Suppression of Human Solid Tumor Growth in Mice by Intratumor and Systemic Inoculation of Histidine-Rich and pH-Dependent Host Defense—like Lytic Peptides

Arik Makovitzki, Avner Fink, and Yechiel Shai

Department of Biological Chemistry, The Weizmann Institute of Science, Rehovot, Israel

Abstract

Previously, we reported that intratumor or systemic inoculation of a cationic 15-mer, innate immunity-like lytic peptide composed of D- and L-amino acids ([D]-K6L9) caused growth arrest of 22RV1 prostate carcinoma xenografts in a mouse model. However, despite its therapeutic potential, this peptide has significant systemic toxicity at concentrations slightly higher than the therapeutic one. Here, we used the acidic environment created by solid tumors as a trigger to activate anticancer lytic peptides by making them cationic only at low pH levels. We achieved this selectivity by substituting lysines (pKa, ~10.5) for histidines (pKa, ~6.1) in the parental peptide [D]-K6L9. Histidine is protonated below pH 7. For that purpose, we replaced either three or all six lysines in the parental peptide with histidines to obtain the peptides [D]-K3H3L9 and [D]-H6L9. Interestingly, in vitro experiments showed pH-dependent activity only with [D]-H6L9 mainly toward cancer cell lines. However, both peptides showed reduced systemic toxicity compared with the parental peptide. Intratumor and systemic inoculation of these peptides resulted in a significant decrease in the 22RV1 prostate cancer tumor volume and systemic secretion of prostate-specific antigen in a xenograft mice model. Moreover, histologic modifications revealed a significant reduction in new blood vessels selectively in tumor tissues after treatment with the peptides compared with the untreated tumors. The lytic mode of action of these new peptides, which makes it difficult for the cancer cells to develop resistance, and their selective and pH-dependent activity make them potential candidates for treatment of solid cancer tumors.

Introduction

The development of an effective therapy for malignant diseases has been hindered by the lack of consistent differences between tumors and normal tissue. Thus, unlike antibiotic treatment of bacteria, it has been difficult to develop therapeutic strategies that have major anticancerous effects without having significant cytotoxicity. One major difference between many solid tumors and the surrounding normal tissue is the nutritional and metabolic environment (1, 2). The functional vasculature of tumors is often inadequate to supply the oxygen demands and nutritional needs of the rapidly proliferating population of cancerous cells. This in turn contributes to the development of anaerobic conditions, under which hydrolysis of ATP results in the production of lactic acid. Moreover, a poor and chaotic tumor vascularization leads to the inefficient washout of the acidic products and further contributes to the development of a chronically acidic extracellular environment (2, 3). The consequence of that phenomenon is that the pH of the extracellular space surrounding solid tumors is significantly lower than the pH of the surrounding normal tissues (1, 3–6). Therefore, in recent years, several attempts have been made to use the solid tumor acidity to develop new pH-dependent approaches to cancer therapy (7–10) or drug delivery (11–14) because this venue seems most promising.

A recent approach under study for cancer treatment is the use of cationic antimicrobial peptides (15–19). These peptides, which have significantly higher toxicity to bacteria than to normal mammalian cells, were shown to exhibit a broad spectrum of cytotoxic activity against cancer cells as well (17, 20, 21). In line with this, we reported that intratumor and systemic administration of a short 15-mer D,L-amino acid peptide ([D]-K6L9-KLLKK1LKKLKLKK-NH2, the underlined letters are D-amino acid) specifically inhibited the growth of primary human prostate carcinomas. The electrostatic attractions between the negatively charged components of the membrane of cancer cells and the positively charged peptide are believed to play a major role in the strong binding of the peptide and its ability to selectively disrupt the membrane of cancer cells (22, 23). However, similar to other membranolytic peptides, higher concentrations of this peptide could damage normal cells and therefore narrow the therapeutic index.

Here, we show the use of the acidic microenvironment created by solid tumors as a trigger to activate anticancer lytic peptides that otherwise are not active under physiologic conditions. For that purpose, we used [D]-K6L9 (22, 23) as a template and substituted either three lysines (pKa, ~10.5) or all of them for histidines (pKa, ~6.1), a change that should make the peptides positively charged and active primarily at acidic pH values.

