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Editors

Stereotactic Radiosurgery and Stereotactic Body Radiation Therapy

A Comprehensive Guide

Springer

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Foreword

There are a large number of books on stereotactic radiotherapy. Some focus on specific techniques, and others on specific indications. Some books try to provide an overview of all available literature, often leaving the reader with many questions on how to do what in their specific situation for an individual patient. Well, this book is different!

After the successful implementation of stereotactic techniques for intracranial lesions in the past century, stereotactic techniques have also become an important treatment option for extracranial tumors, and the role of stereotactic body radiotherapy (SBRT) for lung, liver, and spine lesions is now well established. Moreover, SBRT is being explored and advocated for tumors and functional indications at various other locations.

In this book, the editors have done an excellent job in bringing together the information on all relevant topics in stereotactic radiosurgery (SRS) and SBRT. An impressive line-up of world-renowned experts provides an outstanding and comprehensive review of the biological aspects, radiation physics principles, clinical indications, and the available evidence. Instead of summing up all available literature, the chapters provide clearly written reviews with information which is scientific, but at the same time very practical and immediately applicable to daily clinic.

In the chapters on biology, the rationale for SRS and SBRT and the mechanisms of action of the high-dose hypofractionated treatments are discussed. Latest insights on the interaction of radiotherapy with the immune systems are addressed as well.

The chapters on SRS are partly technique-based, which enables the readers to have easy access to information on the techniques used at their centers. In a separate section, SRS is discussed in detail for the most frequent indications as well.

For SBRT, the general physics aspects including immobilization and motion management are described in detail. A separate chapter focuses on the use SBRT using charged particles. Apart from the important mainstream indications such as lung and spine tumors, newer indications—such as SBRT for head and neck cancer, gastro-intestinal tumors, kidney tumors, and prostate cancer—are addressed in various chapters.

The final chapters of this book provide an excellent overview of some general aspects, such as complication management and integration with other therapies, as well as of future directions of the rapidly emerging fields of SRS and SBRT.

This book is written with the reader in mind who is looking for an up-to-date and state-of-the-art overview of stereotactic radiotherapy for intra- and extracranial indications.

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Preface

Advancements in technology over the past several decades have created an atmosphere of multidisciplinary collaborative care that is very unlike medicine in the early twentieth century. Today, team-based approaches allow us to leverage techniques and modalities for the benefit of the patient. Radiosurgery is a perfect example of multidisciplinary collaboration and the benefit that specialization affords the patient, by blurring the lines between traditional medical specialities. Today, centers across the world have developed skills, techniques, and expertise in use of stereotactic radiosurgery (SRS) and stereotactic body radiation therapy (SBRT) for treatment of many diseases. Perhaps stereotactic radiosurgery can be seen as a budding discipline of its own as it continues to gain distinction that differentiates it from the arenas of conventional surgery and radiation therapy. Unfortunately, the sharing of this practical knowledge has been limited to specialty-specific annual meetings, frequently with conflicting recommendations between medical disciplines.

As a result, it is currently very difficult for a clinician (either in training or in practice) to gain experience and expertise in SRS and SBRT. *Stereotactic Radiosurgery and Stereotactic Body Radiation Therapy: A Comprehensive Guide* was created for the purpose of centralizing the knowledge and experience of experts across a variety of disciplines.

Our vision is that this book be used to serve as a basis for the current state of the art of SRS and SBRT. There is no doubt that advancements are made almost daily and refinements will be made as technology advances. However, for providers seeking to expand their knowledge and grow their radiosurgical skill set, this text will serve as a resource to allow for their development.

We thank our gracious contributors for lending their expertise toward the further advancement of our field and toward the improved care of our patients. Their efforts to improve the care of patients cannot be overstated.

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Part I

Radiobiology of Radiosurgery and Stereotactic Body Radiation Therapy

Vascular-Mediated Mechanisms and SRS/SBRT

Golnaz Farhat, Deepa Sharma, and Gregory J. Czarnota

Abbreviations

ASMase	Acid sphingomyelinase
bFGF	Basic fibroblast growth factors
CSC	Cancer stem cells
Gy	Gray
HIF	Hypoxia-inducible factors
LQ	Linear quadratic
O_2	Oxygen
ROS	Reactive oxygen species
S1P	Sphingosine-1-phosphate
SBRT	Stereotactic body radiotherapy
SM	Sphingomyelin
SRS	Stereotactic radiosurgery
USMB	Ultrasound-stimulated microbubbles
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor

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Introduction

Tumor vasculature plays a significant role in the proliferation and survival of tumor cells. The state of the vascular component determines tumor microenvironmental conditions and the overall tumor response to radiation therapy. Until recently, our understanding of tumor radiobiology was based on conventional fractionated radiotherapy. The role of tumor vasculature was viewed as a modulating factor of the tumor response to radiation through the reoxygenation of hypoxic cells after each fraction of radiation. The engagement of the vascular component with a high dose of radiation per fraction, as seen with stereotactic radiosurgery (SRS) and stereotactic body radiotherapy (SBRT), however, is different. High single doses of radiation cause a severe vascular response, resulting in rapid vascular deterioration. The underlying cellular and molecular mechanisms leading to vascular damage and disruption involve the activation of a cell death pathway mediated by ceramide in vascular endothelial cells. Tumor cells in this response predominantly die as a secondary effect to vascular damage, as opposed to dying by apoptosis resulting from direct radiation damage. Given the significance of the vascular response with high doses of radiation, understanding the effects and underlying mechanisms will play a key role in treatment planning for SRS and SBRT.

This chapter begins with a brief review of tumor vasculature characteristics and their role in determining the radiosensitivity of tumor cells. An overview of recent studies observing the effects of high-dose-per fraction radiation on the vascular component, as well as the cellular and molecular mechanisms of vascular disruption and secondary tumor cell death will follow. Finally, novel methods currently under development for enhancing the vascular response to high doses of radiation will be reviewed.

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Background and History

Tumor Angiogenesis

Angiogenesis, the growth of new capillary blood vessels, is necessary for tumor growth and metastasis. Judah Folkman first suggested, in 1971, that a strong interdependence exists between tumor parenchymal cells and the endothelial cells within the tumor vasculature, which he described as being a "highly integrated ecosystem" [1, 2]. Angiogenesis is a highly controlled process, predominantly regulated by the availability of oxygen. In tumors, the rapid proliferation of cells results in a surge of metabolic activity, increasing the demand for oxygen (O_2). An inability to provide sufficient O_2 to tumor cells results in localized regions of tumor hypoxia [3]. In response to hypoxia, tumors release diffusible angiogenic factors [4], the expression of which are regulated by transcription factors called hypoxia-inducible factors (HIF). It is well established that the HIF pathway is the master regulator of angiogenesis. HIF-regulated proangiogenic factors increase vascular permeability, endothelial cell proliferation, sprouting, migration, adhesion, and tube formation [3, 5].

