SWAINSONINE: A NEW ANTINEOPLASTIC IMMUNOMODULATOR

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Swainsonine, an indolizidine alkaloid with immunomodulatory activity, has been found to be effective in inhibiting metastatic dissemination and growth of primary tumors of both murine and human origins. The unique ability of swainsonine to exhibit antimetastatic, antiproliferative, and immunomodulatory activity imparts this drug a promising future in cancer therapy.

The ability of cancer cells to migrate and colonize distant organs is the major cause of morbidity and mortality among cancer patients. A majority of cancer patients have metastatic disease at the time of clinical presentation. Current treatment modalities, such as surgery, radiotherapy, and chemotherapy, can cure or control cancer in approximately 50% of all cancer patients. The majority of cancer patients who do not respond to standard therapeutic regimens eventually succumb to effects of metastases. Consequently, significant efforts have been made to understand the biology and biochemistry of the metastatic process in order to develop therapeutic probes that can either prevent or destroy metastatic lesions with minimal toxicity to the patient.[1-7]

In this article, the effects of swainsonine—an inhibitor of cell surface oligosaccharides—on several experimental tumor models using animal and human cancer cells are described. Our results suggest that employment of antimetastatic agents, based on improved understanding of cell biology and biochemistry of the metastatic process, may be an effective tool in improving the management of cancer patients.[8-20]

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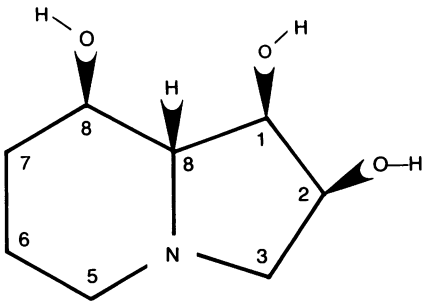
The process of metastasis comprises a sequential series of migratory and invasive events that require the passage of tumor through both connective tissue and vascular elements.[1-7,21] As the primary solid tumor grows, it becomes highly vascularized and rapidly invades the surrounding tissue. Tumor cells spread to distant sites either (1) by direct extension into body cavities where the released tumor cells can implant onto tissue surface and grow, or (2) by release of tumor cells that can invade the blood or lymphatic vessels. Once in circulation, tumor cells form emboli by interacting with other tumor cells and blood platelets. These emboli eventually lodge in capillary beds of distant organs or lymph nodes, where they penetrate the basement membrane to form metastatic colonies.[22-25]

SURFACE OLIGOSACCHARIDES IN CELL ADHESION AND METASTASIS

Specific interactions between carbohydrates and proteins play an important role in mediating biological responses.[26-28] Carbohydrate binding proteins capable of binding to sugar residues have been demonstrated in both plant and animal species.[29] One of the important cell structures that directly mediates the interaction of tumor cells with host environment is the tumor cell surface. Consequently, considerable attention has been focused on the tumor cell surface as a target of antimetastatic therapies. A number of studies using tumor models with experimental metastases have established the importance of cell surface components in certain aspects of metastases. Significant differences in size and structure of asparagine-linked carbohydrate



Swainsonine

Figure 1. Structure of swainsonine.

moieties between normal and transformed cells have been observed in a number of species, including human.[30,31] Compared with normal cells, the transformed cells generally have larger oligosaccharides, which are more highly branched and oversialylated at their chain termini. These alterations result from a quantitative increase in oligosaccharide branching or antennary structure.[32] Tumor cells are characterized by an increase in tri- and tetra-antennary oligosaccharides (containing the GlcNac [β1,6] Man [α1,6] branch) and a decrease in high mannose oligosaccharides; these changes in the expression of β1, 6 branched oligosaccharides are correlated with tumor metastasis.[30,33-35]

Data in the literature indicate that tumor cell oligosaccharides play a significant role in tumor cell invasion and metastasis:

1. The quantity of "exposed" sialic acid, the degree of sialylation of subterminal galactose and N-acetylgalactosamine residues, show a good correlation with metastatic potential[36-38];
2. Tumor cells that are resistant to high concentrations of plant lectins are also less metastatic such lectin-resistant cells show loss of sialylated asparagine-linked oligosaccharides and defective enzymatic pathway of carbohydrate metabolism[30,39]; and
3. Modifications of tumor cell surface carbohydrates also alter their metastatic potential.[10-12,18,40-45]

These data suggest that the extent of processing of oligosaccharide moieties of tumor cell surface glycoprotein is intimately linked to the ability of cells to metastasize.

