northern blot analysis as described below.

RNA Isolation and Northern Blot Analysis. Total cellular RNA was prepared from cultured LNCaP cells or LNCaP/MS chimeric tumors using the guanidinium thiocyanate extraction method (23). Typical yields of total cellular RNA were about 300 μg/200 mg tissue as quantified spectrophotometrically using 40 μg RNA/4500 unit. RNA was denatured in 50% formamide/18% formaldehyde at 55°C and fractionated by electrophoresis in a 0.9% denaturing formaldehyde agarose gel. Samples were transferred onto a Zetaprobe membrane (Bio-Rad) by a capillary method, and the membrane was then baked to 80°C. Following this, the membrane was prehybridized in the presence of 1 x NaCl, 10% dextran sulfate, 1% sodium dodecyl sulfate, and 200 μg/ml salmon sperm DNA for at least 2 h at 65°C. Hybridization was carried out at 65°C overnight with a random-primer-labeled probe. The complementary DNA probe for PSA was a kind gift from Dr. D. Tindall (Mayo Clinic, Rochester, MN) (24) and a complementary DNA probe for TRPM-2 was kindly donated by Dr. R. Buttyan (Columbia University, New York, NY) (25). Finally, the membrane was washed under high stringency conditions (0.5 x standard saline-citrate-1% sodium dodecyl sulfate at 65°C). Autoradiograms were prepared by exposing Kodak X-OMat AR film to the membrane at –80°C with intensifying screens.

RESULTS

Relationship between Tumor Volume and Serum PSA. Coincubation of LNCaP and MS cells s.c. in athymic mice induced tumor formation at approximately 60% of inoculation sites. The tumors first appeared 6–8 weeks postinoculation. More recently, we have been successful in transplanting established LNCaP/MS chimeric tumors from host to host, with an approximate 60% take rate. LNCaP/MS tumors do not form in female hosts or male hosts castrated at the time of inoculation.
Fig. 1 illustrates the relationship between serum PSA and tumor volume. Serum PSA levels increase proportionally to increases in tumor volume with a high degree of correlation ($r = 0.94$; Fig. 1). Serum PSA values ranged from 2.1 ng/ml in a mouse with a tumor volume of 14 mm$^3$ to 672 ng/ml in a mouse with a tumor volume of 2855 mm$^3$. In this model, serum PSA increases 1 ng/ml for every 4.2-mm$^3$ tumor. Conversely, the PSA index, or the change in serum PSA per unit change in tumor volume, is 0.24 ng/ml/mm$^3$.

Effect of Castration on Serum PSA. Castration of male mice bearing LNCaP tumors produced a rapid fall in serum PSA independent of changes in tumor volume (Fig. 2a). A similar but less dramatic and more variable effect was observed using s.c. 17/estradiol dipropionate administration (data not shown). This reduction in the PSA index began 12 h following castration and continued for 2 weeks postcastration after which PSA values stabilized (Fig. 2a). The PSA index in 3 mice gradually increased 21 days after castration without exogenous androgen administration.

During a 4-week postcastration period, no reduction in tumor volume occurred; several tumors remained unchanged in size, but most continued to grow slowly. Therefore, it appears that although initiation of LNCaP tumor growth by coinoculation with MS bone fibroblasts is androgen dependent (because LNCaP/MS tumors do not form in female or castrated male hosts), growth of established tumors occurs in an androgen-independent fashion. This observation corroborates an earlier report by Horoszewicz et al. (17). The decline in serum PSA following castration occurs independent of changes in tumor volume and results in a 3.4-fold reduction in PSA index from 0.24 ng/ml/mm$^3$ tumor to 0.07 ng/ml/mm$^3$ tumor (range, 0.013 to 0.09 ng/ml/mm$^3$) (Fig. 2b).