A tumor may start as an avascular mass obtaining its blood supply through vessel co-option by growing alongside existing, well-established blood vessels. However, it can also grow and develop a new vascular network through various mechanisms. Sprouting angiogenesis is the growth of new capillary vessels from pre-existing ones [6] and occurs because of endothelial cell activation by basic fibroblast growth factors (bFGF) and vascular endothelial growth factors (VEGF). Degradation of the extracellular matrix and basement membrane of the existing vessels allows the proliferation and invasion of endothelial cells into the surrounding matrix. The development of tumor vasculature also occurs through intussusceptive angiogenesis, a rapid process that results in the division of a pre-existing blood vessel into two new vessels through the formation of transvascular tissue pillars [7]. More recently, it has been discovered that entirely new vessels may be formed by the recruitment of endothelial progenitor cells and in situ differentiation of endothelial cells from these precursor cells. These are subsequently organized into a vascular structure. Endothelial progenitor cells have been found in adult peripheral blood [8]. Finally, vasculogenic mimicry – the formation of new blood vessels by tumor cells themselves – has been reported to be a precursor to sprouting angiogenesis and is present in highly aggressive tumors [9].

Characteristics of Tumor Vasculature and Blood Flow

The vascular network and branching patterns in tumors are far from the organized hierarchical branching pattern seen throughout the human body. Normal vasculature is a weblike and well-organized network of capillaries. On a smaller scale, the capillary walls in normal tissue consist of a well-constructed tube composed of endothelial cells, surrounded by a basement membrane and sparsely placed pericytes between the two layers [10]. To meet the needs of highly metabolically active and rapidly proliferating tumor cells, the tumor endothelium is also rapidly proliferating [11]. The resulting vascular network is composed of vessels that are defective and structurally abnormal, tortuous, and often dilated, elongated, and saccular [12]. The vessels are leaky due to the defective vessel lining composed of areas in which the endothelial cells are stacked atop one another and others in which the cells are sparsely distributed. These vessels have uneven diameters due to compression of the poorly formed vessel walls by neighboring tumor cells [10]. The resulting perfusion is poor, intermittent, and often stationary due to the collapse of smaller blood vessels under the high interstitial pressure of tumors caused by poor lymphatic drainage [13]. The tumor vessel network does not follow a regular branching pattern. Instead, it is highly chaotic with poor three-dimensional coverage of the tumor volume. This results in large avascular tumor regions [10] suffering from hypoxia and a highly acidic, nutrient-deprived tumor microenvironment.

Tumor Vasculature and Radiosensitivity

Tumor vasculature has a direct effect on the tumor microenvironment – in particular affecting oxygenation status and acidity. The tumor microenvironment, in turn, affects the viability and proliferative ability of tumor cells. It is well known that oxygenation status has a large influence on the radiosensitivity of tumor cells with the response to radiation therapy being very poor in hypoxic regions [14]. The availability of molecular O_2 is necessary for the cytotoxic effects of radiation, mediated through the formation of reactive oxygen species (ROS) [15]. Radiosensitivity is, therefore, closely related to the state of tumor perfusion and the structure of tumor vasculature.

In 1936, Mottram [16] observed that the well perfused, and thus well oxygenated, tumor rim was more radiosensitive than the hypoxic core. Studies conducted in France in the 1920s and 1930s by Regaud, Ferroux, and Coutard also demonstrated that the therapeutic ratio in radiotherapy could be increased by delivering treatment through multiple small fractions [17]. This was because of reoxygenation that occurred after each radiation fraction. As cells in well-perfused tumor regions were killed, oxygen-deprived cells gained access to previously inaccessible capillaries, in effect reoxygenating them and increasing their sensitivity to the next fraction of radiation [18]. The role played by the vascular effects of radiation was seen, through the lens of fractionated radiotherapy, as an indirect modulator of radiosensitization [15, 19–22]. However, in 2003, Garcia Barros demonstrated that tumor microvascular damage also regulates tumor cell responses to radiation, painting a more

complex picture of radiation-induced tumor cell death. Their work indicated that the vascular endothelial cell, rather than the tumor cell, may be the primary target for radiation therapy [23].

Recent Advances: Vascular-Mediated Mechanisms of Tumor Response

Observed Vascular Effects of High-Dose Radiation

A limited number of human studies have investigated vascular effects of radiotherapy, the majority of which are concerned with conventional fractionated radiotherapy. These studies generally observed that blood flow increased slightly, or remained the same as pre-irradiation levels, early during a course of fractionated therapy and decreased thereafter [24–26]. Recently, Kumar and colleagues reported the results of a pilot study in which 30 patients suffering from spinal metastases received either single-fractionated SRS (24 Gy) or hypo-fractionated stereotactic radiosurgery (3–5 fractions, 27–30 Gy total). Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) scans were acquired before and after radiotherapy to assess perfusion. The plasma volume, V_p, which is related to tumor vascularity, was significantly reduced in patients who were determined to have local tumor control at their 20-month follow-up (−76%) compared to patients with local recurrence (+28%) [27]. The reduction in vascularity following SRS preceded and was predictive of local tumor control with a sensitivity of 100% and a specificity of 98%. An earlier study by the same group reported similar results in a cohort of 12 patients with metastatic sarcoma lesions in the spine [28].

Despite the limited number of published human studies investigating the vascular effects of high doses of radiation from SRS and SBRT, there are several animal studies that can provide insight into the types of effects that can be expected. While these studies, reviewed by Park and colleagues [12], span many different tumor models and have results with some level of heterogeneity, some general trends have been observed. In tumors receiving single, moderately high doses of radiation (5–10 Gy) an initial increase in blood flow is followed by a return to pre-irradiation levels, or slightly below, within a few days. At higher single doses (10–15 Gy), an immediate decrease in blood flow persists for several days, with a return to control levels in some cases. Finally, at very high single doses (15–20 Gy), tumor blood flow decreases rapidly and is accompanied by vascular disruption and, eventually, tumor cell death. Radiation-induced microvascular effects observed in clinical and preclinical studies at various doses are listed in Table 1.

High single-dose radiation effects on tumor vascular permeability have been reported by multiple groups. A large body of work contributing to our understanding of these effects was produced in the 1970s with the investigation of vascular effects in Walker 256 rat mammary carcinoma tumors treated with radiation doses ranging from 2.5 Gy up to 60 Gy in a single dose [29–31]. Vascular permeability was assessed by measuring extravasation of plasma protein via iodine-125-labeled albumin. An increase in vascular permeability peaked at 24 h after irradiation for doses ranging from 2–20 Gy. The changes were dependent on the dose and the number of fractions, with 20 Gy delivered in a single dose causing a more substantial effect than the same dose delivered in four or eight fractions. In all cases, the increase in vascular permeability was transient, returning to pre-irradiation levels within a few days. Dose-dependent decreases in vascular volume were also observed. These were transient, lasting hours, at doses below 2.5 Gy, persisting for several days at doses in the 5–10 Gy range, and were significant and lasting at higher doses. More recent studies have observed similar increases in vascular permeability with single high doses of radiation [32, 33].

