Host Acid Sphingomyelinase Regulates Microvascular Function Not Tumor Immunity

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ABSTRACT

Previous studies provided evidence that MCA/129 fibrosarcomas and B16 melanomas grow 2- to 4-fold faster in acid sphingomyelinase (asmase)−deficient mice than in asmase+/+ littermates and are resistant to single-dose irradiation due to inability to mount an apoptotic response in tumor microvascular endothelium. However, others postulated the differences might be associated with a host antitumor immune response in asmase+/+ mice that is not expressed in asmase−/− mice due to phenotypic deficiency in antitumor immunity. The present studies demonstrate that none of the tumor–host combinations displayed the classic criteria of an immunogenic tumor because they lacked endotumoral or peritumoral infiltrates almost entirely. Furthermore, neither MCA/129 fibrosarcoma nor B16 melanoma tumors showed differences in growth or radiosensitivity when implanted into mutant mouse models (Reg−/− and MEF−/−) lacking functional immune cell [natural killer (NK), NK-T, T, and B cells] populations. Additionally, development and function of B-, T-, and NK-cell populations in asmase−/− mice were normal, indistinguishable from their wild-type littermates. These data provide definitive evidence that MCA/129 fibrosarcomas and B16F1 melanomas do not elicit a host immune response in wild-type mice and that the asmase−/− phenotype is not deficient in antitumor immunity, supporting the notion that the patterns of tumors growth and radiation response are conditionally linked to the ability of the tumor endothelium to undergo ASMase-mediated apoptosis.

INTRODUCTION

Our recent studies (1, 2) have challenged the generally accepted notion that tissue stem cells and clonogens are the determinant targets in normal and tumor tissue responses to ionizing radiation (3, 4), with the host microenvironment providing modulating although not mandatory signals (5, 6). Genetic or pharmacologic inhibition of ceramide-mediated endothelial cell apoptosis, using acid sphingomyelinase (asmase)−deficient mice or wild-type littermates pretreated with the endothelial cell survival factor basic fibroblast growth factor, provided evidence that when exposed to radiation at doses comparable with those used in clinical radiotherapy, expression of damage by intestinal crypt clonogens is conditionally linked to radiation-induced endothelial apoptosis in the affected tissue microvasculature (1). Similarly, implantation of MCA/129 fibrosarcomas or B16 melanomas into mice lacking asmase or the pro-apoptotic Bcl-2 family member Bax rendered tumor clonogens resistant to radiation damage compared with wild-type littermates (2). These studies provided compelling evidence that microvascular endothelial apoptosis was obligatory for induction of tissue or tumor damage in wild-type mice, because its abrogation prevented single-dose radiation damage altogether within the clinical dose range (8–15 Gy).

Whereas our studies reported that the dose required for 50% tumor control (TCD50) in MCA/129 fibrosarcomas grown in asmase+/+ sv129xB6/6 mice was 15 Gy (2), several investigators suggested this TCD50 value is particularly low and virtually unknown for tumor transplants in syngenic hosts (6). Furthermore, MCA/129 fibrosarcomas and B16 melanomas grew 2- to 4-fold faster in asmase−/− than in asmase+/+ littermates. Taken together, these observations raised the speculation that these tumors might be exceptionally immunogenic, leading in wild-type mice to slow tumor growth and hypersensitivity to radiation (6). It was further speculated that the ASMase−/− phenotype may be deficient in one or more antitumor immune functions, leading to an apparent rapid tumor growth relative to wild-type mice and the enhanced resistance to radiation (6).

Tumor immunity proposes that the immune system surveys for nascent malignancies, eliminating many or most tumors, and possibly slowing the growth of others (7). Data from mouse strains lacking distinct immune cell populations or effector molecules demonstrate increased incidence of spontaneous tumors, as well as a higher susceptibility to induced or transplanted tumors (8). Most of these tumors are rejected when transferred into fully immunocompetent hosts, suggesting that the immune system exerts selective pressure on nascent tumors (8).

