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REVIEW

Radiation-Induced Vascular Damage in Tumors: Implications of Vascular Damage in Ablative Hypofractionated Radiotherapy (SBRT and SRS)

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We have reviewed the studies on radiation-induced vascular changes in human and experimental tumors reported in the last several decades. Although the reported results are inconsistent, they can be generalized as follows. In the human tumors treated with conventional fractionated radiotherapy, the morphological and functional status of the vasculature is preserved, if not improved, during the early part of a treatment course and then decreases toward the end of treatment. Irradiation of human tumor xenografts or rodent tumors with 5–10 Gy in a single dose causes relatively mild vascular damages, but increasing the radiation dose to higher than 10 Gy/fraction induces severe vascular damage resulting in reduced blood perfusion. Little is known about the vascular changes in human tumors treated with high-dose hypofractionated radiation such as stereotactic body radiotherapy (SBRT) or stereotactic radiosurgery (SRS). However, the results for experimental tumors strongly indicate that SBRT or SRS of human tumors with doses higher than about 10 Gy/fraction is likely to induce considerable vascular damages and thereby damages the intratumor microenvironment, leading to indirect tumor cell death. Vascular damage may play an important role in the response of human tumors to high-dose hypofractionated SBRT or SRS.

INTRODUCTION

It is well accepted that the intratumor microenvironment, such as oxygenation status, greatly influences the radiosensitivity of tumor cells and that the intratumor microenvironment is closely related to the functional status of tumor microvasculature. Therefore, detailed insight into the radiation-induced changes in tumor microvasculature is important for maximizing the efficacy of radiotherapy against cancer. The radiation-induced changes in tumor blood vessels have been demonstrated to be markedly variable, depending on the total radiation dose, dose rate, fraction size and the number of fractions as well as on biological factors such as the tumor type, tumor site and the stage of tumor growth. In the early years of radiotherapy, tumors were irradiated with single or a few fractionated large doses (*1, 2*). However, after the landmark observations by Regaud and Ferroux (*3*) and Coutard (*4*) about 80 years ago that the therapeutic ratio in treating cancer with radiation could be increased by delivering the radiation in multiple fractions of small doses, fractionated radiotherapy has been an almost universally accepted clinical practice. As early as 1936, Mottram (*5*) reported that the cancer cells in the peripheral tumor volume with good blood supply were more sensitive to radiation than the cells in the central tumor volume. Subsequent studies demonstrated that the response of tumor cells to radiation is closely related to oxygen supply through blood perfusion and that fractionated radiotherapy minimizes radiation-induced vascular damage, thereby allowing reoxygenation of hypoxic tumor cells. In the 1950s, radiosurgery using gamma knives was introduced by Leksell (*6*) to deliver high-dose hypofractionated radiation to confined vascular lesions and malignancies in the brain. As a result of the recent remarkable advances in imaging technology, computer-aided field shaping, treatment planning, dosimetry and radiation delivery systems, it has now become possible to conformally deliver 20–60 Gy to tumors in a single fraction or 2–5 fractions (*7–11*). This is

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referred to as stereotactic radiosurgery (SRS) for the treatment of intracranial lesions and stereotactic body radiation therapy (SBRT) for the treatment of extracranial tumors. A relevant and important question is the fate of tumor blood vessels when tumors are exposed to such high-dose hypofractionated radiation. Another pertinent question is whether the well-established radiobiological principles of conventional fractionated radiotherapy such as reoxygenation of hypoxic cells play any role in the response of tumors to SRS or SBRT. We have briefly discussed these topics in our recent publication (*12*). Numerous reports have described the radiation-induced vascular changes in tumors and their implications for radiotherapy but have often reached conflicting conclusions. The purpose of the present review is to examine the previous studies on radiation-induced vascular changes in both human and experimental tumors to establish an up-to-date view of the subject with greater relevance to the rapidly growing interest in treating tumors with high-dose per fraction radiotherapy, such as SBRT and SRS.

TUMOR BLOOD VESSELS AND BLOOD FLOW

Solid tumors acquire blood vessels by coopting neighboring vessels in normal tissues and angiogenesis, that is, sprouting or intussusceptive microvascular growth from existing arteries or veins (*13, 14*). Tumor blood vessels are also formed by vasculogenesis by adding endothelial progenitor and other stem-like cells that derived from the bloodborne and bone marrow-delivered stem cells to the growing tumor vessels (*15*). As tumors grow and invade normal tissues, the arteries of normal tissues are incorporated into the tumors. Therefore, varying fractions of tumor vascular beds originate from normal tissues. The hastily formed capillary-like tumor microvasculature is comprised of a single layer of endothelial cells often separated by gaps and is devoid of underlying basement membrane or smooth muscle cells (pericytes) and innervations. However, some of the microvasculature of slowly growing human tumors exhibit a thin layer of smooth muscle similar to the capillaries of normal tissues (*13*). In general, tumor blood vessels are irregular in diameter with rather narrow tubes connected next to dilated and often sinusoid-like structures. Compared to the well-organized web-like network of homogeneous capillaries in normal tissues, the tumor capillaries are sharply bent, tortuous and branched with multiple dead ends (*16*). Blood perfusion through such disorganized vascular networks tends to be sluggish and often intermittently stationary. However, it should be stressed that the architecture and morphology of tumor microvasculature and the blood perfusion are markedly heterogeneous and variable, depending on various factors such as tumor type, tumor size and site of tumor growth. Even within a tumor, the vascular distribution and blood perfusion are rather heterogeneous. For example, in some tumors, the central volume is well vascularized and well perfused compared to the periphery, while in other types of tumors the opposite is true. The tumor blood vessels are usually more permeable and leaky compared with the blood vessels in the surrounding normal tissues probably because the tumor vasculatures are morphologically immature (*17*). The tumor vascular permeability is also significantly variable depending on tumor type. For example, the blood-brain barrier in the normal brain tissue is retained in many brain tumors, and thus the blood vessels in brain tumors tend to be less permeable than the blood vessels of other types of tumors. The interstitial fluid pressure (IFP) of certain types of tumors is known to be elevated owing to the high vascular permeability in tumors (*14*).

RADIATION-INDUCED VASCULAR CHANGES IN TUMORS

We have summarized the 43 representative studies on the radiation-induced changes in tumor vasculature reported in the last 60 years in Table 1. Of the 43 reports (*18–60*), the first seven (*18–24*) describe vascular changes in human tumors and the remainder are related to radiation-induced vascular changes in either human tumor xenografts in rodents or transplanted mouse or rat tumors. Various methods, including colpophotography (*18*), ^{133}Xe clearance (*19*), Doppler sonography (*20*), MR imaging (*22*) and CT imaging (*23, 24*), have been used to study the radiation-induced vascular changes in human tumors. All the studies with human tumors are concerned with the vascular changes caused by conventional fractionated radiotherapy. Bergsjö (*18*) studied the vascular changes in cervical tumors caused by radiotherapy with 17 Gy delivered in 10–12 fractions. Colphophotographic examination of the tumor surface indicated that the vascularity on the surface of tumors improved slightly at the end of the therapy. However, it should be realized that the changes in the vasculature on the surface of tumors as determined with colphophotography may not represent the overall vascular changes in tumors. The conclusions of other studies on human tumors (*19–24*) follow a general trend that blood flow remains unchanged or increases slightly during the early period of fractionated radiotherapy but decreases toward the end of treatments. For example, in the study by Mayr *et al.* (*22*), human cervical squamous carcinoma and adenocarcinoma were treated with 40–50 Gy over 4–5 weeks in 5 fractions/week (2 Gy/fraction). Blood flow, as determined by MRI, increased during the first 2 weeks of therapy and decreased thereafter. The authors reported that high cervical tumor perfusion before the treatment and increasing or persistent high perfusion during the early part of the therapy was a favorable sign of the treatment outcome. Pirhonen *et al.* (*20*) reported that a decrease of tumor vasculature during the fractionated radiotherapy of advanced cervical carcinoma was associated with a better treatment outcome and that the

TABLE 1
Summary of Studies on the Radiation-Induced Vascular Changes in Human Tumors, Human Tumor Xenografts Grown in Animals, and Animal Tumors