Mechanisms of Vascular Damage and Vascular Collapse

Vascular effects of radiation are directly related to the death of vascular endothelial cells. Tumor endothelial cells are significantly more radiosensitive than those in normal tissue vasculature [34]. Endothelial cell death widens the junctures between cells in the vessel lining, which, in tumors, is already compromised due to poor structure and uneven distribution of endothelial cells. Eventually, the affected microvessels will rupture or collapse [31]. Erythrocyte concentration in the capillaries will increase due to extravasation of plasma, leading to slow or static blood perfusion [35] and elevation of interstitial fluid pressure in the tumor, causing further vascular collapse [29].

In recent years, the notion that the tumor cell is the primary target of ionizing radiation is being replaced by the notion that tumor microvascular endothelial cell death is required for tumor cure. The interaction between tumor microvascular endothelial cells and tumor parenchymal cells is complex and dose-dependent (Fig. 1). At low doses (1–3 Gy/fraction), tumor cell death is dependent on the presence of reactive oxygen species made newly available after each cycle of hypoxia, reperfusion, and ionization during fractionated radiotherapy [15]. Work by Moeller and colleagues has indicated that the repeated surges of reoxygenation and the presence of ROS may lead to increased HIF-1 activity and the secretion of proangiogenic cytokines, including VEGF and bFGF. These cytokines exert a protective effect on endothelial cells and have the effect of attenuating the apoptotic response of endothelial cells to radiation [36]. Moeller and colleagues further demonstrated that HIF-1 regulates pathways that promote radiosensitization and apoptosis of tumor cells through increased tumor cell proliferation and p-53 activation. The complexity of these interactions makes the net effect of HIF-1 induction difficult to predict [37].

Table 1 Radiation-induced vascular effects observed in clinical and preclinical studies

Dose per fraction	Tumor model	Observed vascular effect	Source
1.9 Gy (fractionated)	Human (advanced cervical carcinoma)	Decrease in tumor vascularity during treatment, which was associated with better treatment outcome	Pirhonen et al. [70]
2 Gy (fractioned daily for 4–5 weeks)	Human (advanced cervical cancer)	Decreased blood perfusion in 50% of patients midtherapy with further decrease in 80% of patients after completion of treatment	Mayr et al. [26]
2.5 Gy (fractionated)	Rat (Walker carcinoma 256)	Transient decrease in vascular volume and increase in vessel permeability	Wong et al. [31]
2.5 Gy	Mouse (neuroblastoma)	Initial increase in functional intravascular volume and extravasation of plasma protein and decrease thereafter	Song et al. [35]
4 Gy/fraction (daily for 5 days)	Rat (BT4C malignant glioma)	Reduction in tumor microvascular density	Johansson et al. [71]
5 Gy	Mouse (mammary carcinoma)	Slight transient decrease in vascular volume with recovery within 4 days	Hilmas et al. [72]
5 Gy	Rat (mammary adenocarcinoma, in a window chamber)	Increase in vascular density and perfusion observed 24 and 72 h after treatment	Dewhirst et al. [73]
5 Gy (once weekly for 4 weeks)	Mouse (MA148 human ovarian carcinoma)	Reduction of 50% in microvessel density	Dings et al. [74]
4.5 Gy/fraction (six fractions over 3 weeks)	Human (advanced non-small cell lung cancer)	Increase in tumor vascular blood volume and permeability, with greater changes observed in tumor periphery compared to the center	Ng et al. [75]
5 Gy/fraction, five consecutive days	Human (nonlocally advanced rectal cancer)	Early increase in tumor perfusion	Janssen et al. [76]
10 Gy and 20 Gy (single doses)	Mouse (neuroblastoma)	Early decrease in vascular blood volume with further gradual decrease thereafter	Song et al. [35]
10 Gy	Mouse (human laryngeal squamous cell carcinoma)	Slight early increase in perfused blood vessels, subsequent significant decrease at 26 h and eventual return to control level by day 11	Bussink et al. [77]
10 Gy	Mouse (human melanoma)	Reduction in tumor blood perfusion of 60% at 72 h after irradiation	Brurberg et al. [78]
10–15 Gy	Mouse (human melanoma)	Loss of function in 35–45% of 5–15 μm diameter vessels within 1 week	Solesvik et al. [79]
12 Gy	Rat (A549 human lung cancer)	Significantly decreased vascular oxygenation within 24 h	Zhou et al. [80]
15 Gy	Mouse (MCF/129 fibrosarcoma)	Significant endothelial cell apoptosis leading to microvascular damage in $ASMase^{+/+}$ mice	Garcia-Barros et al. [23]
15 Gy	Mouse (FSC-1 and T43 tumors)	Significant reduction in functional vascularity leading to tumor growth delay	Ogawa et al. [81]
15 Gy	Mouse (human glioblastoma multiforme)	Decrease in blood perfusion to 10–30% of control within 2 weeks, with restoration of damaged vasculature thereafter	Kioi et al. [82]
15 Gy	Mouse (mammary carcinoma)	Decrease in vascular volume with no recovery	Hilmas et al. [72]
16.5 Gy	Rat (transplanted rhabdomyosarcoma)	Early 35% reduction in blood flow followed by complete recovery within 24 h	Emami et al. [83]
20 Gy	Mouse (adenocarcinoma)	Disruption in blood flow induces indirect cell death in 2/3 of tumor cells beginning 2 days after irradiation	Lasnitzki et al. [84]
20 Gy	Rat (Walker carcinoma 256)	Marked increase in plasma protein extravasation soon after irradiation with abrupt decline thereafter; significant decrease in functional intravascular volume for up to 11 days post irradiation	Song et al. [30]
20 Gy	Mouse (neuroblastoma)	Progressive, significant decrease in vascular volume, transient increase in extravasation of plasma protein, tumor regression accompanied by disorganization, aggregation and condensation of vascular network	Song et al. [35]
20 Gy	Mouse (Lewis lung carcinoma)	Marked decrease in tumor blood flow within 2 days followed by substantial recovery by day 4 after irradiation. Sustained blood flow achieved with second 20 Gy dose delivered 2 days after initial dose	Kim et al. [85]
25 Gy	Mouse (murine prostate TRAMP-C1 tumors)	Progressive, significant decrease in tumor microvascular density, over 3 weeks after irradiation, to 25% of that in unirradiated tumors	Chen et al. [86]
24 Gy	Human (spinal metastases)	Significant decrease in MRI perfusion parameters measured at 20-month follow-up in patients without local recurrence	Kumar et al. [27]
45 Gy	Mouse (mammary carcinoma)	Extensive microvascular damage	Hilmas et al. [72]
60.5 Gy	Rat (transplanted rhabdomyosarcoma)	Early 50% reduction in blood flow reduction that remained decreased at 72 h postirradiation	Emami et al. [83]