Many tumor cells contain endogenous lectins on their cell surface.[46,47] These lectins can recognize and bind complementary glycoconjugates on the cell surface of other tumor cells to form homotypic aggregates, bind cell surface of host cells to mediate heterotypic aggregation, or attach to endothelial cells/basement membrane or the extracellular matrix. These adhesive interactions by tumor cells are specific in that they can be inhibited by specific sugars.[48] In addition, pretreatment of tumor cells with antibodies to endogenous lectins can also inhibit metastases.[49,50]

Even though a number of quantitative and qualitative changes have been observed in tumor cell surface oligosaccharides, it has not been possible to demonstrate that a specific lesion is the cause and not the consequence of malignant transformation. This difficulty has arisen because several features of the complex carbohydrate could contribute to the preferential recognition of highly branched oligosaccharides; some examples include (1) the distribution or density of terminal sugars such as galactose and sialic acid, (2) the tertiary conformation of the oligosaccharide, and (3) the primary structure or specific sequences of the various sugar residues and branching. The present approach of modifying a single characteristic, using an inhibitor of a specific enzyme (Golgi α-mannosidase II) of the processing pathway, may lead to identification of specific oligosaccharide structural features crucial for successful completion of the metastatic cascade.

SWAINSONINE MODIFICATION OF SURFACE GLYCANS AND METASTASIS

Swainsonine, an indolizidine alkaloid, is an inhibitor of Golgi α-mannosidase II, an enzyme responsible for the removal of mannose residues during N-linked oligosaccharide maturation (Figures 1 and 2). In the presence of swainsonine, an unusual hybrid oligosaccharide is synthesized in which maturation of one arm is arrested as the high mannose-type structure and the other arm is trimmed and processed to yield a "complex-type" structure (Figure 2).[51] Treatment of B16-F10 mouse melanoma cells, in monolayer culture, for 24 hours with swainsonine significantly inhibited (>90%) their pulmonary colonization capacity after intravenous injection into C57BL/6 mice.[11,12,52] The results of three independent experiments are shown in Figure 3. This antimetastatic activity was dependent on both the in vitro concentration of

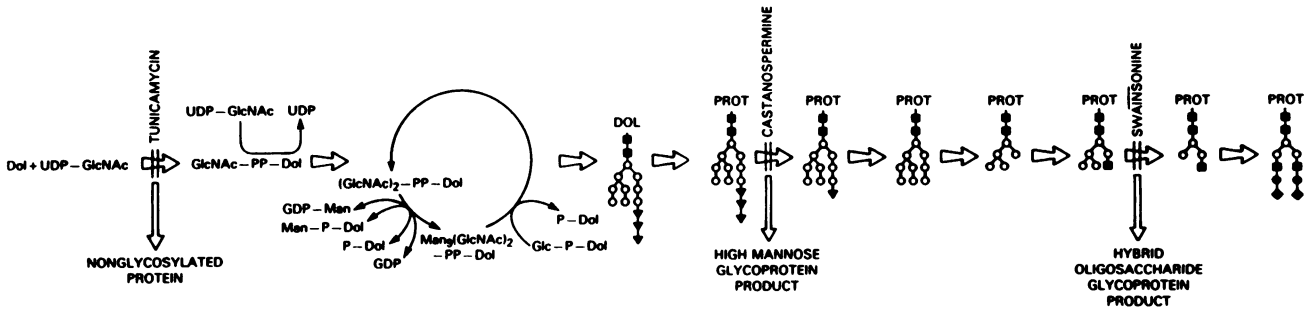


Figure 2. General pathway for glycoprotein processing. The high mannose precursor molecule shown at the left is synthesized on lipid precursor dolichol and then transferred en bloc to the protein backbone. Subsequent processing steps are performed by specific glycosidases and glycosyl transferases located in the endoplasmic reticulum and Golgi apparatus. The reactions inhibited by tunicamycin, swainsonine, and castanospermine are shown.○ mannose, ▼ glucose, ■ N-acetylglucosamine, ● galactose, ♦ sialic acid.

swainsonine and the size of the cell inoculum injected into C57BL/6 mice. Inhibitions in lung colonization were seen with swainsonine concentrations of 100 ng/mL (half maximal inhibition) and 3 μg/mL (80% inhibition). When the dose of cell inoculum was decreased to 5×10^4 cells, inhibition of lung colonization was increased to approximately 95%. Treatment of cells with swainsonine did not increase the frequency of extrapulmonary metastases by redistribution of cells to other sites. Also, swainsonine treatment of cells in vitro was not cytotoxic and did not affect cellular tumorigenicity after subcutaneous implantation.[8,9] However, swainsonine treated cells were cleared from the lungs at a greater rate than control cells, suggesting that swainsonine alters tumor cell retention in target organs.