Tumors and sites	Methods	Vascular changes	Author(s) (year) (ref.)
Human cervical carcinoma (143 patients)	Colpophotography	Irradiation with 1700 R in 10–12 fraction in 15 days, in general, slightly increased the surface vascularity at the end of fractionated radiation therapy.	Bergsjö (1968) (*18*)
Human superficial metastatic tumors (43 patients with 48 tumors)	^{133}Xe clearance	Tumors were irradiated with 1100–3000 rad/week in 5 fraction/week. Blood flow increased during the 1st week of the treatment and decreased from the 2nd week of the treatment. In a longer follow up, the tumor blood flow decreased continuously.	Mäntylä *et al.* (1982) (*19*)
Human advanced cervical carcinoma (14 patients)	Color Doppler, ultrasound	Irradiated with 30–65 Gy at 1.9 Gy/fraction and 5 fraction/week. In 11 out of 14 tumors, vascularity and blood flow decreased significantly. The decrease in tumor vasculature during the treatment was associated with better outcome. Eight of 10 patients with increased tumor vascularity at the end of radiation needed further treatment or died of disease.	Pirhonen *et al.* (1995) (*20*)
Human uterine cervical cancer	Immunostaining of biopsy sections for factor VIII-related antigen	Radiotherapy with 50 Gy in 2 Gy/day for 5 times/week. Endocavitary brachytherapy was delivered in 7–8 fraction to a total dose of 29–34 Gy. No change in vascular density was observed, but vascular damage occurred as indicated by endothelial swelling and hypertrophy during early phase of radiotherapy. Tumor oxygen tension varied depending on various factors beside vascular functions.	Lyng *et al.* (2000) (*21*)
Human squamous carcinoma (14) and adenocarcinomas (3) of cervix	MR imaging	Irradiated with 40–45 Gy/4–5 week, 5 fraction/week. Some tumors showed increased blood perfusion during the first 2 weeks, but the perfusion decreased thereafter. High tumor perfusion before therapy and increasing or persistent high perfusion early during the course of therapy appeared to be favorable signs.	Mayr *et al.* (1996) (*22*)
Human non-small-cell lung cancer (16 patients)	Volumetric perfusion computed tomography	Vascular blood volume and permeability were greater in tumor rim than tumor center. After fractionated irradiation with 9 Gy in 2 fraction, 18 Gy in 4 fraction and 27 Gy in 6 fraction, vascular volume increased significantly in tumor rim and slightly in tumor center. Vascular permeability also increased in tumor rim, but not in tumor center.	NG *et al.* (2007) (*23*)
Human rectal cancer (23 patients)	Perfusion CT imaging	Irradiated with 25 Gy in 5 fraction (5 Gy ’ 5) in 1 week. From 3 days after the hypofractionated treatment, trans-endothelial volume constant (K trans) (permeability) slightly increased. The increased vascular permeability may improve the bioavailability of cytotoxic agents in rectal tumors.	Janssen *et al.* (2009) (*24*)
Human melanoma xenograft in athymic nude mice in the flank	Angiography	In 1 week after irradiation with 10.0–15.0 Gy in a single dose, 35–45% of 5–15-µm-diameter vessels were nonfunctional. The doses required for loss of 50% of the functional vessels with diameters of 5–15, 15–25, and 25–35 µm were 16, 21 and 20 Gy, respectively. In spite of early loss of functional vessels, tumors became supervascularized as tumors regressed after 20 or 25 Gy irradiation. Regrowth of irradiated tumors appeared to be preceded by efficient neovascularization.	Solesvik (1984) (*25*)
Human colon tumor xenografts in the flank (s.c.) of athymic mice	^{99m}TcO4-RBC for functional vascular volume and ^{125}I-plasma protein for vascular permeability	Irradiation with 4–16 Gy in a single dose increased the vascular permeability in 24–72 h and decreased the functional vascular volume in 24 h. The increase in vascular permeability by irradiation is potentially valuable to increase monoclonal antibody uptake by tumors.	Kalofonos *et al.* (1990) (*26*)
Human laryngeal squamous cell carcinoma xenografts in nude mice	Histological imaging of endothelial marker for vessels and Hoechst 33342 injection for vascular perfusion	After irradiation with 10 Gy, the number of perfused vessels slightly increased within 1 day, and then significantly decreased at 26 h followed by recovery to control level in 7–11 days. The hypoxic cell fraction decreased at 7 h after irradiation but significantly increased to pre-irradiation levels at 11 days after irradiation.	Bussink *et al.* (2000) (*27*)
Human MA148 ovarian carcinoma xenografts in nude mice, s.c.	Immunohistochemistry for PECAM (CD31-red fluorescence	After irradiation with 5 Gy/week for 4 weeks, the total vessel density decreased by 50%. Irradiation and anginex synergistically reduced the functional vascularity in tumors.	Dings *et al.* (2005) (*28*)
Human A-07 melanoma xenografts in nude mice	Dynamic contrast-enhanced magnetic resonance imaging using Gd-DTPA	At 72 h after 10 Gy irradiation in a single dose, tumor blood perfusion decreased by 40%. However, intratumor mean pO_2 and pO_2 fluctuation were not altered by irradiation with 5 or 10 Gy, indicating that intratumor microenvironment was not changed by 5–10 Gy irradiation.	Brurberg (2006) (*29*)

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TABLE 1
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Tumors and sites	Methods	Vascular changes	Author(s) (year) (ref.)
Human A549 lung adenocarcinoma xenografts in the hind legs of nude mice, s.c.	Hoechst 33342 for blood perfusion and Dynamic Magnetic Resonance imaging of GD-DTPA for functional vascularization	Analysis with Hoechst 333342 indicated a rich blood vessel perfusion in the peripheral part of the tumors. Irradiation with 20 Gy in a single dose caused no changes in vascular density whereas apoptosis of tumor cells was significant at 10.5 h postirradiation. Blood perfusion, as determined with GD-DTPA imaging increased at 1 h postirradiation. Hypoxic area in the tumors decreased for 30.5 h after irradiation.	Fokas *et al.* (2010) (*30*)
Human U251 glioblastoma xenograft grown s.c. in the back or intracranially (i.c.) in nude mice	Fluorescence imaging of lectin for i.c. tumors and ultrasound analysis of contrast agent for s.c. tumors	Irradiation with 15 Gy in a single dose decreased blood perfusion to 10% of control in i.c. tumors and to 30% of control in s.c. tumors in 2 weeks. In i.c. tumors, CD31-stained cells (endothelial cells) were reduced to 25% of control accompanied by marked increase in hypoxic area (pimonidazole staining). Thereafter, the damaged vasculatures were restored by virtue of vasculogenesis through recruitment of bone marrow-derived cells in both s.c. and i.c. tumors. AMD3100, an inhibitor of vasculogenesis, prevented the recovery of tumor vasculature. Vasculogenesis needs to be blocked for complete control of tumor by radiotherapy.	Kioi *et al.* (2010) (*31*)
Mouse adenocarcinoma of C3H mice in transparent chambers	Transparent chamber. Microsocpic observation	Irradiated with 2,000 or 3,000 R in a single fraction caused pronounced narrowing of microvessels for approximately 1 week. By 2–4 days after irradiation, the circulation was slowed. The retardation of circulation during 2–5 days postirradiation was responsible for tumor cell death. Irradiated vessels were unable to regrow.	Merwin *et al.* (1950) (*32*)
Hamster neurilemmoma in the cheek pouch chambers	Cheek pouch transparent chamber. Microscopic observation	Irradiation with 3,000 R caused variable degrees of edema and extensive reduction in blood flow in 24–30 h, with subsequent restoration toward normalcy accompanied by small focal hemorrhaging. Subsequent tumor growth with neovascularization began in the perimeter of the tumor.	Eddy (1980) (*33*)
Rat R3240 Ac mammary adenocarcinoma in window chambers	Dorsal flap transparent window chamber. Microscopic observation	Irradiation with 5 Gy caused conjoint increase in both vascular density and perfusion during 24–72 h post-irradiation, although the degree of change was variable from one individual to the next. The degree of change in vascular density was inversely related to median pretreatment diameter.	Dewhirst *et al.* (1990) (*34*)
Mouse adenocarcinoma in the thigh	Histological examination	Irradiated with 2400–2600 R in 1 fraction. Slight dilation of blood vessels occurred immediately after irradiation. From 24 h postirradiation, blood vessels dilated markedly and ruptured, and blood extravasated.	Lasnitzki (1947) (*35*)
Mouse malignant tumors in the flank, s.c., 5 different tumors	Angiography	Irradiated with 680–3,000 R in 1 fraction. From 3rd day after 680 R irradiation, the vascularity started to decrease. Abrupt tapering and narrowing of vessels peaked 9–13 days after 680-2000 R irradiation.	McAlister *et al.* (1963) (*36*)
Rat Walker carcinoma and Murphy-Sturn lymphoma in the flank, s.c	Angiography and Histology	After irradiation with 500 R/day for 3 days (1500 R), supervascularization developed. When the tumors were treated with fractionated irradiation with relatively small fraction sizes, progressive destruction of tumor parenchymal cells preceded the regression of the microcirculation leading to development of supervascularization. However, after exposure to 1,500–6,000 R in 1 fraction, tumor vasculatures fragmented.	Rubin *et al.* (1966) (*37*)
Mouse rhabdomyosarcoma in the flank, s.c.	^{133}Xe clearance	Irradiation with 2000 R markedly reduced the blood flow rate between 1 and 9 days post-irradiation.	Robert (1967) (*38*)
Rat Walker carcinoma in the flank, s.c	^{51}Cr-RBC for functional vascular volume and ^{125}I-plasma protein for vascular permeability.	Soon after irradiation with 2 Gy, the extravasation of plasma protein (permeability) increased while the vascular volume remained unchanged. In 2–12 days after irradiation with 10–60 Gy, the vascular volume significantly decreased. Revascularization occurred as the tumor began to regrow about 15 days after 30 Gy irradiation.	Song *et al.* (1971) (*39*)
Rat Walker carcinoma in the flank, s.c.	^{133}Xe clearance	Irradiation with 20 Gy in 1 fraction increased the ^{133}Xe clearance rate in 1–6 days whereas it decreased the vascular volume.	Song *et al.* (1972) (*40*)
Rat Walker carcinoma in the flank, s.c	^{51}Cr-RBC for functional vascular volume and ^{125}I-plasma protein for vascular permeability	While irradiation with 2.5 Gy increased the vascular volume, 5–20 Gy decreased the vascular volume at 24 h post-irradiation. The extravasation of plasma protein increased at 24 h after irradiation with 2.5–20 Gy.	Wong *et al.* (1973) (*41*)