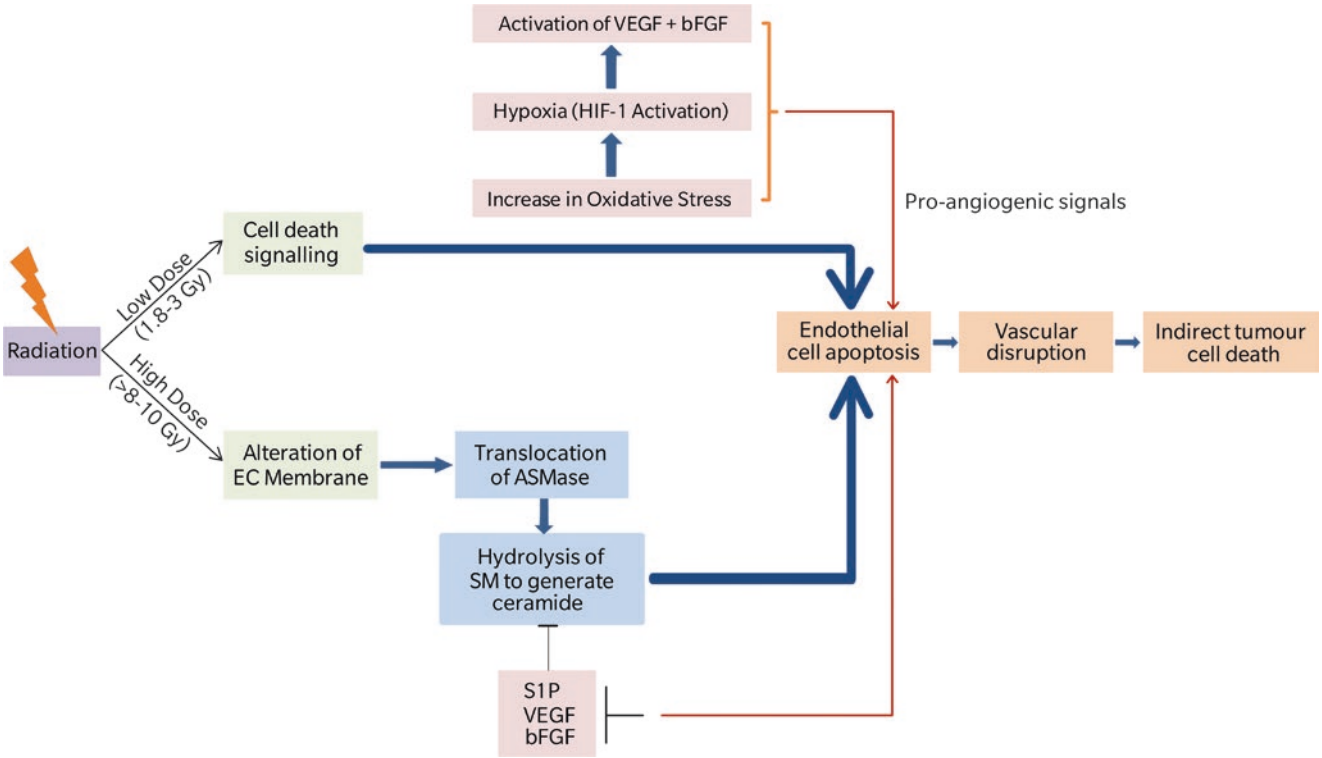

Fig. 1 Model of the dose-dependent microvascular endothelial cell response to irradiation. The vascular response to both low and high doses of radiation is illustrated in this schematic. Low-dose (1.8–3 Gy) fractionated radiation therapy initiates the activation of cell signaling pathways that result in apoptotic endothelial cell death. The generation of oxidative stress through repeated cycles of hypoxia and reoxygenation induces the release of hypoxia-inducible factor 1 (HIF-1), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF). These factors promote endothelial cell survival and have a significant quenching effect on the cell death signals. In response to high doses per fraction of irradiation (>8–10 Gy), endothelial cell ASMase is translocated to the outer leaflet of the cell membrane where it hydrolyzes sphingomyelin (SM) to generate ceramide. Ceramide acts as a second proapoptotic messenger and activates the apoptotic cascade. Proangiogenic factors such as sphingosine-1-phosphate (S1P), VEGF, and bFGF elicit a protective antiapoptotic effect if present in sufficient quantities

In the single, high-dose fraction scenario (>8–10 Gy), endothelial cell death is mediated through an acid sphingomyelinase (ASMase) pathway. Upon stimulation of cell surface receptors, ASMase, a lysosomal enzyme, translocates to the plasma membrane and hydrolyzes sphingomyelin, a phospholipid located on the outer layer of the membrane, to generate ceramide. Ceramide acts as a second messenger, activating downstream signaling pathways that initiate the cell death process [38–41]. Haimovitz-Friedman and colleagues demonstrated that ionizing radiation can interact with the cell membrane to generate an apoptotic signal through this pathway [39, 42]. This contrasts with the classic theory of ionizing radiation-induced cell death occurring through a p53-mediated pathway resulting from damage to cellular DNA. Endothelial cells are particularly vulnerable to radiation-induced apoptosis through the ASMase pathway because they have a 20-fold higher level of secretory ASMase compared to other cell types [38, 43, 44]. The mechanism of endothelial cell apoptosis through the ASMase pathway has been extensively investigated and reported by Kolesnik and Fuks. The window of radiation doses for which ceramide-mediated endothelial cell death occurs starts at 8–10 Gy in a single exposure and peaks at 20–25 Gy [38].