Materials and Methods

Cell culture. The CL1 human prostate carcinoma cell line used is an androgen-independent subclone of LNCaP, which was generated by culturing adenocarcinoma LNCaP cells in charcoal-stripped serum, as previously described (24). The 22RV1 human prostate carcinoma cells are an androgen-independent subclone of the adenocarcinoma prostate adenocarcinoma CWR22 xenograft (25). The CL1 and 22RV1 [American Type Culture Collection (ATCC)] were grown in RPMI 1640 supplemented with 10% FCS (Biological Industries) and antibiotics. NIH 3T3 mouse fibroblast cell lines (ATCC) were grown in DMEM supplemented with 10% FCS.
fetal bovine serum (FBS), t-glutamine, and antibiotics (Biological Industries). Similarly, OL human foreskin fibroblasts (ATCC; a generous gift from Prof. Menachem Rubinstein, Department of Molecular Genetics, The Weizmann Institute of Science, Rehovot, Israel) and murine Lewis lung carcinoma (LLC) cell lines were maintained in DMEM supplemented with 10% FBS, t-glutamine, and antibiotics.

**Peptide synthesis and purification.** Peptides were synthesized by a 9-fluorenylmethoxycarbonyl solid-phase method on Rink amide MBHA resin (Calbiochem-Novabiochem) by using an ABI 4334A automatic peptide synthesizer (Applied Biosystems) followed by peptide cleavage from the resin and purification by reversed-phase high-performance liquid chromatography (>98%). The composition of the peptides was confirmed by electrospray mass spectroscopy and amino acid analysis.

**In vitro cytotoxicity assays.** Aliquots of medium containing 1 × 10⁴ cells (malignant: CL1, 22RV1, and LLC; nonmalignant: NIH 3T3 and OL) were distributed into a 96-well plate (BD Falcon). The following day, the media were replaced with 90 μL fresh culture medium adjusted to pH 7.4 or 5.5 and 10 μL of a solution buffer (adjusted to pH 7.4 or 5.5, respectively) containing different concentrations of the peptides. The plate was then incubated for 24 h before adding to each well 50 μL of 2.3-bis[2-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide inner salt (XTT) reaction solution (Biological Industries). Viability was determined as described previously (22, 23). The LC₅₀ (the concentration at which 50% of the cells die) for each peptide was obtained from the dose-dependent cell viability curves.

**Membrane permeability studies.** Vesicles composed of phosphatidylcholine, sphingomyelin, phosphatidylethanolamine, and cholesterol (4:3.5:4:3.5/1.03/1, w/w) and entrapped with calcein [60 μmol/L calcein, 10 μmol/L HEPES, and 150 μmol/L NaCl (pH 7.4)] were generated by using the extrusion method as described before (22). Peptides were then added to vesicle suspension (2 mL, 2.4 μmol/L liposomes) adjusted to pH 6 or 7.4. Peptide-induced calcein leakage resulted in an increase in fluorescence (λₑx = 485 nm; λₑm = 515 nm; ref. 26), which was monitored.

**Sytox Green uptake assay.** Aliquots of medium containing 1 × 10⁴ CL1 cells were distributed into a 96-well plate. The following day, the media were replaced with 90 μL fresh culture medium adjusted to pH 7.4 or 5.5 and 10 μL of a solution buffer (adjusted to pH 7.4 or 5.5, respectively) containing different concentrations of the peptides. Then, 100 μL Sytox Green (Molecular Probes) was added to a final concentration of 1 μmol/L. After 15 min, the cells were examined with a fluorescent microscope (Eclipse, Nikon) applied to a digital camera (C4742-95; Hamamatsu). Excitation was set at 485 nm and emission at 520 nm.

**Acute toxicity.** Acute toxicity was examined after i.v. injection of the peptides to five male ICR mice (Harlen Co.). Each mouse was injected with a 0.5-mL solution of freshly prepared peptide in Hartmann’s lactate solution (Teva Medical). The doses of peptides administered per mouse were 8, 15, 20, 25, and 30 mg/kg of body weight. Animals were directly inspected for adverse effects for 4 h, and mortality was monitored for 6 d.