Effect of Testosterone Administration on Serum PSA in Castrate Hosts. Administration of 3 mg/kg TP daily in castrate male mice resulted in a marked and rapid rise in serum PSA levels (Fig. 3). Within 24 h of the first injection, PSA levels rose independent of tumor volume and increased up to 20-fold (0.8 ng/ml to 16 ng/ml) above castrate levels. Stimulation of PSA production by exogenous TP returned the PSA index to levels equal to or higher than the precastrate state (range, 0.24 to 0.82 ng/ml/mm$^3$, $n = 5$).

Determination of PSA Half-Life. PSA clearance was evaluated in 3 mice to calculate its serum half-life in this model. Using a log-linear regression model, the half-life of serum PSA was determined to be 11.7 ± 0.3 h (Fig 4). In all cases, the data fit a two-compartment model of first-order elimination kinetics, in which the plot of the natural log of PSA(t)/PSA(0) against time produced a straight line (Fig. 4b). PSA elimination is characteristic of a two-compartment model in which the initial, more rapid elimination (a) phase has a half-life of 6.4 h and the second, more gradual (b) phase has a half-life of 12.8 h.

Histology and Immunohistochemistry of LNCaP Tumors. Tumors from intact and castrate hosts were sectioned and examined for changes in histomorphology and PSA staining. No histomorphological differences were observed in tumors from intact and castrate hosts (Fig. 5, A and B). Specifically, minimal evidence of necrosis or apoptosis was observed in tumors from castrated males. To confirm that castration-related tumor cell death was not responsible for the decrease in serum PSA levels, Northern analysis for TRPM-2 expression was performed. Tumors from intact hosts or from castrated hosts removed on days 3, 4, and 8 following castration did not express TRPM-2, suggesting that cell death resulting from androgen ablation did not occur (data not shown). Immunohistochemical staining for PSA revealed reduced staining for PSA in tumors from castrated hosts compared to tumors from intact hosts (Fig. 5, C and D). Furthermore, tumors from castrate hosts that either received TP or were followed for longer than 21 days also stained strongly for PSA. Although immunohistochemical studies are not quantitative, these observations agree with the results of Northern blot analysis assessing PSA mRNA expression (see below).

Effects of Androgens on PSA mRNA Expression by LNCaP Tumors in Vivo. Tumors from intact and castrate hosts were biochemically analyzed to evaluate changes in PSA mRNA expression associated with castration. Total cellular RNA from LNCaP tumors was isolated (see above) and subjected to Northern blot analysis. PSA mRNA expression decreases 4-fold 3 days following castration and levels are restored to precastrate levels without exogenous TP administration (Fig. 6). However, 21 days following castration, PSA mRNA expression in some tumors returned to precastrate levels without exogenous TP, suggesting that escape from androgen-dependent PSA production has occurred. These data correlate with the gradual increase in serum PSA levels observed in castrate animals bearing LNCaP tumors when these tumors were maintained in castrated hosts for a prolonged period (Fig. 2a).

Effect of Androgens and Growth Factors on PSA Production in Vitro. Northern analysis was performed to assess the effect on PSA mRNA levels by androgens and GFs involved in prostate cancer growth and progression. As determined by densitometry, DHT increased PSA mRNA expression 3- to 4.4-fold in a concentration-dependent manner (Fig. 7a). Testosterone resulted in similar increased expression (data not shown). Surprisingly, the antiandrogen 4-hydroxyflutamide produced similar increases in PSA expression and did not block testosterone- or DHT-induced increases in PSA expression.

We observed that changes in PSA mRNA expression in LNCaP cells exposed to androgens or GFs are not always accompanied by corresponding changes in growth rate of LNCaP cells; androgens increase LNCaP cell growth up to 180% (20) and increase PSA mRNA expression 4-fold, while
DETERMINANTS OF SERUM PROSTATE SPECIFIC ANTIGEN LEVELS

Days Post-Castration

![Graph of PSA Index vs. Days Post-Castration]

Fig. 2. (a) Castration of 5 male mice bearing LNCaP tumors resulted in a rapid decrease in serum PSA levels independent of changes in tumor volume, beginning 12h postcastration and continuing for 2 weeks. (b) Castration reduces the PSA index 3.4-fold from 0.24 ng/ml/mm³ to 0.07 ng/ml/mm³. Data based on serum PSA measurements drawn 7 to 10 days postcastration.