Endothelial Cell Damage Leads to Indirect Tumor Cell Death

Garcia-Barros and colleagues demonstrated that endothelial cell apoptosis regulates tumor cell response to radiation. Fibrosarcoma (MCA/129) and melanoma (B16F1) tumor xenografts were completely resistant to a single 15 Gy exposure when grown in ASMase-deficient mice, whereas the same condition in wild-type mice produced 50% tumor control. Their work further showed that initial rapid endothelial cell apoptosis occurred in these tumors, beginning at 1 hour and peaking at 4–6 hours post-irradiation. Tumor cell death detected during this window was minimal but increased significantly over a period of several days later. Ceramide-mediated endothelial cell apoptosis lead to secondary tumor

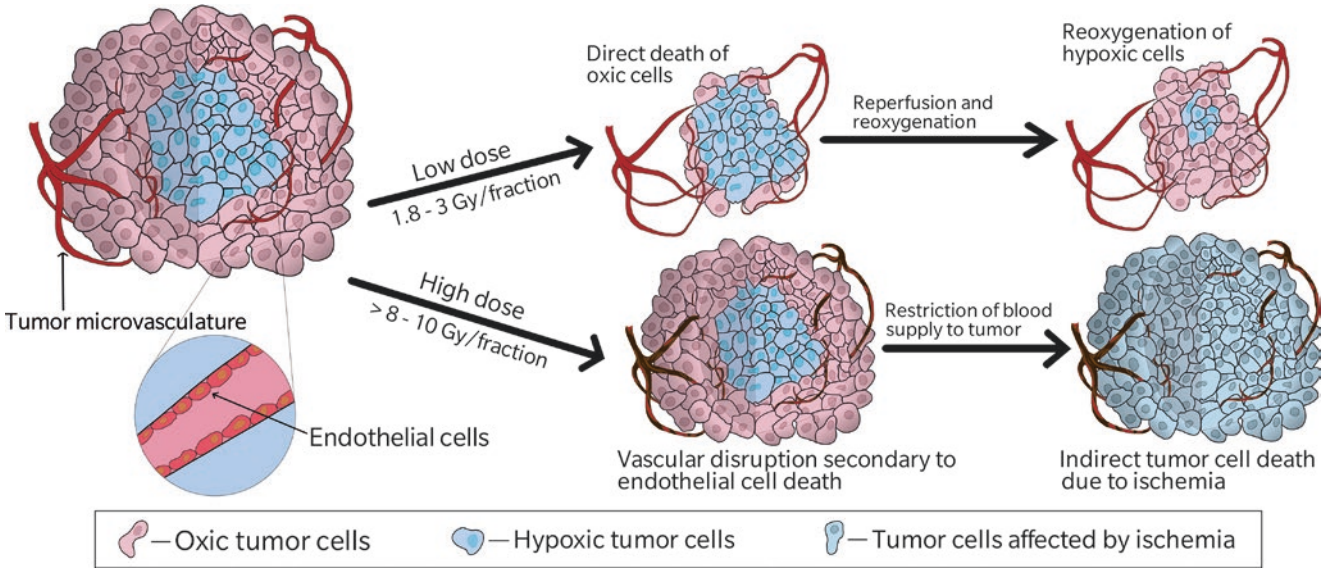

Fig. 2 Tumor cell death in response to single-high-dose or fractionated radiotherapy. Tumor response to low-dose fractionated radiotherapy is dominated by cell death resulting from radiation-induced DNA damage. Radiosensitive, oxygenated cells are preferentially killed, allowing the reperfusion and reoxygenation of hypoxic cells with each fraction of radiation. In contrast, the high-dose response is dominated by secondary tumor cell death resulting primarily from ischemia. Endothelial cell death leads to severe microvascular damage and causes starvation of tumor cells throughout the entire tumor volume

cell death and was proven to be mandatory for tumor cure. Tumors grown in ASMase-deficient mice were resistant to the curative effects of single-dose radiotherapy [23, 45]. Clinical evidence has also demonstrated that patients treated with a single high dose of spatially fractionated radiation of 15 Gy to treat large bulky tumors exhibited elevated serum levels of secretory sphingomyelinase (S-SMase), a protein product of ASMase and ceramide, and that this correlated with the level of tumor response to the treatment.

In clinical studies of cranial and extra-cranial tumors treated with SRS and SBRT, respectively, 80–90% tumor control was achieved with single radiation exposures in the range of 18–24 Gy [46–49]. Brown and colleagues conducted mathematical calculations of the expected level of cell kill using the standard linear quadratic (LQ) model and assuming 20% of cells are hypoxic. Their results concluded that the level of tumor control achieved by Yamada and colleagues at the given doses could not be explained by direct tumor cell death alone [50, 51]. Kocher and colleagues reached a similar conclusion when using a Monte Carlo simulation to fit clinical response data from 90 patients receiving single-dose irradiation (median marginal dose of 20 Gy) to treat brain metastases. The dose-response relationship observed clinically could not be reproduced using the LQ model without introducing significant vascular effects into the model [52]. Given the LQ model implicitly assumes that the underlying mechanism causing tumor cell kill is DNA damage, its inability to predict high-dose-per- fraction effects of radiation points to additional biological mechanisms at play [53]. The evidence suggests that two mechanisms contribute to tumor response to high-dose-per-fraction ionizing radiation. The first mechanism is direct cytotoxic damage to tumor cells caused by DNA damage, which occurs with both low and high doses per fraction. The second mechanism is indirect tumor cell death, preceded by vascular damage and endothelial cell death, which occurs preferentially at higher doses per fraction (Fig. 2).

The fraction of tumor cells succumbing to direct versus indirect death is dose-dependent, with indirect death becoming significant when the radiation dose per fraction reaches levels high enough to cause vascular damage. Park and colleagues, based on the conclusions from numerous clinical and preclinical studies, have estimated the threshold dose for indirect tumor cell death, resulting from a single exposure to ionizing radiation, to be in the range of 10–15 Gy for most human tumors [12]. A hypothetical illustration of the dose-dependence of cell death mechanisms in tumors has been illustrated by Song and colleagues [13]. Assuming 10% of clonogenic cells are hypoxic, direct tumor cell death of oxic cells dominates in the 0–5 Gy range, direct tumor cell death of hypoxic cells dominates in the 5–12 Gy range, and indirect tumor cell death of both oxic and hypoxic cells due to vascular damage dominates at doses greater than 10–12 Gy. The doses quoted are per fraction of ionizing radiation, implying that the relative importance of direct versus indirect cell death in SRS and SBRT is dependent on the size of the fraction rather than the overall dose.

The exact mechanisms leading to indirect tumor cell death are not fully understood. Ischemic cell death, caused by transient local hypoxia and nutrient deprivation resulting from vascular disruption, likely contributes significantly. Each endothelial cell is estimated to subtend a segment of tumor containing approximately 2000 tumor cells [54]. Disruption or collapse of even a small segment of microvasculature can lead to an avalanche of tumor cell death. A second contributing factor is thought to be a bystander effect, secondary to endothelial damage and leakage of circulating factors. Gaugler and colleagues have studied the bystander effect in unirradiated human intestinal epithelial T84 cells in a noncontact co-culture with irradiated endothelial cells [55]. They observed a 29% decrease in cell numbers and a 1.5-fold increase in apoptosis in the T84 cells. When both types of cells were irradiated together, the effects were further amplified, indicating that the bystander effect adds to the direct radiation damage. The bystander effect was specific to endothelial cells as the same effect could not be reproduced when the experiment was repeated with human colon fibroblasts. Finally, the interaction between self-renewing cancer stem cells (CSC) and microvascular endothelial cells is a potential third contributing factor. Recently it has been discovered that a small subpopulation of tumor cells, known as self-renewing cancer stem cells are responsible for tumor recurrence after radiotherapy. These CSC exhibit a higher level of radioresistance than non-stem cells [56]. This fact implies that tumor cure can only be achieved if all cancer stem cells are killed. Stem cells reside within niches composed of microenvironmental cells that regulate their proliferation and self-renewal properties through secreted factors [57–59]. Evidence suggests that the perivascular niche and the interaction of endothelial cells with brain tumor stem cells is critical for their survival [60]. Endothelial cell apoptosis and vascular disruption, resulting in disruption of the perivascular niche due to high single doses of radiation, could, therefore, have a direct effect on tumor control through the eradication of tumor stem cells.