**Inhibition of tumor growth in human prostate carcinoma xenografts.** The s.c. implantation of human prostate carcinoma in mice was done as described previously (27). Briefly, 0.1 mL of androgen-independent 22RV1 human prostate carcinoma cells (5 × 10⁴) in Matrigel (BD Biosciences) was inoculated s.c. into the dorsal side of 5- to 6-wk-old nude male mice with an average weight of 20 to 25 g (Harlen). One week after cell implantation, when the tumor diameter reached −5 mm (we defined this day as day 0), all the peptides or vehicles (Hartmann’s lactate solution; pH 6.5) were injected intratumorally (at 1 mg/kg; 100 μL) or systemically (at 9 mg/kg; 500 μL). This treatment was carried out three times a week (every second day) for a total of nine doses (n = 10 mice per group). Tumor size was measured by a caliper and recorded twice a week. At the end of the treatment, the mice were sacrificed and the tumors were removed, photographed, and weighed.

**Serum prostate-specific antigen levels.** At the end of the treatment, blood samples were collected to heparin-containing tubes and centrifuged, and the supernatants were stored at −20°C. The CanAg prostate-specific antigen (PSA) EIA kit (CanAg Diagnostic) was used to determine total PSA in the mice plasma (27). Tumor size and PSA levels, represented as mean ± SE, were analyzed by Student’s t test. P < 0.05 was considered as statistically significant.

**Histologic and immunofluorescence staining.** Excised tumors were fixed in 4% buffered formaldehyde. Paraffin-embedded 5-μm sections were stained with H&E and examined using a microscope (Eclipse E800M, Nikon) with a digital camera (DXM1200, Nikon). For immunofluorescence staining, the tumors were fixed with 4% buffered formaldehyde for CD34 epitope labeling (new blood vessels) or zinc fixative perfusion for CD31 epitope labeling (old blood vessels). Paraffin-embedded 5-μm tumor sections were overlaid with rat anti-mouse CD31 antibody (Pharmingen) or rat anti-mouse CD34 antibody (Cedarlane) against blood vessels. Sections were incubated with bridging biotinylated rabbit anti-rat antibodies (Vector Laboratories) and visualized with streptavidin-conjugated FITC (Jackson ImmunoResearch Laboratories). For quantitative analysis, capillaries, identified by positive staining for CD34 and CD31, were counted and their density was expressed as the percentage of capillaries of the total section area using Image-Pro Plus 4.1 software. To quantify the vessels, 10 nonnecrotic areas at 100× per field at ×200 were captured using an Olympus BX-40 microscope (Olympus).

**Results**

**In vitro activity of the peptides toward malignant and normal cells at acidic and physiologic pHs.** Using the template of [D]-K₆L₉ (22, 23), we synthesized two histidine-containing peptides: [D]-K₃H₃L₉ and [D]-H₆L₉ (Table 1). The LC₅₀ values of the peptides shown in Table 2 reveal that only [D]-H₆L₉ has pH-dependent activity. However, all three peptides showed selective activity toward cancer cells (Table 2). Note that the histidine-containing peptides are less active than the parental peptide [D]-K₆L₉. Nevertheless, the amount administered to mice is similar to that reported previously for [D]-K₆L₉.

**Membrane disruption induced by the peptides.** Peptides, at increasing concentrations, were added to a suspension of vesicles (lipid composition of cancer cells; refs. 28, 29) encapsulating calcein (2.5 μmol/L phospholipids) at pH 7.4 or 6. Membrane permeability was measured by monitoring the fluorescence recovery. The level of maximum leakage reached, as a function of the peptide-to-lipid molar ratio, is shown in Fig. 1A. [D]-K₆L₉ and [D]-H₆L₉ permeate vesicles at both pHs, whereas [D]-H₆L₉ was highly active only at pH 6.

**pH-dependent permeation of the cancer cell membrane.** Sytox Green can penetrate into cells when their membrane is disrupted and its fluorescence increases drastically when bound to intracellular nucleic acids. Our data reveal that at a concentration of 3.1 μmol/L (LC₅₀ value), [D]-H₆L₉ induced an influx of Sytox

**Table 1. Peptide designations, sequences, and acute systemic toxicities**

<table>
<thead>
<tr>
<th>Peptide designation</th>
<th>Sequence*</th>
<th>Systemic toxicity†</th>
</tr>
</thead>
<tbody>
<tr>
<td>[D]-K₆L₉</td>
<td>LKKLKKLKLLKKLKL</td>
<td>Toxic over 8 mg/kg</td>
</tr>
<tr>
<td>[D]-K₃H₃L₉</td>
<td>LHLLHLKLKLLKLKL</td>
<td>Toxic over 30 mg/kg</td>
</tr>
<tr>
<td>[D]-H₆L₉</td>
<td>LHLLHLHLHLHLHLHL</td>
<td>Toxic over 20 mg/kg</td>
</tr>
</tbody>
</table>