These results suggest a novel requirement for strict carbohydrate specificity, thus strengthening the importance of oligosaccharide-lectin recognition in metastatic colonization.[8,9]

SYSTEMIC ADMINISTRATION OF SWAINSONINE AND METASTASIS

Results with swainsonine treatment of melanoma cells in vitro clearly indicate that inhibition of asparagine-linked oligosaccharide processing causes a dramatic decrease in lung colonization potential following intravenous injection into mice. This inhibition can be enhanced by simultaneous systemic administration of swainsonine to mice.[52]

Swainsonine has potent antimetastatic activity even when administered alone without pretreatment of tumor cells. This antimetastatic effect is maintained up to 5 days after withdrawal of the drug.[13,14] Pharmacokinetic data indicate that swainsonine is cleared rapidly from the body (Humphries MJ. August 1988. Unpublished data. Bowen D. September 1988. Unpublished data). Taken together, these data suggest that swainsonine initiates a cascade of biological events that leads to increased host resistance.

One possible mechanism of action is stimulation of host immune function by swainsonine. Carbohydrate moieties have been shown to modulate immune cell recognition processes,[53,54] specifically, the relationship of cell surface oligosaccharides to natural killer cell function.[55-58] Swainsonine has been shown to restore murine lymphocytic proliferation in animals treated with endogenous immunosuppressive factor.[59] The kinetic profile of natural killer cell activation by other agents shows time course similar to that of swainsonine.[60-63]

It is highly unlikely that the swainsonine-induced inhibition of metastasis is due to alterations in tumor cell oligosaccharides, as the drug is effective long after it has been excreted from the body.[64] Thus, systemic administration of swainsonine to C57BL/6 mice can interfere with pulmonary colonization of B16-F10 melanoma cells. This effect is seen even after a short (6 hr) administration of swainsonine and is independent of the route of administration.[13] This in vivo inhibition requires the presence of natural killer cells; mice depleted of natural killer cell function are not responsive to