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TABLE 1
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Tumors and sites	Methods	Vascular changes	Author(s) (year) (ref.)
Mouse KHT sarcoma in the flank, i.m.	^{133}Xe clearance	Blood flow (^{133}Xe clearance) tended to decrease 3 h after irradiation with >1,000 rad and increased 3–4 days and 7 days after irradiation with 1000 and 2,000 or 4,000 rad. Reoxygenation of hypoxic cells occurred as a result of increased blood perfusion after irradiation. Blood flow decreased after irradiation with 8,000–16,000 rad.	Kallman *et al.* (1972) (*42*)
Mouse neuroblastoma in the flank, s.c	^{51}Cr-RBC for functional vascular volume and ^{125}I-plasma protein for vascular permeability. Histopathology	Irradiation with 2.5 and 5 Gy slightly increased the intravascular volume at 24 h, but it decreased thereafter. Irradiation with 10 and 20 Gy caused progressive decrease in the vascular volume to about 30% of control at days 12. Extravasation of plasma protein increased during 3 days after 2.5–20 Gy irradiation. As the tumors regressed after 20 Gy irradiation, the vascular network became disorganized, aggregated and condensed.	Song *et al.* (1974) (*43*)
Mouse mammary carcinoma in the leg, s.c.	Morphometry (colloidal carbon filling)	After 500 R irradiation, the vascular volume slightly decreased in 1 day and recovered in 4 days. Vascular volume decreased and failed to recover after irradiation with 1,500 R. Nevertheless, the transient vascular changes conceivably rendered the hypoxic cells reoxygenated. Irradiation with 4500 R caused extensive damage to microvasculature.	Hilmas *et al.* (1975) (*44*)
Mouse mammary carcinoma in the leg, s.c	^{51}Cr-RBC for functional vascular volume	After irradiation with 10 and 20 Gy, vascular volume increased within 24 h and subsequently decreased. The numbers of viable tumor cells significantly decreased in 3 days after irradiation. The transitional increase in blood supply and the decline in the oxygen consumption due to death of tumor cells may lead to transitional increase in oxygenation status of surviving tumor cells after tumors are exposed to high dose radiation.	Clement *et al.* (1976) (*45*)
Rat BA-1112 rhabodomyosarcoma in posterior portion of scalp	Photon activation of oxygen and ^{15}O positron decay	After irradiation with 16.5, 38.5 or 60.5 Gy, blood flow decreased by 35–50%. However, the blood flow in the tumor irradiated with 16.5 Gy recovered in 24–48 h while that of tumor irradiated with 60.5 Gy remained decreased at 72 h after irradiation. Irradiation with small doses used in fractionated radiotherapy did not cause vascular changes.	Emami *et al.* (1981) (*46*)
Mouse RIF-1 tumor grown in the leg, s.c.	^{14}C-iodo-antipyrine uptake	Blood perfusion increased 1–2 days after irradiation with 20 Gy but not after irradiation with 2 Gy. Tumor necrosis increased about 3 times at 1 day after 20 Gy irradiation and then declined to about twice its control value at 2 days.	Tozer *et al.* (1989) (*47*)
Rat RIH rhabdomyosarcomas grown in the flank.	Electron micrography of vascular wall of tumor capillaries. Tumor pO2 was measured with polarographic electrodes	Tumors were irradiated with 60 Gy in 3 Gy $\times$ 20 fraction for 4 weeks. After 30 Gy irradiation, inner surface of the lumen became rough, and the endothelial cells and pericytes were swollen. After 60 Gy irradiation, endothelial cells became elongated, sometimes detached from each other and from basal lamina. In the early phase of the fractionated treatment (up to 30 Gy) tumor oxygenation slightly improved, but it distinctly decreased in the later phase of treatment.	Zywietz *et al.* (1996) (*48*)
Rat BT4C glioma grown intracerebrally	Immunohistochemical staining for blood vessels	Whole brains treated with 20 Gy in 4 Gy $\times$ 5 fraction tumor volume decreased to 77% of original volume and the microvascular density decreased to 72% of original value at 5th after the treatment.	Johansson *et al.* (1999) (*49*)
Mouse KHT fibrosarcoma in the hind limb, i.m.	Immunohistochemical staining for blood vessels and fluorescent DiOC7 injection for perfused vessels	Many tumor vessels were not perfused before irradiation. The density of perfused vessels decreased at 24 h after 10 Gy irradiation and recovered to control level by 72 h. Despite the decrease in the perfusion, O_2 availability appeared to be increased due to reduction in O_2 consumption and yet the radiobiologically hypoxic cell fraction did not change.	Fenton *et al.* (2001) (*50*)
Mouse glioblastoma in the thighs, s.c.	Power Doppler ultra sound for vascularity (vascular index) and Immunofluorescence staining of tissue sections	The majority of the functional vasculatures were in the periphery of tumor. Irradiation with 10 Gy in 2 fractions reduced the tumor vascularity to 37% of control in 3 days. The immunofluorescence staining for endothelial cell demonstrated a similar pattern to that of the quantified power Doppler US study.	Donnelly *et al.* (2001) (*51*)

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TABLE 1
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Tumors and sites	Methods	Vascular changes	Author(s) (year) (ref.)
Rat autochthonous mammary tumors induced by s.c injection of N-nitroso N-methyl urea	Power Doppler sonography for Doppler index (PDI)(vascularity)	Irradiation with 18 Gy in a single fraction caused variable reduction (12–63%) in PDI (vascularity). The degree of reduction in PDI at 7 days after irradiation was correlated with the subsequent tumor regression. The early changes in tumor perfusion after irradiation appeared to precede the long-term tumor regression.	Denis *et al*. (2003) (*52*)
Mouse SCCVII tumors grown in the hind legs of C3H mice, s.c.	Selective irradiation of tumor vessels endothelial cells with BNCT using BSH-liposome	Selective irradiation of tumor vessel endothelial cells with 30–33 Gy irradiation with $^{10}B(n\alpha)^7Li$ reaction completely suppressed the tumor growth without direct irradiation of tumor cells. Destruction of tumor vasculatures alone can cause tumor regression.	Ono *et al*. (2003) (*53*)
MCF/129 fibrosarcoma were induced in apoptosis sensitive Amase$^{+/+}$ or apoptosis resistant Amase$^{-/-}$ mice, and B1F1 melanomas were induced in Bax$^{+/+}$ or Bax$^{-/-}$ mice	Endothelial cell apoptosis in tumors was determined by TUNEL. Radiation-induced tumor growth delay was related to endothelial cell apoptosis.	Irradiation of tumors with 15 Gy in a single dose caused far more tumor endothelial cell apoptosis in Amase$^{+/+}$ mice than in Amase$^{-/-}$ mice. Tumors grown in apoptosis-resistant Amase$^{-/-}$ mice and Bax$^{-/-}$ mice were much more radioresistant than the tumors grown in Amase$^{+/+}$ mice or Bax$^{+/+}$ mice. Conclusion: Microvascular damage regulates tumor response to radiation at the clinically relevant dose range.	Garcia-Barros *et al*. (2003) (*54*)
Mouse squamous cell carcinoma (SCCVII) in the hind legs of C3H mice, s.c.	Dynamic magnetic resonance imaging of G8-Gd-D (13 nm) for vascular permeability	After irradiation with 15 Gy, vascular permeability increased to about 1.4 times of control occurring between 7 and 24 h after irradiation. Irradiation with 5 Gy × 5 decreased the vascular permeability whereas 2 Gy × 5 and 3 Gy × 5 did not.	Kobayashi *et al*. (2004) (*55*)
Mouse K1735 melanoma grown in C3H/HeN mice	Doppler ultrasound for blood flow. Immunostaining for vascular density, HIF-1 and VEGF. TUNEL staining for endothelia cell (EC)apoptosis	Tumors were exposed to 12 Gy. Blood flow decreased significantly and the histological preparations of tumors grown 10-fold in volume after irradiation showed the following: prominent regions of hypoxia (EF5 positive), HIF-1α and VEGF, and decreased microvascular density. Significant EC death occurred next day after irradiation.	Tsai *et al*. (2005) (*56*)
Lewis lung carcinoma of C57BL/mice. Grown s.c. in the hind limb and dorsal skin window chamber	Doppler ultrasound for blood flow, microvascular density, endothelial cells apoptosis, proliferation of tumor cells and tumor growth	Irradiation of tumors in window chambers with 20 Gy completely obliterated tumor vessels. The blood flow and vascular density in the s.c. tumors markedly decreased at 2 days after 20 Gy irradiation but blood flow recovered substantially at day 4. Second 20-Gy irradiation 2 days after the 1st 20 Gy was far more effective than that 4 days after the 1st 20 Gy for sustained reduction of blood flow and also for suppression of tumor growth.	Kim *et al*. (2006) (*57*)
Rat colorectal tumor grown s.c. in the hind legs of WAG/Rij rats	DCE-MRI with macromolecular contrast agent (P792)	Irradiation with 5 × 5 Gy decreased the neovascular leakage (endothelial transfer constant), fractional interstitial space, and microvessel density in the tumor rim at day 5 and increased the tumor pO_2. No correlation was found between the DCE-MRI and the histological parameters. Tumor PO_2 is related to vascular leakage and fractional interstitial space.	Ceelen *et al*. (2006) (*58*)
Radiosensitive FSC1-3 (DNA-PK$^{-/-}$) and radioresistant T53(DNA-PK$^{+/+}$) tumors in nude and SCID mice, s.c.	Labeling of endothelial cells in situ by i.v. injection of biotinylated lectin followed by histological staining	Irradiation with 15 Gy in a single dose markedly reduced the functional vascularity in the tumors grown in SCID mice but only moderately in the tumors grown in nude mice. Tumor cell radiosensitivity was the major determinant of radiation-induced growth delay in nude mice while both tumor cell death and vascular damage contributed to the tumor growth delay in SCID mice.	Ogawa *et al*. (2007) (*59*)
Mouse TRAMP-C1 prostate tumors in the thigh of C57BL/6J mice, i.m.	Immunohistochemistry and molecular assays	Irradiation with 25 Gy in a single dose or 60 Gy in 15 fraction decreased the tumor microvascular density over a 3-week period to nadirs of 25% and 40% of control, respectively. Consequently, chronic and persistent hypoxia regions infiltrated with CD68^{+} tumor-associated macrophages developed in the irradiated tumors. Central dilated vessels developed surrounded by avascularized hypoxic regions.	Chen *et al*. (2009) (*60*)