*Bold and underlined letters are D-enantiomers.
†Systemic toxicity was measured by i.v. injection of the peptide dissolved in Hartmann’s lactate solution.
Table 2. Lethal concentration (LC50 in μmol/L) of the peptides at physiologic and acidic pHs detected by XTT assay

<table>
<thead>
<tr>
<th>Peptide</th>
<th>CL1 prostate carcinoma pH 7.4</th>
<th>CL1 prostate carcinoma pH 6</th>
<th>22RV1 prostate carcinoma pH 7.4</th>
<th>22RV1 prostate carcinoma pH 6</th>
<th>LLC lung carcinoma pH 7.4</th>
<th>LLC lung carcinoma pH 6</th>
<th>NIH 3T3 mouse fibroblasts pH 7.4</th>
<th>NIH 3T3 mouse fibroblasts pH 6</th>
<th>OL foreskin fibroblasts pH 7.4</th>
<th>OL foreskin fibroblasts pH 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>[D]-K3H3L9</td>
<td>2.3</td>
<td>2.3</td>
<td>3.1</td>
<td>3.1</td>
<td>6.2</td>
<td>6.2</td>
<td>25</td>
<td>12.5</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>[D]-K3H3L9</td>
<td>9.3</td>
<td>9.3</td>
<td>12.5</td>
<td>12.5</td>
<td>25</td>
<td>12.5</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>[D]-H6L9</td>
<td>12.5</td>
<td>3.1</td>
<td>75</td>
<td>9.3</td>
<td>50</td>
<td>12.5</td>
<td>100</td>
<td>12.5</td>
<td>50</td>
<td>25</td>
</tr>
</tbody>
</table>

Note: Results are the mean of three independent experiments, each performed in duplicate.

Green into CL1 cancer cells only at acidic pHs. No significant influx was observed at physiologic pHs (Fig. 1B).

**Animal studies.** All animal experiments were performed according to regulations approved by the Institutional Animal Care and Use Committee.

**In vivo acute systemic toxicity.** Acute systemic toxicity was observed after a single i.v. injection of [D]-K3H3L9 at concentrations above 8 mg/kg (n = 5 mice). In contrast, both [D]-K3H3L9 and [D]-H6L9 did not indicate any acute systemic toxicity at doses of up to 30 and 20 mg/kg, respectively (n = 5 mice for each group; Table 1).

**Inhibition of solid prostate cancer growth by intratumor administration of the peptides.** 22RV1 cells were implanted s.c. in mice. One week after tumor implantation, [D]-H6L9 and [D]-K3H3L9 were injected intratumorally at a dose of 1 mg/kg (Fig. 2). A significant inhibition in tumor growth volume was observed during the course of the experiment in mice that were treated with the peptides (Supplementary Fig. S1). In addition, a significant reduction (81%) in dissected tumor weight was measured on the last day of the experiment (Fig. 3 and 4A and B). The reduction in tumor size was accompanied by a marked reduction in 22RV1-secreted PSA levels during the course of the experiment in mice treated with the peptides (Supplementary Figs. S2A and S3A). Histologic examination of dissected tumor sections stained with H&E and CD34 (Figs. 3 and 4C and D, respectively) revealed similar results as those obtained by intratumor administration (see above). Note that CD31 staining used for detection of established microvessels was not present in the dissected tumors. Other typical phenomena observed in the treated tumors were hematomas and proteinaceous liquids within the tissue. Immunohistochemistry of newly formed microvessel tube densities (CD34 staining) within and around the cancerous tissue revealed that the untreated tumor contained a branched net of newly formed capillary tubes, whereas the treated tumors barely contained newly formed capillary tubes inside the tumors (Fig. 2D). However, the newly formed capillary tubes in the adjacent connective tissue were not damaged from the treatment.

**Inhibition of solid prostate cancer growth by systemic administration of the peptides.** 22RV1 cells were implanted s.c. in mice. One week after implantation, [D]-K3H3L9 and [D]-H6L9 were injected systemically at a dose of 9 mg/kg (Figs. 3 and 4).