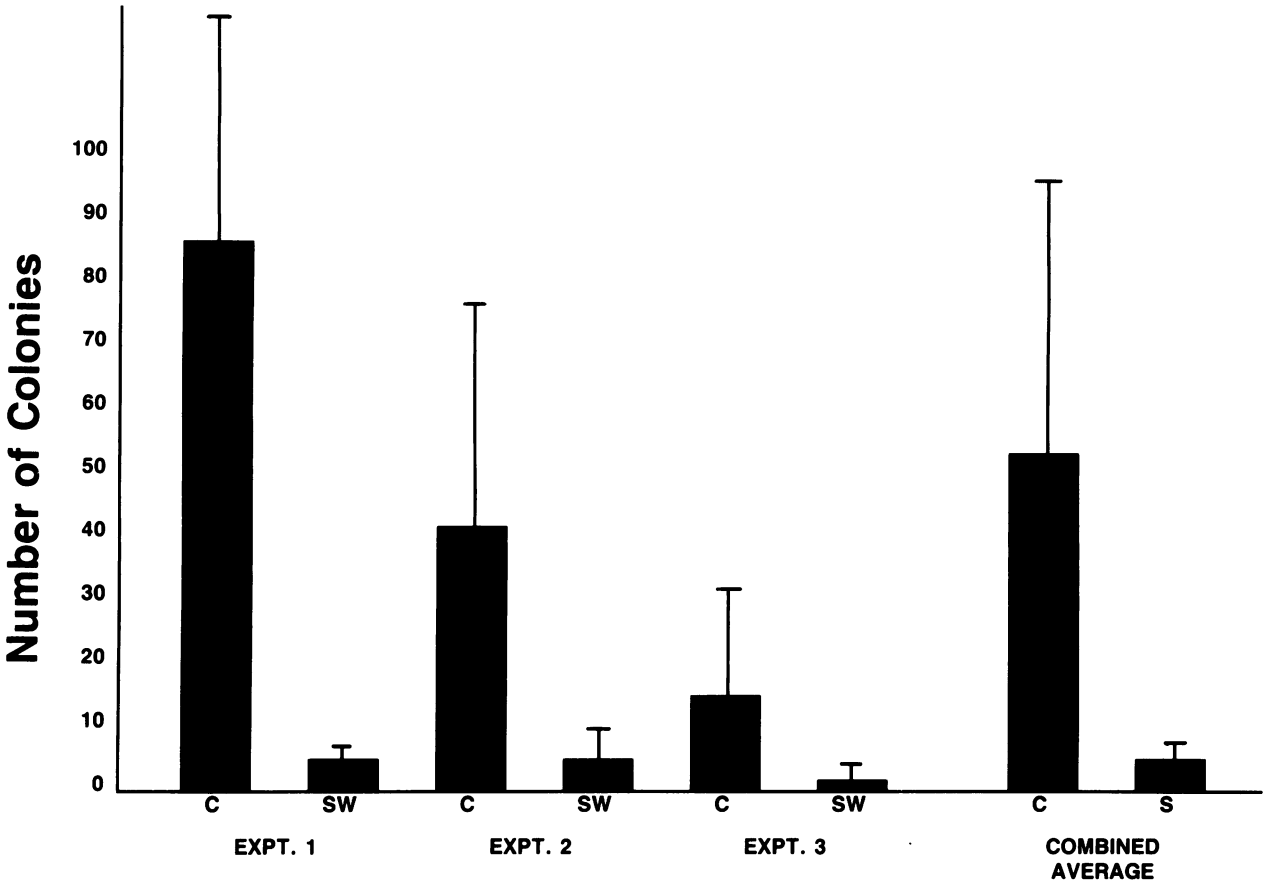


Figure 3. Effect of swainsonine on experimental metastasis of B16-F10 cells. Water containing 3 μg/mL of swainsonine was supplied ad libitum for 24 hours to a group of 12 C57BL/6 mice. A second group of 12 mice (the control group) were given regular drinking water. Aliquots of 8 × 10^4 viable cells were then injected into the lateral tail vein, and both groups of animals were returned to regular or unsupplemented drinking water. Two weeks later, surface melanotic colonies were counted. The results shown represent the findings of three different investigators. Values are mean ± standard error.

vivo can cause enhanced lymphoproliferation as measured by incorporation of ^{3}H-thymidine into DNA.[14] The mitogenic response of spleen cells from mice maintained on swainsonine (given in drinking water) for 48 hours is four- to sixfold greater than that of spleen cells from control mice maintained on water without swainsonine. Moreover, this enhanced mitogenic response persists up to 72 hours after withdrawal of swainsonine-supplemented water.[13] Initial studies suggest that T cells are one of the primary subpopulations of lymphocytes responding to swainsonine. This hypothesis is based on the following evidence:

1. The mitogenic effect of swainsonine is dramatically reduced in athymic nude mice, which have a T cell deficiency.
2. The effect of swainsonine on lymphocytic cell proliferation was only slightly decreased in mice that were depleted of natural killer cells by anti-asialo-GM1.
3. Systemic administration of swainsonine stimulated a two- to sixfold increase in the synthesis of the lymphokine interleukin-2 (IL-2) and directly stimulated the proliferation of T helper cell clones (White SL. October 1988. Unpublished data). IL-2 is a glycoprotein produced by T helper cells that promotes the clonal expansion of activated T-lymphocytes.

Recent data using flow cytometry indicate that swainsonine treatment also enhances the concanavalin A-induced IL-2 receptors. Swainsonine has no effect on interferon production, suggesting that its immunomodulatory properties are independent of any action by interferon (White SL. October 1988. Unpublished data).

The therapeutic effects of swainsonine were enhanced by simultaneous administration of polyinosinic acid:polycytidylic acid,[66] interferon inducer,[66] and IL-2 (Humphries MJ. August 1988. Unpublished data). Under appropriate conditions, combination therapy prolongs survival of recipient animals (Humphries MJ. August 1988. Unpublished data). Swainsonine may therefore be classified as a new immunomodulator that acts in combination with other stimulants of the immune system.