The molecular basis for the recognition of tumor cells by the immune system is not fully understood. The host mounts a cellular immune response, with natural killer (NK) cells serving as the main effectors of innate antitumor responses (9), whereas cytolytic T cells and to a lesser extent B cells provide adaptive antitumor immunity (9). Two main mechanisms are used by NK cells to eliminate nascent tumors. Tumor cells often down-regulate expression of class I major histocompatibility complex (MHC) molecules, enabling NK-cell activation and killing processes that are normally inhibited by the presence of class I MHC molecule expression on normal, nontransformed cells (8, 9). Tumors can also express tumor-specific ligands that stimulate receptors expressed by NK cells, T cells, and macrophages (NK2G2D), leading to tumor rejection in vivo (8). These mechanisms not only activate the innate immune system directly, but they can also induce and enhance adaptive immune responses. In the adaptive immune system, antigen-specific T- and B-cell responses are initiated by dendritic cells, which capture antigens secreted or shed by tumor cells or upon cell lysis (9). Processing and presentation of these antigens by the MHC class I and class II molecules of dendritic cells allows for the priming and activation of CD8+ and CD4+ T cells, respectively (9). Ultimately, activated antigen-specific CD8+ T cells differentiate into cytotoxic lymphocytes that lyse tumor. Intra- and peritumoral infiltration with NK cells, tumor-specific lymphocytes, and other mononuclear cells constitutes a hallmark of tumor immunity (10).

The present studies provide direct evidence that the low TCD50 and the slow tumor growth observed in asmase−/− mice do not result from host antitumor immune response and that the accelerated tumor growth and radioresistance in asmase−/− mice does not reflect defi-
cient host immune response. Histopathologic, immunopathologic, and immune function studies of MCA/129 fibrosarcomas and B16 melanomas implanted into wild-type mice failed to demonstrate classic criteria of an immunogenic tumor, while B-, T-, and NK-cell development and function in asmase−/− mice tested normal. These data invalidate the speculation that a host antitumor immune effect, rather than primary microvascular dysfunction, regulates tumor growth and the radiation response in our experimental tumor systems.

MATERIALS AND METHODS

Mice. asmase+/+ and asmase−/− mice maintained in an sv129xB6 background and MEF+/+ and MEF−/− mice in a C57BL/6 background were propagated using heterozygous breeding pairs and genotyped as described previously (11, 12). Rag2−/− mice in a C57BL/6 background were propagated using homzygous breeding pairs and genotyped as described previously (13). Eight- to 12-week-old C57BL/6 male mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were housed at the animal core facility of Memorial Sloan-Kettering Cancer Center. This facility is approved by the American Association for Accreditation of Laboratory Animal Care and is maintained in accordance with the regulations and standards of the United States Department of Agriculture and the Department of Health and Human Services, NIH.

Tumor Growth. MCA/129 fibrosarcoma and B16F1 melanoma cells were maintained in DMEM high glucose supplemented with 10% fetal calf serum, 100 U of penicillin per milliliter, and 100 μg of streptomycin per milliliter in 10% CO2 at 37°C. Cells (105) resuspended in PBS cells were injected subcutaneously into the right hind limb as described previously (2), and tumor volume, based on caliper measurements, was calculated daily according to the formula of Kim et al. (14).

Tumor Irradiation. Whole body radiation (WBR) was delivered with a Shepherd Mark I unit (model 68; SN643) operating 137Cs sources at a dose rate of 1.5 Gy/min as described previously (1). Tumors were irradiated at a volume of 100 to 150 mm3 as described previously (2). Mice, lightly sedated with ketamine (0.1 mg/g) and xylazine (0.02 mg/g), were irradiated using an X-ray unit (Philips, Eindhoven, The Netherlands) at 105.5 Gy/min (50-cm source to skin distance). Only tumor, surrounding skin, and subcutaneous tissues were exposed; the rest of the mouse was shielded using a specialized lead jig.

Bone Marrow Transplantation. For autologous bone marrow transplantation, 107 bone marrow cells harvested from femur and tibia of donor mice by flushing medullary cavities with Hank’s balanced salt solution were injected into the tail vein of recipient mice 16 hours after 12 Gy WBR as described previously (1).

Flow Cytometry Analysis. Anti-T-cell receptor-β (mAb H57-579), anti-CD19, and anti-DX5 were obtained from PharMingen (San Diego, CA). Single-cell suspensions were preincubated with anti-CD16/CD32 (PharMingen) and stained with the indicated antibodies, and 30 × 106 cells/sample were analyzed using a FACScan Cytometer (Becton Dickinson, Mountain View, CA). Data were then analyzed using CellQuest 3.1 software.