Future Directions: Enhancing the Vascular Response with Combination Therapies

With recent advances in our understanding of the vascular component in tumor responses to high single fractions of radiation, it would be reasonable for future directions to take advantage of this interaction by combining radiotherapy with other treatment modalities that enhance the vascular response and increase the possibility of tumor cure, while de-escalating the overall delivered radiation dose. Czarnota and colleagues [61] have induced an enhancement of the vascular response to radiation using biophysical means to selectively target tumor vascular endothelial cells. This approach consists of using acoustic stimulation of microbubbles to mechanically injure the plasma membrane of endothelial cells. Microbubble solutions are currently in clinical use as ultrasound contrast agents and are comprised of gas spheres stabilized by a biocompatible lipid or protein shell. Their 3–4 micron diameter allows them to circulate freely within the microvasculature when injected intravenously. When placed within an ultrasound field at or near their resonant frequency, microbubbles may oscillate, cavitate, and even collapse, generating shear stresses on the membranes of nearby cells. This physical perturbation can have effects ranging from transient membrane permeabilization to complete destruction of the cell. In vitro and in vivo studies have demonstrated that ultrasound-stimulated, and microbubble-mediated endothelial cell perturbation can significantly enhance radiation therapy. In experiments with bladder, breast, and prostate tumor xenografts, mice were treated with ultrasound-stimulated microbubbles (USMB), followed by a single dose of 2–8 Gy of radiation. Significant tumor cell death (40–70%) was detected within 24 hours of treatment and demonstrated a whole tumor effect resulting in tumor regression [62–64]. The increase in cell death in tumors receiving a combination of USMB and radiation was significant and synergistic. A 2 Gy radiation treatment delivered to prostate tumor (PC-3) xenografts resulting in 4 ± 2% tumor cell death was converted to 40 ± 8% cell death when the treatment was combined with USMB [63]. Similar results were achieved in breast and bladder cancer xenografts [64–66]. Immunohistochemistry of tumor specimens identified endothelial cells as the primary target of the microbubble perturbation. Vascular leakage (detected using Factor VIII staining) and vascular collapse (detected using cluster of differentiation 31 (CD-31) staining) appeared to occur secondary to endothelial cell apoptosis resulting from the treatment. Significant differences in high-frequency power Doppler signals (drop in vascular index of 65% versus 20%), detected in tumors receiving the combined therapy versus radiation therapy alone, further confirmed the vascular effects. When delivered as multiple treatments, there was no evidence of a viable rim within the tumors, as seen with conventional fractionated radiotherapy. Instead, vascular disruption and cell death were observed across the whole tumor. Areas left with partially functioning vasculature responded after multiple treatments. Most importantly, survival studies demonstrated that mice receiving a 24 Gy dose (BED10 = 28.8) combined with USMB had better survival than mice receiving a much higher dose of radiation (BED10 = 58.5) alone. This method, thus, has the potential to convert a noncurative radiation dose into a curative one [63]. In vitro experiments with human umbilical vein endothelial cells, acute myeloid leukemia cells, murine fibrosarcoma (KHT) as well as breast (MDA-MB-231) and prostate (PC-3) cancer cells demonstrated that the synergy between USMB treatment and radiation is caused by mechanical damage to the endothelial cell

membrane, which activates the same cell death pathways activated by high-dose fractions of radiation. When combined with USMB, the activation of the ceramide apoptosis pathway was achieved with radiation doses as low as 2 Gy [67]. Manipulation of the ASMase pathway, either chemically or genetically, suppressed the radiation enhancement effect of USMB. A schematic of the treatment method and representative in vivo results are presented in Fig. 3.

Antiangiogenic approaches may be a viable avenue for further enhancing the vascular response to high-dose irradia-