Note. The tumor types studied, methods for the determination of vascular changes, radiation-induced vascular changes, and the authors of the studies are shown.

patients with increased tumor vascularity at the end of treatment needed further treatment. One may assume that, during the course of fractionated radiotherapy, tumor microvasculature beds gradually become nonfunctional as the demands for nutrients, including oxygen, decline due to radiation-induced death of tumor cells.

In the seven studies with human tumor xenografts (*25–31*), mostly large-dose irradiations were used in either a single dose or several fractions. An angiographic study by Solesvik *et al.* (*25*) showed that 35–45% of 5–15-μm-diameter vessels in human melanoma xenografts became nonfunctional within a week after irradiation with 10–15 Gy in a single exposure, as shown in Fig. 1A. In a xenograft of human squamous cell carcinoma of the larynx (*27*), irradiation with 10 Gy in a single dose caused a slight increase in the functional vascularity soon after irradiation and a significant decrease at 24 h followed by a gradual recovery to the preirradiation level by 11 days after irradiation. The vascular density in human ovarian carcinoma xenografts irradiated with 20 Gy in 5 Gy/week for 4 weeks was about one-half of that in the control xenografts, as shown in Fig. 1B (*28*). In a recent study with human glioblastoma xenografts grown in the brain of nude mice (*31*), functional vascular density and blood perfusion markedly decreased and hypoxic regions increased at 17–18 days after irradiation with 15 Gy in a single dose. However, the tumor blood perfusion began to recover from 3 weeks after irradiation and the tumors started to grow, but the recovery of blood perfusion could be suppressed by inhibiting vasculogenesis by preventing the influx of bone marrow-derived cells.

Mouse or rat tumors grown in transparent window chambers have been used to directly observe vascular changes after treatment. Irradiation with 5 Gy increased both vascular density and perfusion during 24–72 h postirradiation in R3230 mammary adenocarcinoma grown in window chambers placed in the back of rats (*34*). On the other hand, irradiation of mouse adenocarcinoma in window chambers with 20–50 Gy in a single exposure caused progressive narrowing of blood vessels (*32*). Irradiation of Lewis lung carcinoma of mice grown in window chambers in dorsal skin with 20 Gy in a single exposure led to uniformly complete destruction and hemorrhage of the tumor blood vessels in 2 days (*57*).

The results and conclusions of studies with mouse and rat tumors reported over the last several decades are quite variable (*35–60*) (see Table 1). We can attribute such variable observations to the differences in the method to determine the vascular changes, tumor type used, and the site where tumors were transplanted. Nevertheless, the conclusions of the numerous studies may be generalized as follows. After a single exposure to moderately high doses of radiation, e.g. 5–10 Gy, tumor blood flow initially increases, returning to preirradiation levels or slightly below the preirradiation levels in 2–3 days. After irradiation with 10–15 Gy/fraction once or twice, tumor blood flow

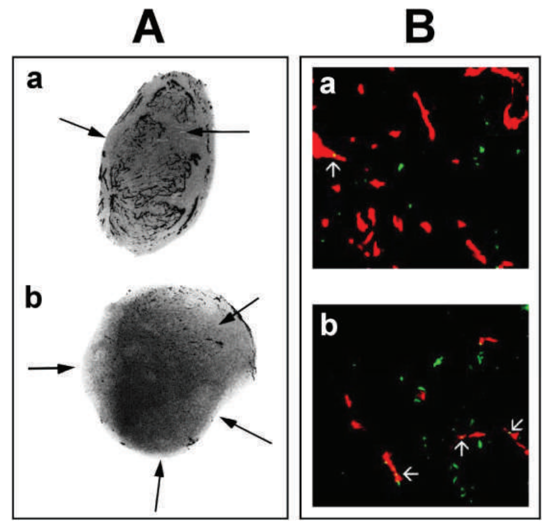

FIG. 1. Panel A: X-ray images of 720-μm-thick sections from (a) an unirradiated human melanoma grown in athymic nude mice and (b) a tumor exposed to a single dose of 10 Gy 1 week earlier. The vessels were filled with a radio-opaque medium administered via the abdominal aorta of mice. Vascular structures are not seen in areas confirmed to be necrotic (indicated by arrows) (*25*). Panel B: Immunohistochemistry for PECAM-1 (CD31 red fluorescence) as a marker for vessel density in MA148 human ovarian carcinoma xenografts that were left untreated (a) or were treated with 5 Gy once per week for 4 weeks before staining (b). Vessel density in the irradiated tumors was found to be approximately half of the untreated tumor value as assessed by digital quantification of PECAM-1 signal (*28*). Arrows point to positive TUNEL staining to assess the amount of apoptosis occurring in the tumor at the time of staining. Colocalization of red and green indicates an endothelial cell or vessel component undergoing apoptosis.

decreases soon after irradiation, remains reduced for varying lengths of time, e.g. 1 to several days, and occasionally recovers to the control levels. In most cases, after tumors are irradiated with doses higher than 15–20 Gy in a single exposure, tumor blood flow decreases rapidly followed by deterioration of the vasculature as the tumor volume decreases. Some of the representative studies on the radiation-induced vascular changes in rodent tumors are discussed below. Emami *et al.* (*46*) reported that the blood flow in rhabdomyosarcomas grown in the scalp of rats declined by 40–50% within 2 h after irradiation with 16.5–60.5 Gy in a single dose, but the blood flow in the tumors treated with 16.5 Gy recovered by 24 h. In a recent study by Chen *et al.* (*60*) with mouse prostate tumors grown in the thigh of mice, irradiation with 25 Gy in a single exposure decreased the vascular density to 25% over a 3-week period, thereby increasing chronic and persistent hypoxic regions in the tumors. Whereas most of the studies with rodent tumor models were conducted using tumors grown subcutaneously in either the thigh or back of animals, Johansson *et al.* (*49*) studied the radiation-induced vascular damage in glioma grown in rat brains. Irradiation of tumors with 20 Gy in 5 daily fractions of 4 Gy decreased the vascular intensity to 72% of original levels and also decreased the tumor volume

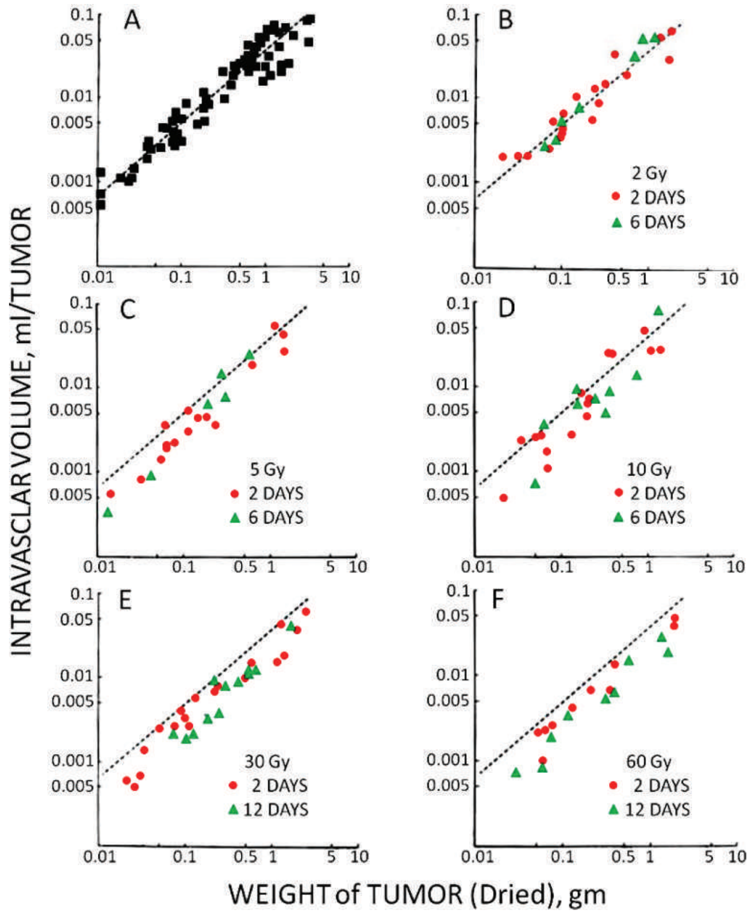

FIG. 2. Effects of radiation on the functional intravascular volume in Walker 256 tumors (s.c.) grown in the legs of Sprague-Dawley rats (*39*). Panel A: Vascular volume in the control tumors. Panels B–F: Vascular volume in the tumors irradiated with various doses of X rays in a single exposure. The dotted line in panel A represents the vascular volume of 68 control tumors of different weights, and it is shown in panels B–F for comparison with the vascular volume of the irradiated tumors. The distributions of the marks representing functional vascularity in individual tumors shifted to below the dotted line after irradiation with >5 Gy, indicating that radiation caused vascular damage. The statistical significances between the control group and 10-Gy group as well as the 30-Gy group were analyzed using linear regression. The vascular volumes at 2 days and 6 days after 10 Gy irradiation were combined, and the vascular volumes at 2 days and 12 days after 30 Gy irradiation were combined. The vascular volumes in the tumors irradiated with 10 Gy as well as 30 Gy were significantly smaller than that in the control tumors with $P < 0.001$.