Proliferation Assay. Splenocytes from asmase−/− and asmase+/+ mice were collected as described previously (12). Cells were stimulated by either T-cell receptor cross-linking or lipopolysaccharide (LPS) (from Escherichia coli; Sigma, St. Louis, MO) treatment as described previously (12). To cross-link the T-cell receptor, sterile polyvinyl chloride microtiter plates (Fisher Scientific, Pittsburgh, PA) were coated overnight at 4°C with 200 μL per well of anti-CD3 antibody [clone2C11 at the concentrations indicated in carbonatebicarbonate buffer (pH 9.6)]. Plates were then washed and blocked with complete medium, and 1 × 105 spleen cells were added per well in the presence of anti-CD28 (3 μg/mL) obtained from PharMingen and incubated for 3 days. Proliferation was measured by [3H]thymidine uptake for 10 additional hours. Cells were harvested, and thymidine incorporation (12) was determined by scintillation counting using a top-count Packard β counter (Packard Instruments, Downers Grove, IL). LPS was added at a concentration of 10 μg/mL and incubated with cells for 3 days before performing the proliferation assay. Incorporation was measured in sextuplicates.

Fig. 1. Hematoxylin–eosin staining detects only rare peritumoral or endotumoral immune cell infiltrates. Representative 5-μm histologic tumor sections of MCA/129 fibrosarcoma (top panel) and B16 melanoma (bottom panel) were stained with hematoxylin–eosin (magnification, ×200).

Natural Killer-Cell Cytotoxic Assay. NK-cell analysis was performed using cells isolated from the spleen of mice that had previously received intraperitoneal injections of 100 μg of poly(LC) (Sigma) or PBS vehicle as described previously (12). Target cells (2 × 105/well) were labeled with 100 μCi of sodium [51Cr]chromate for 1 hour at 37°C and washed three times before being plated in the presence of different concentrations of spleen cells. After 4 hours at 37°C, target cell lysis was measured by counting the supernatant using a Packard β counter. The percentage of specific lysis was calculated as [experimental 51Cr release − spontaneous release]/[maximum release − spontaneous release]. Maximum release was measured by adding 1% Triton X-100, whereas spontaneous release was measured by omitting effector cells (15).

Histologic Analysis. Tumor specimens were fixed in 4% formaldehyde and embedded in paraffin, and 5-μm sections were stained with hematoxylin–eosin or incubated with anti-CD45 (anti-leukocyte common antigen, 1:200 dilution), anti-CD3 (anti-T-cell receptor, 1:1000 dilution) or anti-B220 (anti-B-cell antigen, 1:100 dilution; PharMingen). Staining was developed using biotinylated rabbit-antirabbit antibodies (1:100 dilution), the avidin–biotin peroxidase complex (1:25 dilution; Vector Laboratories, Burlingame, CA), and True Blue Peroxidase substrate as the final chromogen (KPL Laboratories, Gaithersburg, MD).

TCDS90 Studies. Serial dilutions of tumor cell suspensions (1.0 × 105–1 × 106 cells) were inoculated subcutaneously in 0.1 mL as described above. At least five asmase+/+ and asmase−/− mice were used for each dose. Tumor take was scored when implanted tumors reached 8 mm in diameter (8–10 days post-implantation). Animals not displaying tumors were followed for at least 2 months.
RESULTS

MCA/129 Fibrosarcomas and B16F1 Melanomas Do Not Elicit Host Immune Responses. To explore whether MCA/129 fibrosarcomas and B16F1 melanoma tumors are immunogenic in the sv129xBl/6 background, histopathologic and immunopathologic studies were conducted in tumors implanted in asmase−/− mice and asmase+/− control littersmates before and after exposure to 15 Gy. Hematoxylin–eosin staining detected only rare peritumoral or endotumoral mononuclear, lymphocytic, or inflammatory infiltrates in any of the tumor–host combinations (Fig. 1A and B), regarded as hallmark features of tumor immunogenicity (10). The lack of immune cell infiltrates was confirmed using immunohistochemical markers. A representative 129/MCA fibrosarcoma stained with anti-CD45 (leukocyte common antigen) shows only occasional positive peritumoral cells (Fig. 2A, positive cells in brown). A more detailed analysis of these rare positive cells indicates immunoreactivity to the B-cell antigen B220 (Fig. 2B) but not to T-cell markers (Fig. 2C). Positive controls for each specific marker were performed in parallel in sections from a mouse axillary lymph node, as shown in Fig. 2D for anti-CD45 antibody. Similar results were obtained with B16 melanomas either before or after irradiation. These results are inconsistent with the hypothesis that 129/MCA fibrosarcomas or B16 melanomas elicit a substantive immune response in these mouse systems.