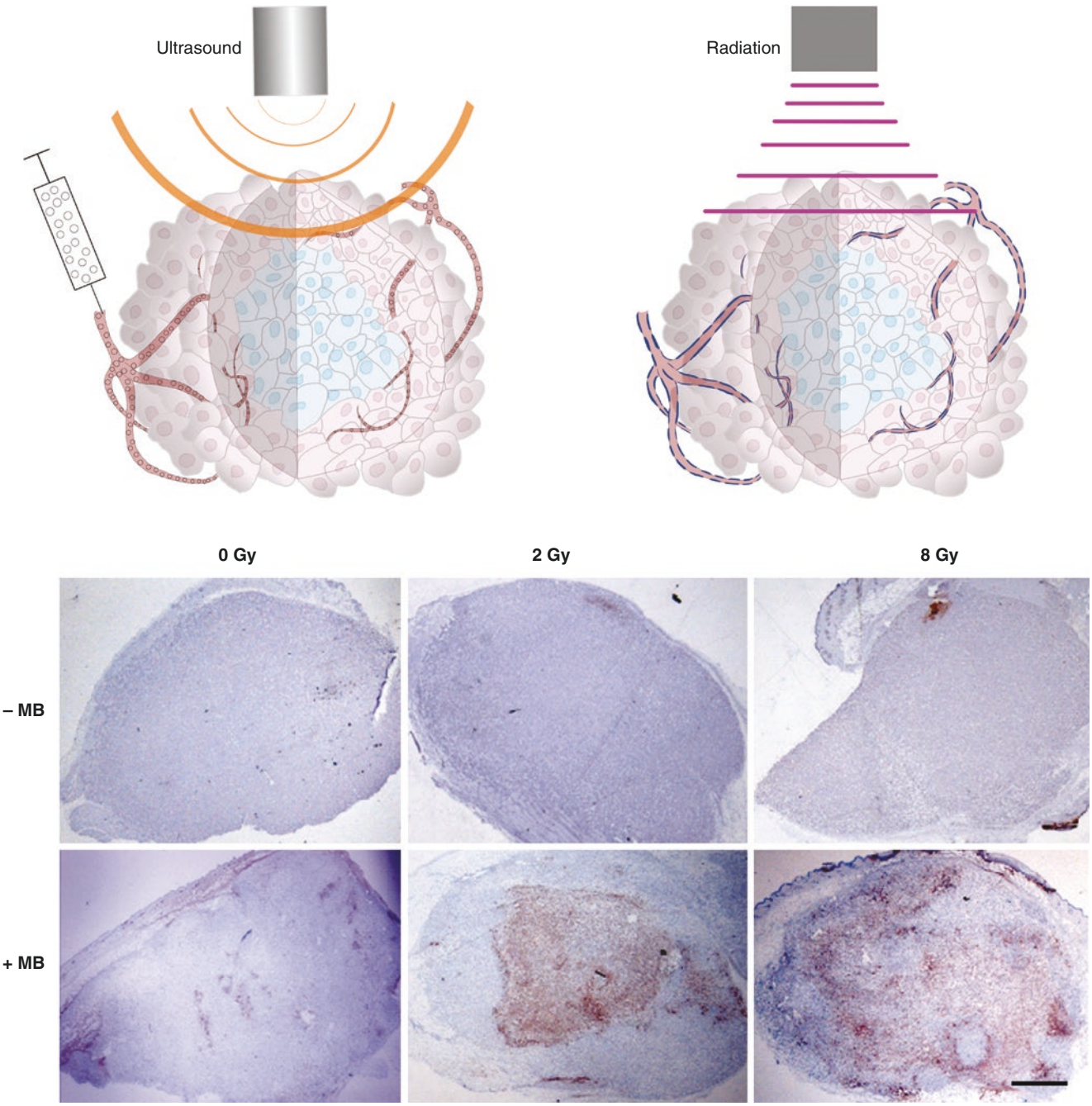

Fig. 3 Treatment schematic and representative in vivo results of ultrasound-stimulated microbubble radiation enhancement. Microbubbles are injected intravenously and circulate freely throughout the tumor microvasculature (top left). The tumor volume is subsequently targeted with ultrasound to stimulate microbubble cavitation. Endothelial cell membranes are perturbed (dark blue dashed outline, top right) by the microbubble therapy. The tumor microvasculature is radiosensitized and responds to radiation doses that are not normally sufficient to cause significant tumor cell death. The bottom panel shows representative images from in situ end labeling (ISEL)-stained sections of prostate cancer (PC3) tumors treated with radiation and/or ultrasound-activated microbubbles. Columns represent 0, 2, and 8 Gy of radiation exposure from left to right. Rows indicate the absence (–MB) or presence of microbubbles (+MB). Exposure to radiation alone (top row) resulted in no appreciable cell death as detected by ISEL-staining. Exposure to microbubbles alone resulted in minor cell death. The combination of ultrasound-stimulated microbubbles and radiation led to significant detectable cell death

tion. Antiangiogenic agents have been used in the context of radiosensitization by normalizing dysfunctional tumor vasculature, improving perfusion and, thus, the response to fractionated radiotherapy [68]. In contrast, Truman and colleagues have proposed the use of antiangiogenic therapy to enhance ceramide signaling. Their work has demonstrated that local ceramide levels within the outer leaflet of the plasma membrane dictate whether endothelial cells are in an antiapoptotic (proangiogenic) or proapoptotic (antiangiogenic) state [44]. Restoration of ceramide levels exogenously in cells where the ASMase pathway was previously inhibited by VEGF/bFGF reestablished apoptosis, even in the continued presence of VGEF/bFGF. An acute, yet transient inhibition of the vascular endothelial growth factor receptor (VEGFR) proved sufficient to evoke synergy with SBRT, indicating that the timing of antiangiogenic drug delivery is important – these agents should be delivered immediately prior to irradiation. In a preclinical study with fibrosarcoma tumors in mice, the delivery of axitinib (Pfizer), a VEGFR-selective small molecule inhibitor, enhanced tumor endothelial cell death and tumor cure when delivered immediately prior to single-dose radiosurgery [69]. The type of synergistic enhancement of the vascular response to radiation seen with USMB endothelial membrane perturbation or VEGFR inhibition using antiangiogenic therapy has the potential to allow for dose de-escalation in SRS and SBRT. Their implementation could reduce normal tissue toxicity while significantly improving treatment outcomes.

Conclusions

Studies have indicated that radiation delivered in high doses per fraction or in high single doses leads to severe vascular damage, vascular permeability, disruption, and deterioration. These effects result from vascular endothelial cell apoptosis caused by activation of the ASMase pathway through the interaction of radiation with the endothelial cell membrane. Endothelial cells are particularly sensitive to apoptosis via this pathway due to their 20-fold higher amount of secretory ASMase compared to other cells. The severe and rapid vascular deterioration leads to an ischemic event, causing secondary tumor cell death. This effect is observed across the whole tumor and is not limited to the viable tumor rim, as with conventional fractionated radiotherapy. Recent studies have investigated methods to synergistically increase tumor response to radiation by manipulating the ASMase activated apoptosis pathway in endothelial cells. Ultrasound-stimulated microbubbles can mechanically perturb the endothelial cell membrane, and antiangiogenic agents, such as axitinib can enhance ceramide signaling to achieve a similar effect. However, the presence of proangiogenic molecules, such as VEGF and bFGF, can dampen the effects of such therapies. Further investigation to determine optimal sequencing and timing of combination therapies is key to their successful clinical implementation. If implemented correctly, combination therapies may allow de-escalation of doses required to achieve tumor control or cure, thus, minimizing normal tissue toxicity.

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Radio-Immunology of Ablative Radiation

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Tumor Immunity Is Critical for Local Control of Tumors After Ablative Radiation

There were significant advancements in radiation technology over the last few decades, including the advent of image guided radiation therapy. With the introduction of stereotactic radiosurgery (SRS) stereotactic body radiation therapy (SBRT) or stereotactic ablative radiotherapy (SABR), high ablative doses of radiation can be safely delivered to a small, well-defined target with high accuracy and steep dose gradients, with local control rates similar to surgery. The use of computed tomography (CT) and multiple coplanar and noncoplanar radiation fields allows for the treatment of targeted tissue with minimal toxicity to surrounding normal tissue. Although conventional fractionation schedules in radiotherapy were considered beneficial in terms of reoxygenation and redistribution of cancer cells to more radiosensitive points of the cell cycle, fractionation with lower dose per fraction also allows for the survival of cancer stem cells, enabling repopulation and tumor regrowth. Several clinical studies have recently demonstrated >90% local control of the irradiated tumor with a short course (1–5 fractions) of ablative fractionation of RT. For example, SBRT with three 18 Gy fractions had a 3-year primary tumor local control rate of 97.6% and 3-year overall survival of 55% in inoperable lung cancer [1]. In another study, a single fraction of 24 Gy to metastatic spinal lesions led to a 90% local control rate [2]. Subsequent studies by these investigators showed that single-dose SBRT can effectively control extracranial metastases, irrespective of the histologic type and target organ, provided sufficiently high doses (>22 Gy) of radiation are delivered [3, 4].