A significant inhibition of tumor growth was observed during the course of the experiment in mice treated with the peptides (Supplementary Figs. S2A and S3A). In addition, a significant reduction (75% and 72% for [D]-K3H3L9 and [D]-H6L9, respectively) in dissected tumor weight was measured on the last day of the experiment (Figs. 3 and 4A and B). The reduction in tumor size was accompanied by a marked reduction in 22RV1-secreted PSA levels during the course of the experiment in mice treated with the peptides (Supplementary Figs. S2A and S3A). Histologic examination of dissected tumor sections stained with H&E and CD34 (Figs. 3 and 4C and D, respectively) revealed similar results as those obtained by intratumor administration (see above). Note that CD31 staining used for detection of established microvessels was not present in the dissected tumors. Other typical phenomena observed in the treated tumors were hematomas and proteinaceous liquids within the tissue. Immunohistochemistry of newly formed microvessel tube densities (CD34 staining) within and around the cancerous tissue revealed that the untreated tumor contained a branched net of newly formed capillary tubes, whereas the treated tumors barely contained newly formed capillary tubes inside the tumors (Fig. 2D). However, the newly formed capillary tubes in the adjacent connective tissue were not damaged from the treatment.

**Figure 1.** A. Calcein release induced by the peptides. The peptides, at different concentrations, were added to the buffer containing small unilamellar vesicles at pH 6 and 7.4. ▲, [D]-H6L9 at pH 7.4; ▲, [D]-H6L9 at pH 6; ■, [D]-K3H3L9 at pH 7.4; □, [D]-K3H3L9 at pH 6; ●, [D]-K3H3L9 at pH 7.4; ○, [D]-K3H3L9 at pH 6. Treatment with Triton X-100 was used as 100% activity, and the leakage rate of vesicles alone was <1% in 5 h. B, pH-dependent influx of the vital dye Sytox Green into CL1 membranes induced by [D]-H6L9.
capillary tubes revealed no significant difference in the densities of these capillary tubes in treated and untreated tumors (data not shown).

Discussion

The important findings in this study are the following: (a) the histidine-containing peptides have significantly reduced systemic toxicity and (b) the entirely substituted histidine peptide has pH-dependent activity. The peptide damaged membranes and cells only at acidic pH (Table 2; Fig. 1). We have shown previously that the selective activity of the parental peptide [D]-K6L6 toward cancer cells results predominantly from differences between the membranes of malignant and normal cells (23). Whereas normal cells have mainly zwitterionic membranes on their outer leaflet (30), cancer cells express approximately 3% to 9% more of the acidic phospholipids, phosphatidylserine, in their outer leaflet (31–33). These cells also contain a higher density of negatively charged O-glycosylated mucins compared with normal cells (34, 35). Thus, electrostatic interactions between cationic peptides and the anionic cell membrane components are believed to be a major factor in the selective killing of cancer cells by these peptides. Because the histidines in [D]-H6L9 are protonated at acidic pHs, the peptide is not active at physiologic neutral pHs. Indeed, [D]-H6L9 is the only peptide that could not permeate model membranes and cancer cells at physiologic pH (Fig. 1). Interestingly, [D]-K3H3L9 has no pH-dependent activity, although it contains a significant number of histidines. This differs from several natural antimicrobial peptides that have pH-dependent activity, although they contain only a few histidines in their sequence (36–47).

The membranolytic activity of the peptides toward model membranes (Fig. 1A) and cell membranes (Fig. 2) supports our assumption that the histidine-containing peptides kill cancer cells by disrupting the cell membrane similarly to [D]-K6L6 and other antimicrobial peptides (20, 22, 23, 29). Importantly, substituting lysines for histidines significantly reduced the systemic acute toxicity of the peptides.
toxicity of the peptides (Table 1). Furthermore, topical and systemic administration of these two peptides significantly inhibited 22RV1 prostate carcinoma tumor xenograft growth and lowered the level of PSA known to be secreted by 22RV1 xenografts (25). Note that [D]-K3H3L9 was less cytotoxic than [D]-H6L9. This might be due to the relatively elevated toxicity of the latter in acidic organs besides the tumor.