to 77% of original size by 5 days after completion of the treatment. Using Doppler sonography, Kim *et al*. (*57*) noninvasively determined the blood flow in Lewis lung tumors of mice grown s.c. in the hind limbs after irradiation with 20 Gy once or twice separated by 2 or 4 days. The tumor blood flow decreased significantly by 2 days after 20 Gy irradiation, but it recovered substantially by 4 days after irradiation. Such recovery of blood flow after the initial 20-Gy irradiation could be successfully suppressed by irradiating the tumors again with 20 Gy at 2 days after the initial irradiation. Reirradiation at 4 days after the first irradiation was less effective than that at 2 days after the first irradiation for sustained reduction of tumor blood flow and for suppressing tumor growth. Tsai *et al*. (*56*) also used Doppler sonography and immunohistochemistry to determine the blood flow and intratumor microenvironment in murine melanoma tumors irradiated with 12 Gy in a single exposure. The authors reported that there were marked defects in vascular perfusion and decline in tumor vascularity accompanied by prominent regions of hypoxia, necrosis and hemorrhage when the tumor volume increased 10-fold after irradiation.

We have extensively investigated the radiation-induced vascular change in the Walker 256 tumors grown subcutaneously in the hind legs of rats (*39–41*). In general, irradiation with doses smaller than 2.5 Gy caused a slight decrease in the functional vascular volume for 6–12 h, followed by a return to preirradiation levels. Irradiation with 5–20 Gy in a single exposure decreased the vascular volume in dose- and time-dependent manner. As shown in Fig. 2, the functional intravascular volume in Walker 256 tumors decreased for 2–6 days after irradiation with 5–10 Gy in

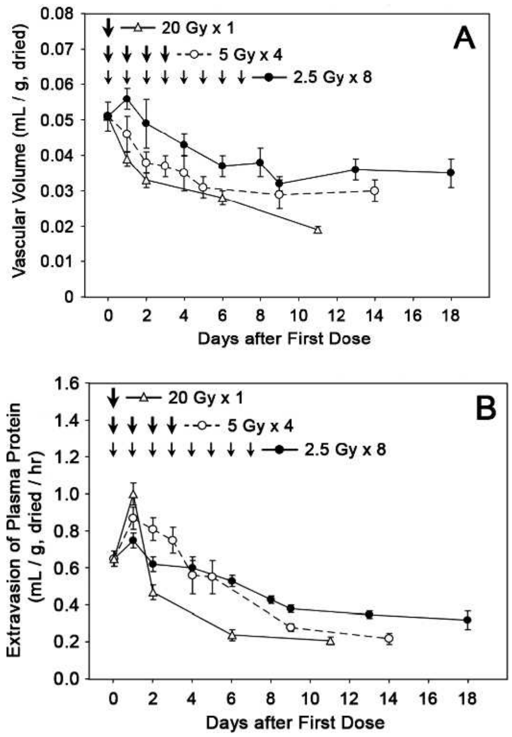

FIG. 3. Panel A: Effects of 20 Gy radiation given in a single fraction, 4 daily fractions of 5 Gy, or 8 daily fractions of 2.5 Gy on the vascular volume in Walker 256 carcinomas (s.c.) grown in the legs of Sprague-Dawley rats. Each data point represents the mean ± SE for 8–10 tumors. Panel B: Effects of 20 Gy radiation given in a single fraction, 4 daily fractions of 5 Gy, or 8 daily fractions of 2.5 Gy on the rate of extravasations of plasma protein (vascular permeability) measured simultaneously with the vascular volume in the same tumors. Each data point represents the mean ± SE for 8–10 tumor-bearing animals (*61*).

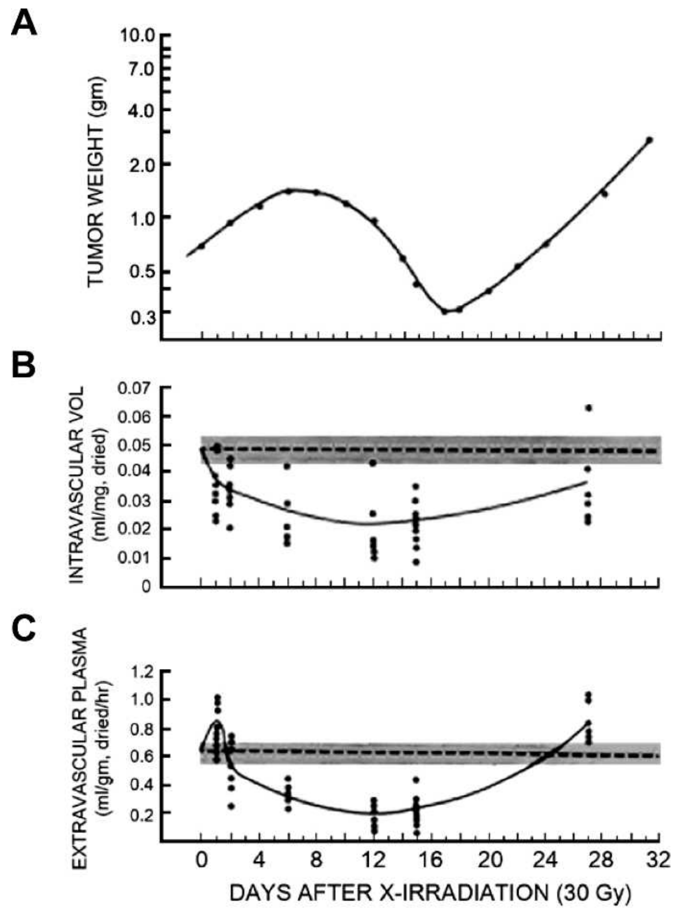

FIG. 4. Effects of 30 Gy radiation given in a single dose on the tumor size and vascular functions in Walker 256 tumors (s.c.) grown in the legs of Sprague-Dawley rats. Panel A: Dried tumor weight. Panel B: Intravascular volume. Panel C: Extravasation rate of plasma protein. The solid lines in each panel indicate the means of 6–10 tumors used at the different times indicated. The dotted lines in each panel are the mean values of 15 control tumors weighing 0.3–2.0 g. The shaded areas show the range of standard error of the mean (*39*).

many but not all tumors (*39*). However, irradiation with 30 or 60 Gy in a single dose caused marked and lasting decreases in functional vascular volume or vascularity in the tumors. The decrease in the vascular volume by irradiation with doses higher than 10 Gy was statistically significant ($P < 0.001$). The vascular permeability in the Walker 256 tumors of rats, as assessed by the extravasation of ^{125}I-labeled albumin, was 20–30 times greater than that in the muscle (*17*). This report was probably the first demonstration that tumor blood vessels are highly permeable compared with the blood vessels of normal tissue. The vascular permeability in Walker 256 tumors increased immediately after irradiation throughout the delivered dose range of 2–20 Gy and then returned to preirradiation levels in 2–3 days (*39–41*). Others have observed similar increases in vascular permeability in tumors or normal tissues after irradiation (*23, 24, 26, 41, 43, 55*). Figure 3 shows the effects of irradiation with 20 Gy in 1, 4 or 8 daily fractions on functional vascular volume and vascular permeability in Walker 256 tumors (*61*). Clearly, the rapid drop in the functional vascular volume after 20 Gy irradiation in a single dose was more substantial than that caused by 20 Gy given in 4 fractions (Fig. 3A). Furthermore, when tumors were exposed to 20 Gy in 8 fractions (8 × 2.5 Gy), the vascular volume initially increased slightly and then decreased as the number of fractions increased. Unlike the rapid decline in intravascular volume observed after a single 20-Gy irradiation, the extravasation of plasma protein (vascular permeability) increased significantly at 24 h after irradiation with 20 Gy (Fig. 3B). It was evident that the radiation-induced increase in vascular permeability at 1 day after irradiation was dose-dependent, with the largest and smallest increases occurring by 20 Gy and 2.5 Gy, respectively. However, the vascular permeability in the tumors irradiated with 20 Gy decreased markedly at 2 days after irradiation. Taken together, it may be concluded that irradiation of Walker 256 tumors with doses exceeding about 10 Gy/fraction causes considerable vascular damage. Figure 4A shows that when Walker 256 tumors grown in the legs of rats were irradiated with 30 Gy in a single dose, the functional intravascular volume declined rapidly and remained decreased until 15–16 days after irradiation while the tumors grew continuously for 7–8 days and then regressed (*39*). Most of the regressed tumors (80%) started to grow again from 15–16 days after irradiation, and the functional intravascular volume also gradually recovered. It should be stressed that the vascular volume decreased much sooner than the tumor began to regress. In a recent investigation by Brown and his associates (*31, 62*), irradiation of human brain tumor xenografts with 15 Gy or 20 Gy caused profound vascular damage and regression of tumors, but the tumors began to recur 2–3 weeks after irradiation accompanied by vasculogenesis caused by bone marrow-derived $CD11b^+$ myelomonocytes. It was further observed that the expression of hypoxia-inducible factor-1 (HIF-1) was upregulated in the irradiated tumors and that HIF-1 enhanced the recruitment of bone marrow-derived cells for vasculogenesis (*31*). In this context, Dewhirst *et al.* (*63*) reported that reoxygenation of hypoxic cancer cells after irradiation increased the level of HIF-1, which then upregulated the level of vascular endothelial cell growth factor (VEGF) and other proangiogenic factors that are known to protect the tumor microvasculature. Effective inhibition of revascularization caused by angiogenesis and vasculogenesis during radiotherapy or after tumors have regressed may enhance the response of tumors to radiotherapy and prevent the recurrence of tumors after treatment.