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Tumoricidal effects of ionizing radiation is primarily attributed to dose-dependent DNA damage that results in growth arrest and senescence, as well as cell death via mitotic catastrophe, apoptosis, and necrosis of irradiated tumor cells. The lethal effects of irradiation on the tumor stroma have also contributed to tumor control. The high local control rates of single fraction SBRT have been attributed to the ablation of the tumor endothelium due to acid sphingomyelinase-mediated generation of ceramide in cell surface lipid rafts that signals the induction of apoptosis in the microvascular endothelium of the irradiated stromal tissues [5]. Although lethal effects of radiation were directly linked with the radiation-induced DNA damage in irradiated cells, numerous preclinical [6, 7] and clinical studies [8–10] have shown that an intact immune system, including cytotoxic T cells and antigen presenting dendritic cells, is not only necessary for immune surveillance but also required for efficient tumor control. In a multi-institutional report, chronically immunosuppressed patients had higher rates of cutaneous squamous cell carcinoma of the head and neck, and despite being treated with surgery and postoperative RT, these patients had poor outcomes, compared to immunocompetent patients with similar disease [9]. In a matched pair analysis of patients with prostate cancer who were treated with external beam RT, there was an increase in 3- and 5-year biochemical failure in immunocompromised patients. In another retrospective review of 244 consecutive patients with early stage non-small cell lung cancer (NSCLC) who were treated with SBRT, patients on chronic immunosuppressive therapy had poor local control and progression-free survival, compared to historic controls [10]. Although these clinical reports were all retrospective studies with small number of patients, the poor clinical outcome seen in immunocompromised patients support the hypothesis that immune response plays a critical role in tumor control after RT. Ablative radiation promotes the release of tumor antigens and damage-associated molecular pattern (DAMP) molecules from irradiated tumor cells for activation of dendritic cells (DCs). DCs engulf, process, and cross-

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present tumor antigens on class I major histocompatibility complex (MHC) for activating CD8+ cytotoxic T cells (CTLs) that are responsible for eradicating surviving clonogens in the irradiated tumor. In murine models of melanoma [6], colorectal cancer [7], and hepatocellular cancer [11, 12], ablation of immune effector cells, especially CD8+ T cells, abrogated control of both local and systemic disease and cure. These studies suggest that RT can generate an autologous in situ tumor vaccine and induce antitumoral immunity that contributes to the high rates of local tumor control, usually seen after SABR or SRS. However, despite evidence of the induction of antitumoral immunity after local tumor irradiation, RT usually fails to control systemic metastases. This suggests that therapeutic strategies to enhance antigen presentation from irradiated tumor cells, targeting the immunosuppressive features of the irradiated tumor microenvironment (TME) and reversing T cell anergy and exhaustion will be critical to realize the potential of RT-enhanced in situ tumor vaccines. This review focuses on the immunological consequences of ablative radiation and proposes a road-map for combination of RT with immunotherapy to induce a strong antitumoral immunity for both local and systemic tumor control.

Radiation-Enhanced Antigen Presentation (REAP)

The radiation-enhanced antigen presentation (REAP) would be an integral component of the proposed tumor vaccination strategy for solid tumors. Since cancer is a chronic disease, induction of the body's own immune system to fight distant microscopic metastatic disease would be highly beneficial in prolonging patient survival and eventual eradication of distant micrometastatic disease in liver cancer patients. Cancer cells express unique tumor antigens that include viral proteins, mutated oncoproteins, such as, p53 and ras, unique hybrid proteins expressed from translocated oncogenes, such as, BCR-ABL and proteins that are expressed during embryogenesis, but are not expressed by normal adult tissues [13]. Some of these "oncofetal" proteins serve as epitopes for host humoral and cellular immune response, which could potentially eradicate cancer cells. The immune system has the potential to recognize and eliminate cells with mutated proteins that are precursors to tumor. During the evolution of tumors, mutated cells lose the expression of proteins that participate in the antigen processing and presentation machinery, such as the antigen transporter gene product, TAP-2, and class I MHC molecules [14, 15]. This adaptive evasion of immune surveillance involves the selection of less immunogenic clones of tumor cells and is frequently mediated by acquisition of loss-of-function mutations and epigenetic regulation of the transcription of genes that are involved in the immune recognition and effector pathways of the adaptive tumor immunity.

Although, vaccination with defined tumor antigens and peptides has obvious appeal, natural immuno-variation, MHC polymorphism, and expected emergence of antigen-loss variants would require an ever-changing mixture of potential tumor antigens in vaccine formulations. Instead of individualized vaccines, a radiation-mediated, autologous in situ vaccination approach (Fig. 1) has been designed, whereby circulating DCs can be stimulated to infiltrate irradiated tumors and harvest tumor antigens released from dying tumor cells after RT treatment [16]. Modulation of the professional antigen presenting cells (APCs) such as DC may determine the efficacy of tumor immunity following primary tumor RT. DCs have been shown to acquire antigen from both apoptotic and necrotic cells. Localized RT by inducing tumor cell death would conceivably increase the tumor antigen available for presentation by DC. However, DCs are rare cells (<1%) in normal peripheral blood. The number of circulating DCs can be increased by administration of Flt3L (fms-like tyrosine kinase 3 ligand), which is a naturally occurring glycoprotein that stimulates the proliferation and differentiation of DCs [17, 18]. Thus, it was hypothesized that following local tumor irradiation, systemic administration of Flt3L would induce DC proliferation and infiltration of irradiated tumors by naïve circulating DCs that will readily endocytose tumor antigens released from dying tumor cells. Irradiated tumor cells could also provide "danger" signals that are necessary for DC activation. In murine models of lung cancer and hepatocellular carcinoma, it has been demonstrated that systemic administration of Flt3L, following ablative fractionation of primary tumor RT, generates effective tumor immunity that eradicates systemic metastases and cures mice with metastatic lung [16, 19] and liver cancer [11, 12].

Irradiated tumors can potentially serve as a source of tumor antigens *in vivo*, where dying tumor cells would release various tumor antigens slowly over time. Upon exposure, radiation initially increases the degradation of cellular proteins and eventually stimulates translation of novel proteins due to activation of the mammalian target of rapamycin pathway [20]. Radiation also increases the cell surface expression of Class I MHC molecules and cell death receptors, such as Fas in a dose-dependent fashion, thereby increasing peptide production, antigen presentation, and susceptibility to T cell-mediated cytotoxicity [20, 21]. Irradiation induces transcription and variant splicing of human endogenous retrovirus K (HERV-K) transcripts in human prostate and breast cancer cells, thereby raising the possibility that aberrant HERV-K peptides could also contribute to enhanced immunogenicity after RT [22]. In fact, HERV-K triggers a T cell response in breast cancer patients and chimeric antigen receptor-expressing T cells targeting HERV-K peptides have been designed that can inhibit tumor growth and metastases [23, 24]. Another source of