Tumor Growth and Radioresponsiveness of 129/MCA Fibrosarcomas and B16F1 Melanomas Are Unaffected by Implantation into Rag−/− or MEF−/− Mice. To provide additional evidence that the tumors tested in our systems were nonimmunogenic, a genetic approach was undertaken. It was reasoned that if immunogenicity of the 129/MCA fibrosarcomas or B16F1 melanomas was the cause of the slow tumor growth or enhanced radioresponsiveness observed in wild-type mice, this phenotype would be reversed when tumors were implanted into animals incapable of mounting an immune response. For these studies, tumors were implanted into mice manifesting the Rag−/− genotype, which lacks B and T cells (13), and into wild-type littersmates. Fig. 3 (left panel) shows that the growth rate and radiosensitivity of B16F1 melanomas grown in Rag−/− are similar to those found in Rag+/+ littersmates. Because the Rag colony is maintained in the C57BL/6 background and 129/MCA fibrosarcomas grow only in the sv129xBl/6 background, wild-type mice (sv129xBl/6) were treated with 12 Gy WBR to sterilize the bone marrow and were reconstituted with 107 bone marrow cells from Rag−/− or Rag+/+ littersmates. The growth pattern and radiation responsiveness were evaluated in stable chimeras 4 weeks after marrow take. As observed with the B16F1 melanomas, the growth pattern and radiosensitivity of 129/MCA tumors were insensitive to the Rag genotype of the immune system (Fig. 3, right panel).

Similar studies were performed using MEF−/− mice, which lack NK and NK-T cells (12). B16F1 melanoma tumors, implanted into MEF−/− and MEF+/+ mice, manifested no differences in growth or radiosensitivity as shown in Fig. 4 (left panel). Because the MEF colony is also maintained in the C57BL/6 background, sv129xBl/6 mice were reconstituted after 12 Gy WBR with bone marrow from MEF−/− or control MEF+/+ littersmates. As observed with the B16F1 melanoma, the growth pattern and radiosensitivity of 129/MCA tumors were unaffected by the MEF genotype of the immune system (Fig. 4, right panel). Taken together, these data support the notion that the growth patterns and radioresponses of 129/MCA fibrosarcomas and B16F1 melanomas in our experimental systems were not modified by host NK-, T-, or B-cell antitumor immune functions.

Development and Function of B, T, and Natural Killer Cells Are Normal in asmase−/− Mice. To study whether the accelerated tumor growth and lack of tumor response to radiotherapy in asmase−/− mice are associated with a defective host antitumor immune response, we first compared by fluorescence-activated cell sorting analysis the numbers of B, T, or NK cells in spleens obtained from asmase−/− and asmase+/+ littersmates (Fig. 5A). The percentage of T cells detected by anti-T-cell receptor antibody in asmase−/− splenocytes (46.7%) was similar to that of controls (45.0%). The level of B cells detected by anti-CD19 was also similar at 33.8% in asmase−/− and 32.2% in asmase+/+ splenocyte populations. T- and B-cell pop-

Fig. 2. The lack of immune cell infiltrates is confirmed using immunohistochemical markers. Representative 5-μm sections of MCA/129 fibrosarcoma tumor stained with anti-CD45 antibody (A; leukocyte common antigen) showing only occasional peritumoral and endotumoral positive cells (positive cells manifest brown signal), anti-B220 (B; B-cell antigen) showing occasional peritumoral and endotumoral positive cells (positive cells manifest brown signal), or anti-CD3 (C; T-cell receptor), which did not display positive staining. D, lymph node used as positive control for anti-CD45 (magnification, ×200).
Fig. 3. Tumor growth and radioresponsiveness of 129/MCA fibrosarcomas and B16F1 melanomas are unaffected by implantation into Rag−/− mice. Left panel shows the tumor growth and radioresponsiveness of B16 melanomas implanted into Rag+/+(wild-type) and Rag−/− mice (C57BL/6 background). Right panel shows growth pattern and radioresponsiveness of 129/MCA fibrosarcomas implanted into asmase−/− mice (sv129xC57BL/6 background) harboring Rag−/− or Rag+/+ bone marrow (C57BL/6 background). For autologous bone marrow transplantation, 10⁷ bone marrow cells harvested from femur and tibia of donor mice were irradiated on day 10, and MCA/129 fibrosarcoma tumors on day 14. Number of mice per group is indicated in parentheses. Values are means ± SEM.