Previous studies have reported on the use of various techniques to activate anticancer drugs in the presence of the low pH. However, the majority of these techniques focused on drug delivery systems that are pH dependent. Examples include the controlled, pH-dependent release of chemotherapy agents from polymers (12–14) and liposomes (48) as well as by using cell-penetrating peptides (11, 49). Here, [D]-H6L9 possesses three functions: targeting, activation at low pHs, and cytotoxic activity. We expect that owing to the strong membranolytic effect of the peptides, it would be difficult for the tumor cell to select resistant variants. This is similar to what has been found in many cases with bacteria treated with cationic innate immunity lytic peptides (15, 50).

In addition to the potent inhibition of tumor growth by [D]-H6L6 and [D]-K6H6L3, the histology of the tumors treated both intratumorally and via systemic inoculation revealed a significant decrease in vascularization of new capillary tubes compared with untreated mice (Figs. 2D, 3D, and 4D). This could be the result of either the reduced cancer cell density or the direct activity of the peptides toward the sensitive new capillary tubes. The latter is supported by the recent finding that endothelial cells forming the branched vasculature supporting solid tumors express enhanced amount of phosphatidylserine molecules on the outer leaflet of their plasma membrane (51, 52). Treatment of the tumors with the peptides was also accompanied by a reduction in the density of cancer cells and an increase in the amount of newly formed connective tissue within the tumor. This phenomenon correlates with an increased number of cells containing necrotic debris, comprehensive hematomas, and pertinacious liquid found within the cancerous tissue after treatment. Taken together, these results suggest that the observed reduction in the tumor size is only an underestimation of the combined destructive effect of the peptides on the tumor tissue and cells.

In summary, the histidine-rich peptides offer a new class of membranolytic agents for efficient anticancer treatment with...
reduced systemic toxicity. Moreover, the pH-selective activity of [D]-H,L,
may provide a new strategy for treatment of solid tumors within naturally occurring low-pH extracellular environments.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

References
1. Vaupel P, Kallinowski F, Oliemieff P. Blood flow, oxygen and nutrient supply, and metabolic microenviron-
49:4373–84.
4. Martin GR, Jain RK. Noninvasive measurement of interstitial pH profiles in normal and neoplastic tissue
of lactic acid is not the only cause of tumor acidity, Proc Natl Acad Sci U S A 1990;90:1227–31.
tumors: contribution to the preservation of methotrexate-pharmacologic activity in HeLa cells lacking the reduced
8. Wang Y, Zhao R, Goldman ID. Characterization of a futile transporter in HeLa cells with a low pH optimum
49:4373–84.
Prostate 2002;51:823–32.
15. Allen TM, Cleland LG. Serum-induced leakage of liposome contents. Biochim Biophys Acta 1980;597:
17. Kichler A, Mason AJ, Bechinger B. Cationic amphiphilic helix motif preferentially kill tumor cells in
9346–54.
20. Kuczynski Z. Changes in electric charge and phospholipid asymmetry in blood cells. Biochim Biophys Acta
21. Ran S, Thorpe PE. Phosphatidylserine is a marker of the diagnosis of pancreatic cancer]. Tumori 1999;85:
51:3062–6.
22. Ran S, Downes A, Thorpe PE. Increased exposure of phosphatidylserine on the surface of tumor blood
23. Ran S, Thorpe PE. Phosphatidylserine is a marker of tumor vasculature and a potential target for cancer

Acknowledgments
Received 8/5/08; revised 1/14/09; accepted 1/30/09; published OnlineFirst 4/7/09.
Grant support: Israel Cancer Research Funds and Prostate Cancer Research Foundation (United Kingdom). Y. Shai is the incumbent of the Harold S. and Harriet B. Brady Professorial Chair in Cancer Research.

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.
Suppression of Human Solid Tumor Growth in Mice by Intratumor and Systemic Inoculation of Histidine-Rich and pH-Dependent Host Defense–like Lytic Peptides

Arik Makovitzki, Avner Fink and Yechiel Shai


Updated version
Access the most recent version of this article at: doi:10.1158/0008-5472.CAN-08-3021

Supplementary Material
Access the most recent supplemental material at: http://cancerres.aacrjournals.org/content/suppl/2009/04/06/0008-5472.CAN-08-3021.DC1

Cited articles
This article cites 52 articles, 20 of which you can access for free at: http://cancerres.aacrjournals.org/content/69/8/3458.full#ref-list-1

Citing articles
This article has been cited by 5 HighWire-hosted articles. Access the articles at: http://cancerres.aacrjournals.org/content/69/8/3458.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.