MECHANISMS OF RADIATION-INDUCED VASCULAR DAMAGE

Radiation-induced changes in blood perfusion, functional intravascular volume and extravasations rates (vascular permeability) are directly related to the functional integrity and viability of vascular endothelial cells. In a previous study with bovine endothelial cells *in vitro* (*64*), the radiation survival curve could be characterized with a D_0 of 1.01 Gy, a D_q of 0.65 Gy, and an extrapolation number (*n*) of 1.9. In a study with endothelial cells of normal tissues (*65*), the D_0 of the radiation survival curve was found to be in the range of 1.2–2.0 Gy and 1.7–2.7 Gy *in vitro* and *in vivo*, respectively (*65*). The radiation survival curve of umbilical cord vein endothelial cells had a D_0 of about 1.65 Gy with a moderate initial shoulder (*66*). Note that these studies were concerned with the radiosensitivity of normal tissue endothelial cells and not with the tumor-associated endothelial cells. Park and her associates recently developed a novel method for harvesting endothelial cells from cancer and normal tissues of human breast cancer patients and expanding the cell populations *in vitro*. Figure 5 shows the *in vitro* radiation survival curves of endothelial cells derived from tumor and normal tissues obtained from two different breast cancer patients (unpublished observations). It is clearly demonstrated that the endothelial cells from breast cancer tissue were significantly more radiosensitive than the endothelial cells from normal breast tissue. Relevant to this conclusion, a recent study by Grabham *et al.* (*67*) using human vessel models demonstrated that developing vessels are more radiosensitive than mature vessels.

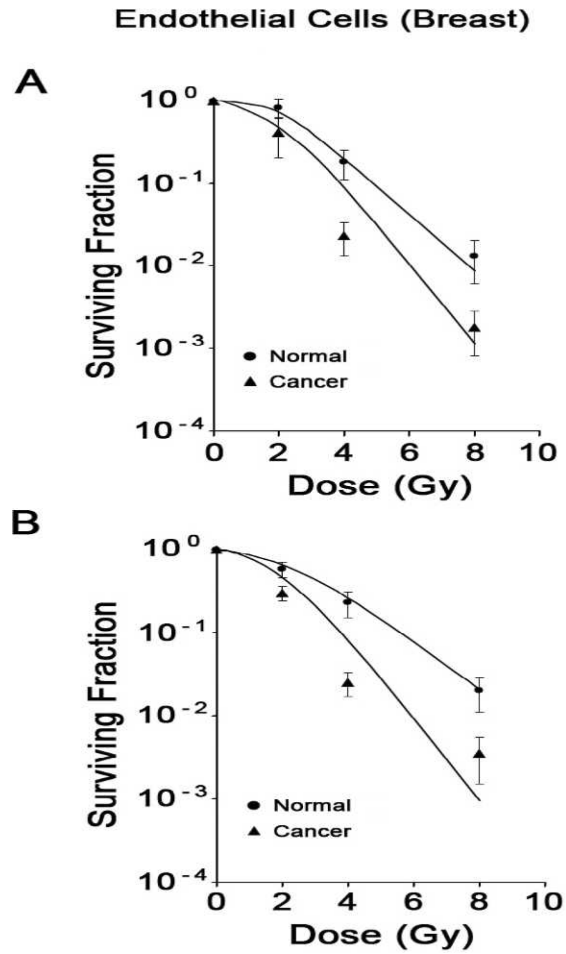

FIG. 5. Clonogenic survival of endothelial cells of breast cancer tissues and normal breast tissues obtained from two breast cancer patients (panels A and B). To obtain the endothelial cells from the tissues, 0.1–0.2-cm^3 pieces of cancer or normal tissue were placed in culture dishes, covered with Matrigel containing VEGF or bFGF, allowed to solidify, and cultured in ECM. After culturing for 2 weeks, the Matrigel containing glowing endothelial cells was separated from the explants and dispersed to single cells by trypsin treatment, and the endothelial cell population was expand in gelatin-coated culture dishes with ECM. Endothelial cells were plated onto collagen-coated 60-mm dishes, incubated overnight, exposed to different doses of γ rays in a single fraction, and cultured in a humidified incubator under a 95% air/5% CO_2 atmosphere at 37°C for 20 days. The colonies were stained with 0.5% crystal violet and the numbers of colonies containing more than 50 cells from triplicate dishes were counted. The data are means of 4 experiments $\pm$ SE.

The death of endothelial cells as a result of direct radiation damage in irradiated tumors would cause focal microscopic or macroscopic vascular damage and eventual malfunction and collapse of the affected capillary-like vessels. As noted above, vascular permeability in tumors increases rapidly after irradiation, probably due to damage in the endothelial cells followed by widening of the gaps between endothelial cells (Fig. 3B, Fig. 4C) (*23, 24, 39, 41, 43, 48, 55, 68*). The increase in extravasation of plasma due to the increase in vascular permeability may increase the erythrocyte concentration within the narrow capillaries, thereby leading to retardation or stasis of blood perfusion. In addition, the increased permeability of capillaries may increase the extravascular or interstitial plasma protein concentrations, thereby elevating interstitial fluid pressure. The elevation of interstitial fluid pressure above the intravascular blood pressure will cause vascular collapse. Therefore, it is probable that the early decline in functional vascularity after irradiation in tumors may be caused at least in part by collapse of blood vessels as a result of elevation of interstitial fluid pressure. When tumor volume shrinks due to death of parenchymal cells after irradiation, the tumor vascular beds may become further disorganized, aggregated, condensed and fragmented (*43*). Figure 3 shows that the extent of vascular damage in tumors treated with fractionated radiation was less than that caused by high-dose single fractions, in accordance with the reports by others (*55, 60*). It is likely that sublethal radiation damage in endothelial cells is repaired during fractionated irradiation and thus the functional integrity of tumor vessels is less impaired.

It is noteworthy that in most of the previous studies on the effects of radiation on tumor vascular functions using rodent tumors or human tumor xenografts, the tumors and varying volumes of the surrounding normal tissues were irradiated simultaneously. Since tumor vascular beds are connected to the vascular networks of normal tissues, it is likely that the vascular damage in the adjacent normal tissues significantly influenced the tumor blood perfusion in the previous studies. Therefore, the inconsistent results on the radiation-induced vascular changes observed in the previous studies with experimental tumors may be attributed in part to the differences in the type and site of tumors studied and also the differences in the volume of normal tissues irradiated. Kioi *et al.* (*31*) reported that irradiation of human U251 glioblastoma xenografts growing in the brain and in the back of nude mice reduced blood flow to 10% and 30% of the original value, respectively. In this respect, it is known that tumors growth is significantly retarded when tumors are transplanted into previously irradiated tissues rather than unirradiated tissues, which is commonly known as the tumor bed effect (*69*). It is believed that angiogenesis in tumors, which originates from existing normal tissue blood vessels, is retarded due to radiation-induced vascular damages in the surrounding normal tissues, and thus the supply of oxygen and other nutrients essential for the growth of tumors is limited. It remains to be investigated whether the vascular changes in tumors treated with conformal irradiation such as SBRT or SRS significantly differ from those in the tumors treated with adjacent normal tissues.