SPONTANEOUS METASTASIS AND SYSTEMIC ADMINISTRATION OF SWAINSONINE

Although studies using the experimental metastasis model are relevant in elucidating the biochemical events associated with metastasis, important differences exist between experimental and authentic or spontaneous metastasis.[67] Spontaneous metastasis tumor models closely mimic the patterns observed clinically with patients in whom metastases develop from primary tumors. In experimental metastasis, tumor cells are injected directly into the circulation and therefore bypass early events of the metastatic cascade. Experimental metastasis may also provide an opportunity for tumor cells to form metastatic foci, which normally may not have metastasized from the primary tumor.

To test the effect of swainsonine on spontaneous metastases, two murine tumor cell lines were used: (1) M5076, a highly invasive reticulosarcoma cell line, which metastasizes from subcutaneous implants to visceral organs including the liver and spleen[68-70]; and (2) B16-BL6 melanoma, which can metastasize from an intramuscular foot pad injection to colonize the lungs.[2] Both B16-BL6 and M5076 cell lines have been used by other investigators as models of spontaneous metastasis. In addition, earlier work from this laboratory on the effect of swainsonine on experimental metastasis used the related B16-F10 melanoma.

Systemic administration of swainsonine effectively inhibited the spontaneous metastases of B16-BL6 to lungs by 88%. Swainsonine-induced inhibition was dose-dependent with maximum inhibition observed at 3 μg/mL.[71] Swainsonine treatment given in drinking water for 28 days also inhibited hepatic metastases induced by interscapular subcutaneous injection of M5076 cells; the mean colony number of liver metastases decreased from more than 300 in controls to 16 in swainsonine-treated mice.[71] These results indicate that swainsonine is highly effective in inhibiting experimental as well as spontaneous metastases—an important, clinically relevant finding.

EFFECT OF SWAINSONINE ON GROWTH RATE AND LUNG COLONIZATION OF HUMAN BREAST CANCER CELLS

Swainsonine has been shown to induce changes in tumor cell proliferation and differentiation. For example, transformed NIH 3T3 cells grown in the presence of swainsonine for 4 to 10 days lose their ability to grow in soft agar and acquire a morphology characteristic of nontransformed cells.[72] In addition, swainsonine given in combination with the interferon-inducer polyriboinosinic:polyribocytidylic acid (poly I:C) inhibits the growth of MDAY-D2 solid murine tumors, whereas neither agent alone was an effective inhibitor.[52]

To determine whether swainsonine had tumor antiproliferative activity in vivo, the human breast carcinoma cell line MDA-MB-231 was used. These cells are devoid of both estrogen and progesterone receptors, are highly malignant, and form poorly differentiated tumors when injected subcutaneously into athymic nude mice. Swainsonine treatment given in drinking water for 60 days resulted in an approximately 70% inhibition of tumor volumes (from 361 mm^3 in controls to 108 mm^3 in swainsonine-treated mice). More important, when swainsonine was withdrawn, the slower growing tumor implants continued their slower growth, suggesting a permanent alteration (Mohla S. September 1988. Unpublished data). In contrast, when swainsonine treatment was given intermittently, growth inhibition was less when compared with the group in which swainsonine was administered continuously for 60 days. Further, withdrawal of swainsonine resulted in a rapid increase of tumor growth comparable to that of untreated controls. Thus, intermittent treatment with swainsonine can inhibit tumor growth as long as swainsonine treatment is continued. Similar results were obtained when an estrogen-receptor positive human breast carcinoma cell line MCF-7 was used. Systemic administration of

swainsonine for 45 days significantly inhibited (>85%) the growth of MCF-7 cells that were injected subcutaneously in athymic nude mice. Thus, systemic treatment with swainsonine was effective in inhibiting in vivo growth of both estrogen-receptor-negative and -positive human breast carcinoma cells.

The MDA-MB-231 cell line can also colonize lungs when injected intravenously. The extent of pulmonary metastases depends on the number of cells injected. Injection of as few as 2.5×10^4 cells can form lung metastases within 3 weeks. For most experiments, the number of cells injected ranged from 5×10^4 to 1×10^5. Swainsonine was administered in drinking water at 3 μg/mL 1 day before injection of tumor cells. Our results show that continuous administration of swainsonine for 21 days significantly inhibited the lung colonization of human breast carcinoma cell line MDA-MB-231 (Mohla S. September 1988. Unpublished data). Thus, swainsonine treatment can block the experimental metastasis as well as inhibit tumor growth rate of human breast carcinoma cells.