Fig. 4. Tumor growth and radioresponsiveness of 129/MCA fibrosarcomas and B16F1 melanomas are unaffected by implantation into MEF−/− mice. Left panel shows the tumor growth and radioresponse of B16 melanomas implanted into MEF+/+ (wild-type) and MEF−/− mice (C57BL/6 background). Right panel shows the growth pattern and radioresponse of 129/MCA fibrosarcoma implanted in asmase−/− mice (sv129xC57BL/6 background) harboring MEF−/− or MEF+/+ (C57BL/6 background) bone marrow as described in Fig. 3. For the irradiated groups, B16F1 melanoma tumors were irradiated on day 10, and MCA/129 fibrosarcoma tumors on day 14. Number of mice per group is indicated in parentheses. Values are means ± SEM.

Fig. 5. Showed the tumor growth and radioresponsiveness of MCA/129 fibrosarcoma tumors on day 14. Number of mice per group is indicated in parentheses. Values are means ± SEM.
asmase−/− mice. The TD50 for the MCA/129 fibrosarcoma and B16F1 melanoma was 7 × 10^5 cells each in either the asmase+/+ or asmase−/− background.

**DISCUSSION**

The present studies provide definitive evidence that MCA/129 fibrosarcomas and B16F1 melanomas do not elicit a host immune response while growing in wild-type asmase+/+ mice. Hematoxylin–eosin staining and immunohistochemical studies using CD45, B220, and CD3 antibodies detected only rare intratumoral or peritumoral immune mononuclear or inflammatory infiltrates, the hallmarks of tumor immunogenicity (10), before or after 15 Gy. Furthermore, genetic studies showed that tumor growth and radioresponsiveness were unaffected by implantation into Rag−/− mice, which lack functional T and B cells, or into MEF−/− mice, which have severe numeric and functional NK- and NK-T–cell defects.

The present studies also show that the asmase−/− mice are not defective in B-, T-, or NK-cell development or function. However, recent studies by Utermöhlen et al. (17) reported that macrophages of asmase−/− mice are deficient in the intracellular control of *Listeria monocytogenes* growth, perhaps due to the loss of ceramide-mediated maturation of the phagolysosome (18). Other essential macrophage stress responses appeared normal, however, including bacterial uptake, production of both classes of major antimicrobial effector molecules, generation of nitric oxide and reactive oxygen species, and heat-killed *L. monocytogenes* (HKLM)-induced interleukin (IL)-1 and IL-6 secretion. Similarly, Manthey and Schuchman (19) showed that tumor necrosis factor, IL-1β, and interferon-γ signaling was
intact in murine macrophages from asmase−/− mice. As macrophages may promote tumor initiation and accelerate tumor progression and metastasis (16), the potential impact of altered macrophage function on development of 129/MCA fibrosarcoma or B16F1 melanoma tumors was assessed. This possibility seems highly unlikely given the TD50 experiments showing no differences for either 129/MCA fibrosarcomas or B16F1 melanomas implanted into asmase+/+ or asmase−/− mice. 