Intact
PSA Index =
0.24 ng/ml/mm³

Castrate
PSA Index =
0.07 ng/ml/mm³

Tumor Volume (mm³)

Fig. 3. Administration of testosterone propionate (T) to castrate mice produced a marked and rapid rise in serum PSA levels. Up to 20-fold increase in the PSA index was observed, returning the PSA index back to levels equal to or higher than the precastrate state (0.24–0.82 ng/ml/mm³).

DISCUSSION

PSA is a serine protease produced exclusively by human prostatic epithelial, but not stromal, cells (19). This M, 34,000 glycoprotein is a single polypeptide chain of 240 amino acids that shows strong homology with human tissue kallikreins (26, 27). Normally PSA is secreted in high concentrations into the seminal fluids where it functions to liquefy the seminal coagulum. In patients who have prostate cancer, PSA has been studied extensively as a tissue-specific tumor marker, where its discovery has raised hope for early detection of prostate cancer at a potentially curable stage. Serial serum PSA measurements are the most specific and reliable indicator to monitor response to therapy, and to signal residual and recurrent disease (2, 3, 27). Preoperative PSA levels correlate statistically with capsular penetration, seminal vesicle invasion, and lymph node metastasis but are not sufficiently reliable to predict clinical stage (27). Because of considerable overlap of PSA concentrations in patients with early stage prostate cancer and BPH, it is difficult to select a cutoff value that would enable earlier detection of cancer. Furthermore, the wide range of PSA values in patients with the same clinical stage suggests that factors other than tumor volume alone determine final PSA levels.

An improved understanding of the pharmacokinetics (regulation of production, metabolism, and excretion) of PSA would help clinicians better to interpret PSA levels in individual patients. Circulating PSA levels represent a steady state between total PSA production and its rate of metabolism and excretion. Routes of excretion of PSA are unknown, and the consequences of liver and renal dysfunction on serum PSA levels remain undefined. However, factors that determine total PSA production are becoming better defined. For instance, prostate cancer volume is one of the most important tumor factors affecting the amount of PSA produced (7, 8, 28). However, prostatic adenocarcinomas are heterogeneous tumors with variable PSA production throughout the tumor mass depending on vascular supply, hormonal milieu, and grade. Variable PSA levels may result partially from decreased cellular PSA production by poorly differentiated tumors compared to normal, hyperplastic, or well differentiated tumors (29, 30). However, serum PSA levels may be higher on a g-for-g basis for cancer than BPH, despite lower cellular production, because a higher percentage of cancer cell PSA enters the circulation as a result of more distorted architecture and obstructed ducts. Furthermore, because it is difficult to accurately measure tumor volume, interpretation of the PSA index in patients with prostate cancer is impaired.

Several host factors independent of prostate cancer, including bFGF stimulates LNCaP cell growth 180% but decreases PSA mRNA expression by 50% (Fig. 7b). TGFβ inhibits LNCaP cell growth by 70% and yet increases PSA mRNA expression 180%. Taken together, these data suggest that PSA mRNA expression and growth are independent of one another and influenced by various factors such as androgens and GFs.
Previous reports have documented that PSA expression in vitro is stimulated by androgens (10, 11). However, this is the first report to document a similar increase in PSA mRNA expression and circulating protein levels in vivo independent of changes in tumor volume. Similarly, androgen ablation results in rapid and dramatic decreases in serum PSA levels independent of tumor volume. No evidence of cell death, as assessed by the absence of post-castration tumor volume reduction, histomorphological evidence of cell necrosis, and TRPM-2 expression on Northern analysis, was observed to account for the fall in serum PSA. The PSA index decreased 3.4-fold following castration from 0.24 ng/ml/mm³ of tumor to 0.07 ng/ml/mm³ tumor. The observation that the antiandrogen, 4-hydroxyflutamide, increases PSA expression in vitro similar to that produced by androgens contrasts with findings observed by Bilhartz et al. (10). LNCaP cells are known to have a structurally aberrant androgen receptor with a broad binding specificity which may result in abnormal responses to androgen receptor antagonists (35).