The in vitro treatment of MDA-MB-231 cells with swainsonine for 21 days completely abolished the cells' ability to form lung colonies. Such swainsonine-treated cells were not able to spread on plastic dishes coated with laminin or fibronectin. These data suggest that treatment of human breast carcinoma cells in vitro with swainsonine may alter the cells' ability to adhere to an extracellular matrix—an important initial step for successful metastasis (Mohla S. September 1988. Unpublished data).

SWAINSONINE PHARMACOKINETICS

As mentioned earlier, swainsonine has been shown to be cleared rapidly from the body.[64] Blood concentration of swainsonine versus time was fitted to a classic two-compartment open-pharmacokinetic model. Following an intravenous delivery, the rapid equilibration of swainsonine in blood is most striking. The pharmacokinetic properties of swainsonine suggest that this agent is readily distributed throughout the body (time 1/2 $\alpha = 0.85$ min); blood clearance (0.66 mL/min) and elimination (time 1/2 $\beta = 36$ min) are moderately fast (Humphries MJ. August 1988. Unpublished data. Bowen D. September 1988. Unpublished data). Systemic exposure to 10 μg of swainsonine—as determined by area under the curve (AUC) values for intravenous, intraperitoneal, subcutaneous, and oral routes—was essentially the same for all routes (85.9 to 90.5 nmol/mL/min). The peak concentration for 10 μg swainsonine was: intraperitoneal 2.1 nmol/mL, subcutaneous 1.6 nmol/mL, and oral 1.9 nmol/mL (Humphries MJ. August 1988. Unpublished data. Bowen D. September 1988. Unpublished data). The highest tissue levels were found in kidneys, and the lowest levels were in the brain and the spinal cord. Urinary excretion is rapid and accounts for the majority (>85%) of the exposed swainsonine dose over the first hour. Metabolites of the drug are found in neither the plasma nor most of the body organs other than the kidney. Rapid swainsonine clearance may be associated with lack of toxicity in vivo.

CONCLUSION

The results presented in this article demonstrate that swainsonine is effective in inhibiting tumor cell metastasis as well as tumor cell growth of a number of tumor cell lines, both murine and human in origin. Swainsonine was effective in blocking experimental and spontaneous metastases and in preventing tumor cells from colonizing the lung and liver. These effects were observed with minimal toxicity to the tumor-bearing mice, even at concentrations as high as 0.6 to 1.2 mg/kg per day given continuously for over 65 days.

Whether the antimetastatic effects of swainsonine will be observed in other human tumor models remains to be determined. However, when swainsonine was administered in vivo, several tumor cell lines were effectively inhibited, including murine melanoma (B16-F10, B16-BL6), murine reticulum sarcoma (M5076), human breast carcinoma (both estrogen-receptor-positive [MCF-7] and estrogen-receptor-negative [MDA-MB-231]), human colon carcinoma (HT-29), and human osteosarcoma. In addition, the unique ability of swainsonine to be antimetastatic, antiproliferative, and immunomodulatory imparts this drug a promising future as an effective agent for clinical trials. The efficacy of swainsonine in combination with other biological response modifiers (eg, interferon or IL-2) will allow this drug to be tested as an adjuvant chemotherapeutic agent. For example, clinical trials on patients with colorectal cancer have shown that when other antimetastatic agents are used as adjuvants to surgery, the disease-free interval is significantly increased in treated patients compared with controls.[73]

Finally, these studies re-emphasize the tenet that cell proliferation is not the only exploitable property of a tumor cell in the development of chemotherapeutic

agents. Because metastasis and invasion are unique to cancer cells (unlike cell division), the development of agents that interfere with these processes might be a more realistic approach to controlling human malignancies.

During the last two decades, limited success has been achieved in suppressing tumor growth by use of immunomodulatory agents, such as IL-2 and interferon. The use of nonspecific immunomodulatory agents, such as swainsonine, has been rare and less in vogue compared with the use of more specific cytokines. However, the ability of swainsonine to elicit a broad range of immune responses rather than a single effector mechanism (with minimal toxicity) indicates a potential clinical advantage of such molecules.

Acknowledgments

We thank Traci Kelly, Emily Reeves, and Kenneth Foster Olden for allowing us to use their results shown in Figure 3. This work was supported in part by PHS grants CA-14718 and CA-34918; American Cancer Society Grant PDT-312, The Ella A. Latham Trust Fund, and The Howard University Faculty Research Support Grant. The technical assistance of Walter Griffin, Vandana Narang, and Camille D. Bowen is gratefully acknowledged.

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