VASCULAR CHANGES AND OXYGEN TENSION IN IRRADIATED TUMORS

The intratumor oxygen tension is controlled by the oxygen supply through blood perfusion and the oxygen consumption rate mainly by the tumor cells. Therefore, the radiation-induced vascular changes may affect the tumor oxygenation. Surprisingly, however, there have been only a few studies that simultaneously measured the radiation-induced changes in tumor microvasculature and the intratumor oxygen tension. In the 1960–1970, Carter and Silver (*70*), Evans and Naylor (*71*), Kolstad (*72*), Bergsjo and Evans (*73*), and Badib and Webster (*74*) pioneered investigations of the effects of radiotherapy on the oxygen tension in various human tumors. Unfortunately, the results of these early studies are rather inconsistent and difficult to interpret because the studies were conducted with equipment and methods with limited accuracy and reliability (*75*). For example, Badib and Webster (*74*) reported that radiotherapy increased tumor oxygenation, but in this study, the tumor pO_2 was measured at only a single point in each tumor. In recent years, using more advanced and reliable methods, investigators determined the changes in pO_2 in various human tumors caused by conventional fractionated radiotherapy. Dunst *et al.* (*76*) determined the pO_2 in human cervical cancer treated with fractionated radiotherapy and reported that the median tumor pO_2 increased significantly when the total dose reached 20 Gy, particularly in tumors that had low baseline pO_2 values. However, the tumor pO_2 declined at the end of treatment, and this appeared to be due to vascular damage. Cooper *et al.* (*77*) also reported that fractionated radiotherapy increased median pO_2 in human cervical cancer. On the other hand, Lyng *et al.* (*21, 78*) and Fyles *et al.* (*79*) observed no significant changes in pO_2 in cervical cancer, and Brizel *et al.* (*80*) also reported no changes in pO_2 in head-neck tumors during the course of conventional fractionated radiotherapy. Interestingly, in the study by Lyng *et al.* (*21*), little changes occurred in pO_2, while there was a clear evidence of vascular damage in the cervical cancer treated with fractionated radiation. In a well-designed study by Stadler *et al.* (*81*), the pO_2 in head-neck tumors decreased significantly by the end of a first course of split-course radiotherapy with 30 Gy, recovered during a 2-week break, and then decreased again by the end of the second course of treatment with 40 Gy. These studies showed that fractionated radiotherapy of human tumors may increase, cause no significant change, or decrease in tumor oxygen tension. As pointed out by Molls *et al.* (*75*), the only trend observed in studies was that the pO_2 in human tumors decreased by the end of the course of fractionated radiotherapy. It is entirely unclear why the direction and magnitude of changes in tumor pO_2 are so inconsistent among different studies and even in the same tumor types, e.g. cervical cancer (*76–79*) and head-neck cancer (*80, 81*). Tumor size, oxygen measurement technique, pO_2 level before treatment, radiation dose and different time-dose schedules are some of the many factors that may control the direction of changes in tumor pO_2. Importantly, unlike the changes in tumor pO_2 during treatment, the tumor pO_2 prior to fractionated radiotherapy has been shown to be related to the outcome of the treatment. The human cervical tumors with high pO_2 before receiving fractionated radiotherapy responded better than those with low pO_2 to the treatments (*82*).

Radiation-induced changes in pO_2 in human tumor xenografts or animal tumors have also been investigated. Brurberg (*29*) studied possible relationships between vascular changes and pO_2 in human melanoma xenografts in nude mice. Irradiation with 10 Gy in a single dose caused no changes in pO_2 in the xenografts in 72 h, while the irradiation reduced the blood perfusion by as much as 40%. In the study by Ceelen *et al*. (*58*), irradiation of rat colorectal tumors grown in the hind legs of rats with 5×5 Gy significantly reduced the microvascular density but slightly increased the intratumor pO_2. Zywietz *et al*. (*48*) treated rhabdomyosarcoma in rats with a total dose of 60 Gy in 20 fractions over 4 weeks and observed that tumor pO_2 increased slightly in the early phase of the treatment but declined as the treatment progressed. The investigators attributed the decrease in tumor pO_2 at the end of treatment to damage in the tumor capillary endothelial cells. The pO_2 in mouse adenocarcinoma decreased significantly in 6 h after 20 Gy irradiation in a single exposure, recovered to control levels by 48 h, and then gradually declined (*83*). Vaupel *et al*. (*84*) reported that irradiation of mouse mammary adenocarcinoma with a single dose of 60 Gy markedly increased tumor pO_2 at 72–74 h after exposure. However, Endrich and Vaupel (*85*) later suggested that a single large dose of radiation would destroy the tumor microvasculature and lead to parenchymal cell death. In a study by Koutcher *et al*. (*86*), irradiation of mouse mammary carcinoma with single doses of 32 or 65 Gy significantly increased the mean tumor pO_2 and reduced the frequency of pO_2 values lower than 2.5 mmHg at 3–4 days after radiation exposure. Unfortunately, in those two studies (*84, 86*), the tumor pO_2 was measured within 3–4 days after irradiation, where as tumor pO_2 may decline later as the radiation-induced vascular damage becomes significant. In this regard, Goda *et al*. (*83*) reported that tumor pO_2 underwent dynamic changes after irradiation with 10, 20 and 40 Gy in a mouse tumor model, and they concluded that repeated monitoring is necessary to know the precise changes in tumor oxygenation in irradiated tumors. Nevertheless, it is rather curious that the tumor pO_2 increased after irradiation with 60 Gy or 65 Gy in view of the possibility that irradiation with such large doses would cause severe damage in the tumor microvasculature, as discussed in the previous section. One may speculate that, in the tumors irradiated with 50–60 Gy, the oxygen demand in tumors is drastically diminished due to rapid death of tumor cells or severe damage to tumor cells that would reduce oxygen consumption before vascular damage is fully expressed (*12*). Another conceivable explanation is that a single large dose of radiation causes a transient vascular normalization by preferentially destroying the most immature and abnormal portions of the vascular bed, allowing for a reorganization of perfusion through the remaining functional, more mature vasculature. However, it is highly likely that an increase in tumor pO_2 after irradiation with doses as high as 60 Gy is a transitional phenomenon because marked increases in the hypoxic areas could be observed in the immunohistochemical preparations of human tumor xenografts 2–3 weeks after irradiation with 15 Gy or 20 Gy (*31, 62*) or in mouse prostate tumors after irradiation with 25 Gy in a single dose (*60*). Likewise, necrotic and hypoxic areas increased significantly in human squamous cell carcinoma xenografts after irradiation with 10 Gy (*27*) and in mouse melanoma irradiated with 12 Gy (*56*). Fractions of hypoxic cells in rodent tumors have been demonstrated to be reoxygenated after an exposure to doses as high as 10–20 Gy. It should be noted that the reoxygenation of hypoxic cells refers to an improvement of oxygenation status of hypoxic cells that survived the initial high-dose irradiation, and it does not necessarily indicate that the overall oxygenation status in the irradiated tumors is increased. Furthermore, it does not indicate the extent of cell death including hypoxic cells after the initial high-dose irradiation (*45*). To our knowledge, the oxygen tension in human tumors treated with high-dose hypofractionated SBRT or SRS has not been investigated.

ROLE OF VASCULAR DAMAGE IN THE RESPONSE OF TUMORS TO RADIOTHERAPY

There have been considerable discussions in the radiotherapy community as to whether the primary effect of ionizing radiation in destroying tumors is directly killing cancer cells or indirectly killing cancer cells via vascular damage. Cramer (*87*) reported as early as 1932 that interference with tumor blood flow caused by radiation damage to tumor stroma played an important role in the overall response of tumors to radiation. Denis *et al*. (*52*) reported that the radiosensitivity of rat mammary tumors correlated with early vessel changes. In support of the notion that the major target of radiotherapy is tumor endothelial cells or vasculature and not tumor parenchymal cells, investigators reported that irradiation caused rapid apoptosis in tumor endothelial cells by promoting acidic sphingomyelinase (ASMase)-mediated generation of ceramide, a proapoptotic second messenger (*54, 88, 89*). Garcia-Barros *et al*. (*54*) concluded that ceramide-mediated apoptosis in tumor endothelial cells leads to secondary death in tumor cells and that radiation-induced endothelial cell death is thus the major player in the response of tumors to radiation at the clinically relevant dose range. Fuks and Kolesnick (*90*) reported that irradiation of tumors with doses higher than 8–10 Gy in a single exposure causes

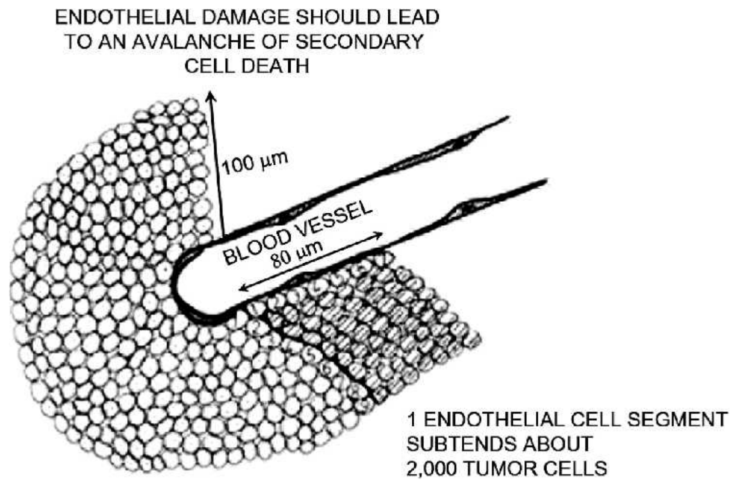

FIG. 6. Schematic illustration of how many tumor cells would be at risk if even a small segment of a capillary is occluded, so that their nutrient supply is completely lost (*94*).