The absence of gross, histological, or biochemical evidence of castration-induced LNCaP cell death suggests that the initial decrease in PSA results from reduction in androgen-regulated PSA production. The gradual return to a normal PSA index in castrated mice suggests that a subpopulation of LNCaP cells adapts to an androgen-deprived environment, a phenomenon consistent with a process of progressive state selection (36) whereby different subpopulations within a heterogeneous tumor differentially respond when confronted by environmental change. Following castration, some LNCaP cells continue to produce PSA, while others variably decrease PSA expression on Northern analysis, which may result in abnormal responses to androgen receptor antagonists (35).

In addition to studying control of PSA by endocrine factors, we also investigated whether autocrine or paracrine GFs, implicated as possible mediators of androgen-induced growth and prostate cancer progression (37-40), affect PSA production. PSA mRNA expression was increased by TGF/β and decreased by bFGF. Changes in PSA expression did not parallel changes in LNCaP cell growth rate produced by these GFs, demonstrating that changes in PSA production do not simply reflect
DETERMINANTS OF SERUM PROSTATE SPECIFIC ANTIGEN LEVELS

changes in growth rate. Because we have not observed changes in the expression of TGFβ, TGFα, or bFGF in response to in vitro androgen stimulation, these observations suggest that PSA production and LNCaP cell growth rate are independent and that GFs do not mediate androgen-induced changes in PSA expression. Furthermore, differential PSA production in response to androgens, GFs, and extracellular matrix (10, 11) suggests that serum PSA levels are influenced by various factors such as hormonal and stromal milieu and imply that tumor cell PSA production may vary depending on its metastatic site.

Finally, we should address how the findings in this study can be extrapolated to the human situation. The major limitation of this study is the use of the immortalized cell line (LNCaP) to produce a relatively homogeneous and uniform PSA-producing carcinoma. This contrasts with the clinical situation where prostate cancers are heterogeneous, composed of cells with a spectrum of grade, hormonal responsiveness, and PSA produc-

Fig. 5. LNCaP tumor PSA staining is reversibly androgen sensitive. No histomorphological differences were visible in tumors from intact (A) and castrate (B) hosts. Specifically, areas of necrosis were similarly distributed in tumors from intact and castrate hosts. Immunohistological staining using anti-PSA antibodies revealed increased staining for PSA in tumors removed from intact hosts (C) compared to those from third day postcastrate hosts (D). Furthermore, tumors from castrate hosts treated with TP (E) and tumors from mice followed for more than 21 days postcastration (F) also stained strongly for PSA. H & E.

4 J. T. Hsieh, unpublished observations.
TP (Lane 6). By 3 weeks postcastration, however, PSA mRNA expression decreases 4-fold 3 days following castration (Lanes 4 and 5), or 17β-estradiol analysis to determine whether decreases in PSA protein production were accompanied by concomitant changes in PSA mRNA expression. PSA mRNA expression results to precastration levels with exogenous TP (Lane 6). By 3 weeks postcastration, however, PSA mRNA expression returns to precastration levels without TP (Lanes 8 and 9), suggesting that an escape from androgen-dependent PSA production has occurred.

DETERMINANTS OF SERUM PROSTATE SPECIFIC ANTIGEN LEVELS

ACKNOWLEDGMENTS

We acknowledge and thank C. Davis and D. Evans for their excellent secretarial and editorial assistance, respectively.

REFERENCES

DETERMINANTS OF SERUM PROSTATE SPECIFIC ANTIGEN LEVELS


Serum Prostate Specific Antigen Levels in Mice Bearing Human Prostate LNCaP Tumors Are Determined by Tumor Volume and Endocrine and Growth Factors

Martin E. Gleave, Jer-Tsong Hsieh, Hsi-Chin Wu, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/52/6/1598

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.