Taken together, these data preclude the possibility that the low TD50 of 129/MCA fibrosarcoma in wild-type mice results from an exceptionally active host antitumor immune effect. The TCD concept is a widely accepted approach to assess curability of tumors with radiation, although experience with transplantable tumors in mice has also demonstrated that this method is extremely sensitive to experimental manipulation. The high TD50 values (30–100 Gy) reported in the literature may have resulted in many cases from host manipulations [i.e., pretransplant whole body irradiation (20–23)] or tumor manipulations [i.e., clamp hypoxia (21, 23–25)] before tumor irradiation. Whole body radiation, used in many studies before tumor implantation to “inactivate” antitumor host immune systems (20–23), may artificially increase TD50 values, at least in part by modifying the function of radiosensitive host bone marrow–derived endothelial precursors required for development of tumor neovascularization (2, 26, 27). Clamp hypoxia, often used to normalize for variations in tumor oxygenation, significantly increases TD50 values (21, 23–25). Experimental data demonstrated that clamp hypoxia, followed by clamp release and reoxygenation, initiates extensive cellular signaling, including activation of protein kinase C (28); the mitogen-activated protein kinases p38, c-Jun kinase, and extracellular signal-regulated kinase (28, 29); JAK/STATs (30); nitric oxide synthase (31); and nuclear factor-κB (32). These activated signaling pathways engage Cyclin D1 (33), induce transcription of COX-2 (30), increase the Bcl-2/Bax ratio (32), and up-regulate DNA repair enzymes (33, 34). These adaptive responses provide anti-apoptotic protection to endothelium (32), enhance radioresistance of tumor cells in vitro (35–38), and increase the TCD50 of experimental tumors (39, 40). Finally, the cultured tumor cell lines injected typically into mice to produce experimental tumors frequently represent highly selected clones with inherent resistance to stress required to survive tissue culture conditions: their cross-resistance to radiation may contribute to the high TCD50.

The relatively low TD50 value for 129/MCA fibrosarcoma is consistent with values recently reported for single-dose irradiation in human tumors. Multiple recent clinical studies using stereotactic radiotherapy for metastatic tumors to the brain (i.e., from lung, breast, melanoma, renal, colorectal, testicular, gynecologic, and thyroid tumors) reported 80 to 95% permanent local control after high single doses of radiation, regardless of tumor type (41–44). A study by Shiau et al. (44) reported a TD50 value of 15 Gy and a TCD50 value of ≥18 Gy for 261 metastatic lesions treated in 119 patients followed for a median of 1 year after therapy. Similar results have been reported in 60 primary and metastatic (colorectal, breast, lung, sarcoma, pancreatic, melanoma, and renal) tumors to the liver treated in 37 patients with stereotactic single-dose radiotherapy. At 10 months, all of the lesions treated with <20 Gy relapsed locally, compared with 80% local control for similar tumors after doses of 20 to 26 Gy (P < 0.0001). Koong et al. (45) reported in a phase I study of stereotactic radiotherapy in patients with locally advanced pancreatic cancer that three of eight patients receiving 15 to 20 Gy relapsed locally within a median period of 5 months, compared with zero of seven patients receiving 25 Gy. These studies demonstrate that the single-dose levels required to control the local growth of tumors classically considered radiosistant to fractionated radiotherapy (i.e., renal cell and pancreatic carcinoma, soft tissue sarcoma, and melanoma) are similar to those required to control relatively radiosensitive tumors (i.e., colorectal, breast, and lung).

Although our observations on the radiosensitivity profiles of MCA/129 fibrosarcoma and B16F1 melanoma are compatible with the clinical TCD values for single-dose radiotherapy, they both are distinctly different from the radiosensitivity profiles of human tumors treated with fractionated radiotherapy. Okunieff et al. (46) reviewed 62 published series on dose-response data of different human tumors treated with fractionated radiotherapy. The median TCD50 value for gross tumors of all types (excluding lymphomas) was 51.9 Gy (range 21.71–90.31 Gy). Unlike single-dose radiotherapy, the fractionated radiation TCD50 were highly sensitive to tumor type and the biological phenotype of the tumor, such as tumor size and stage, suggesting different mechanisms of action for these two modes of clinical radiotherapy.

Although the mechanism for single-dose radiation is unknown, it likely involves, at least in part, an endothelial tumor-clonogen–linked death mechanism, as described by us in murine MCA/129 fibrosarcoma and B16F1 melanoma. Such a mechanism is unlikely to prevail in fractionated radiotherapy, because our studies showed that the threshold for induction of apoptosis in angiogenic tumor endothelium is 8 Gy (2), which is significantly higher than the daily 1.8 to 2.5 Gy per fraction used nearly universally in fractionated radiotherapy of human cancer. Hence, the fractionated radiation scheme likely bypasses the endothelial-clonogen–linked death mechanism, switching to the more resistant, reproductive (also known as mitotic or post-mitotic) cell death mechanism. This hypothesis, which may have significant implications for the design of optimal radiotherapy of human cancer, is currently being investigated.

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REFERENCES


A. Narayana, manuscript in preparation.
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