ceramide-mediated apoptosis in endothelial cells, thereby causing indirect death of parenchymal cells, whereas fractionated irradiation with 1.3–3.0 Gy per fraction induces apoptosis in endothelial cells through other signaling pathways. However, above contention that radiosensitivity of endothelial cells dictates the response of tumor to radiotherapy has been strongly rebuffed by other investigators (*91, 92*). Budach and his coinvestigators in Suit's laboratory (*93*) previously reported that the TCD_{50} (radiation dose that cures 50% of tumors treated) for human or murine tumors transplanted into two different strains of mice with different radiosensitivities was dependent on the radiosensitivity of the tumor cells and not the radiosensitivity of the host stromal cells. To further assess the relationship between the intrinsic tumor cell radiosensitivity and tumor response, Gerweck *et al*. (*94*) transplanted radiosensitive DNA-$PKcs^{-/-}$ and radioresistant DNA-$PKcs^{+/+}$ tumor cells into the same strain of nude mice and studied the radiation-induced tumor growth delay. The growth delay of the tumors derived from DNA-$PKcs^{-/-}$ cells were significantly longer than that of the tumors derived from radioresistant DNA-$PKcs^{+/+}$ cells, indicating that the radiosensitivity of tumor cells, not that of stromal cells, dictates the response of tumors to radiotherapy. In a subsequent study by the same group of investigators (*59*), DNA-$PKcs^{-/-}$ and DNA-$PKcs^{+/+}$ tumor cells were transplanted into nude mice and radiosensitive SCID mice, and the resultant tumors were irradiated with 15 Gy in a single exposure. Whereas the irradiation reduced the functional vascularity only modestly in the tumors induced in the nude mice, the irradiation caused considerable reductions in the number of functional vessels in the tumors grown in SCID mice regardless of the intrinsic radiosensitivity of the transplanted tumor cells. An analysis of the radiation-induced growth delay of the tumors indicated that whereas direct killing of tumor cells was the major determinant of tumor response in nude mice, both direct killing and indirect killing of tumor cells as a result of vascular damage contributed to tumor response in SCID mice. It may be concluded that the contribution of radiation-induced vascular damage to the response of tumors to radiation will be significant only when tumors are irradiated with doses high enough to cause substantial vascular damages in the tumors. Therefore, it is likely that the radiosensitivity of endothelial cells is relatively insignificant in the conventional fractionated radiotherapy using 1.5–2.0 Gy/fraction.

Denekamp (*95*) estimated that one endothelial cell subtends a segment of a tumor volume containing as many as 2000 tumor cells (Fig. 6). Given that blood vessels are serial tissues, sectional damage in a vessel may induce cessation of blood perfusion throughout the affected vessel. Unless the blood circulation through the affected vessel is reestablished soon, severe deprivation of oxygen and nutritional supply will develop along the damaged vessel leading to avalanche of tumor cells death. Clement *et al*. (*45, 96*) reported some time ago that irradiation of rodent tumors with 20 Gy in a single exposure caused marked vascular damages leading to massive killing of tumor cells.

In recent years, increasing numbers of cancer patients have been treated with SBRT or SRS, which delivers 20–60 Gy of radiation in 1–5 fractions (*7–11*). It would be quite reasonable to expect that in human tumors, like in animal tumors, irradiation with high doses in a single or several fractions over a short period will cause severe vascular damage and make the intratumor microenvironment hypoxic, acidic and nutritionally deprived, thereby inducing indirect tumor cell death. Kirkpatrick *et al*. (*97*) and Kocher *et al*. (*98*) suggested that the total cell death in tumors receiving high-dose hypofractionated radiotherapy is the product of the direct cytotoxicity of radiation to tumor cells and the indirect tumor cell death caused by radiation-induced vascular damage. An important aspect of the cell death due to vascular damage is that, unlike the direct death, the indirect death caused by vascular damage can occur

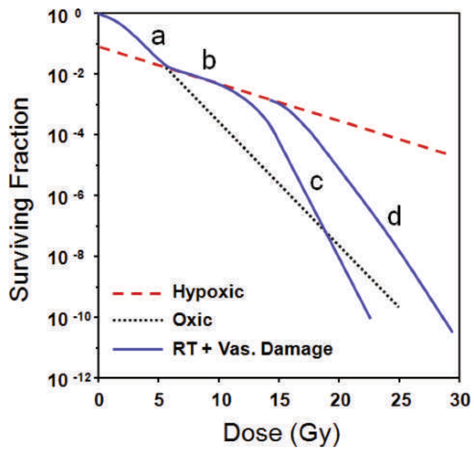

FIG. 7. Hypothetical cell death mechanism in the tumors by an exposure to various doses of ionizing radiation in a single dose assuming 10% of clonogenic cells in the tumors are radiobiologically hypoxic. The initial part of the radiation survival curve a shows the death of fully oxygenated cells. With the increase in radiation dose to higher than about 5 Gy, death of hypoxic cells dominates the cell death, as indicated by curve b. As the radiation dose is increased further to about to 12 Gy, vascular damage begins to occur in the tumors in which endothelial cells are relatively radiosensitive, thereby causing indirect tumor cell death, as shown by curve c. In the tumors in which endothelial cells are radioresistant, indirect cell death due to vascular damage begins when the radiation dose is increased to about 17 Gy, as indicated by curve d (*12*).

regardless of the oxygenation status prior to radiation exposure. Fowler *et al*. (*99*) reported that 3 fractions of 23 Gy (69 Gy) will be necessary to achieve a good chance of eliminating malignant cells in 1–10 g of tumors, assuming that 10% of the tumor cells are hypoxic. Brown *et al*. (*100*) concluded that irradiation with 60 Gy in 3 fractions will reduce the survival of tumor cells by 7.7 logs when 20% of the tumor cells are assumed to be hypoxic, and thus 60 Gy irradiation in 3 fractions would not be able to eradicate all clonogenic cells including hypoxic cells in 1–2-cm-diameter tumors. However, high local control of small tumors has often been achieved in clinical trials for SBRT/SRS with doses apparently insufficient to directly kill all the tumor cells, including hypoxic cells (*7–11*). Based on computer simulation, which was fitted to 90 sets of clinical data, Kocher *et al*. (*98*) concluded that the therapeutic effect of a single radiosurgery in malignant brain tumors cannot be explained without the consideration of vascular effects. The hypothetical cell death mechanism in two tumor types with different endothelial cell radiosensitivity is illustrated in Fig. 7. It is assumed that 10% of clonogenic tumor cells are radiobiologically hypoxic in both tumor types. It is shown that, as the radiation dose is increased, fully oxygenated tumor cells are killed initially and then some hypoxic cells are killed as denoted by ''a'' and ''b'', respectively. With the further increase in radiation doe to about 12 Gy, vascular damage is evoked in the tumors with radiosensitive endothelial cells. Consequently, hypoxic tumor cells are indirectly killed, as denoted by ''c''. In the tumors with radioresistant endothelial cells, vascular damage and resultant indirect death of hypoxic cells begin to take place when the radiation dose is increased to about 17 Gy, as denoted by ''d''. Based on the results of the numerous preclinical and clinical studies, it may be reasonable to suggest that the threshold radiation dose in a single exposure for indirect death of tumor cells in most of the human tumors may fall in the range of 10–15 Gy.

It should be note that the indirect death of tumor cells as a result of vascular damage may not be the only mechanism that accounts for the response of human tumors to SBRT or SRS. It has been suggested that local high-dose irradiation evokes immune reactions and thereby eradicates the tumor cells that escaped the radiation-induced death (*101*). In support of such notion, a recent report showed that ablative radiotherapy dramatically increased T-cell priming in graining lymphoid tissues, leading to reduction/eradication of the primary tumor or distant metastasis in a $CD8^+$ T-cell-dependent fashion (*102*). A possible role of cancer stem cells in the response of tumors to SBRT or SRS has also been suggested. The frequent failure of radiotherapy of tumors due to recurrence has been suggested to be caused by cancer stem cells that are able to survive conventional fractionated radiotherapy. Cancer stem cells have been reported to reside in the perivascular niche in tumors (*103*). It is probable that high-dose irradiation disrupts the niche for the survival of cancer stem cells leading to eradication of radioresistant cancer stem cells.

It is evident that further investigation is warranted to obtain better insights into the vascular damage caused in tumors by high-dose hypofractionated irradiation and the implications of such vascular damage for the response of human tumors to SBRT and SRS. An important question is how the vascular damage caused by high-dose hypofractionated irradiation and the ensuing deterioration of intratumor microenvironment such as the hypoxic, acidic and nutritionally deprived environment would affect the well-established radiobiological principles such as the 4Rs (reoxygenation, repair, repopulation and redistribution) and the linear-quadratic equation.

SUMMARY

An analysis of the studies reported over the last several decades indicates that the functional vascularity in human tumors remains unchanged or improves slightly during the early period of conventional fractionated radiotherapy with 1.5–2.0-Gy daily doses but gradually diminishes during the later part of treatment. Numerous studies with experimental tumors indicated that irradiation with doses higher than 10 Gy in a single fraction or 20–60 Gy in limited numbers of fractions causes severe vascular damage leading to the deterioration of the intratumor microenvironment and indirect death of tumor cells. It is highly likely that similar vascular damage would occur in human tumors irradiated with high-dose hypofractionated radiation. We conclude

that the radiation-induced vascular damage and the resulting indirect death of tumor cells play important roles in the response of tumors to high-dose hypofractionated SBRT and SRS. In addition, enhanced immune reactions and increased eradiation of cancer stem cells might be involved in the response of tumors to SBRT or SRS. Further studies to gain better insights into the effects of high-dose hypofractionated irradiation on tumor vasculature are warranted. In addition, whether the 4Rs and the linear-quadratic equation are applicable for SBRT or SRS remains to be investigated.

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