PERSPECTIVES: CANCER

# What Does Radiotherapy Do to Endothelial Cells?

Judah Folkman and Kevin Camphausen

Radiotherapy has been used to successfully treat a variety of solid tumors. The conventional explanation for why radiotherapy is so effective is that tumor cells are the principal target of ionizing radiation, which damages their DNA causing them to undergo programmed cell death (apoptosis) (*1*). Similarly, it has been presumed that the side effects of radiotherapy are caused by radiation damage to the DNA of normal cells. For example, the severe gastrointestinal side effects of radiotherapy have been attributed to the radiation-induced death of epithelial stem cells residing in the crypts of the gut (see the figure).

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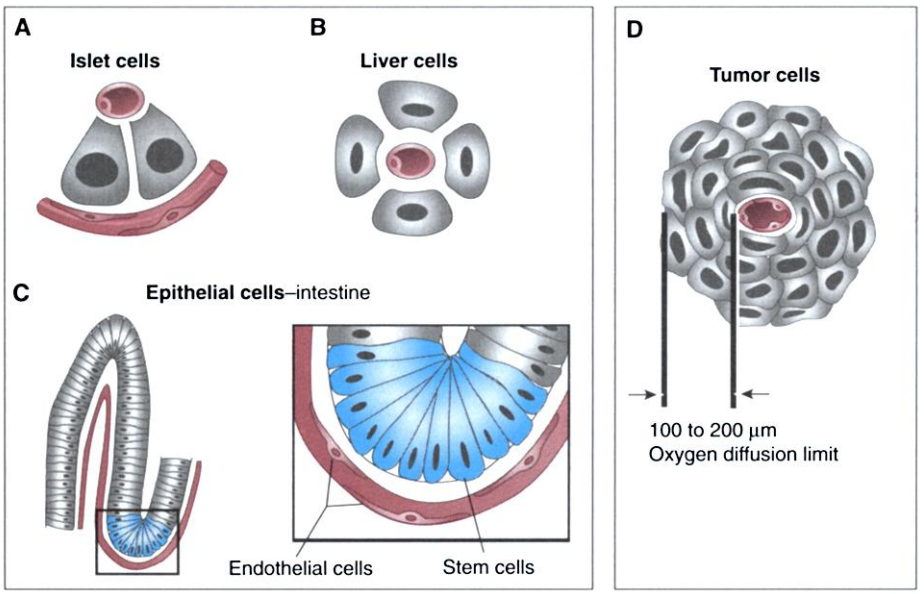
On page 293 of this issue, Paris *et al.* (*2*) turn this scenario on its head. They report that a single large dose of radiation administered to the mouse gastrointestinal tract preferentially damages the endothelial cells of the gut microvasculature. The investigators conclude that the death of epithelial stem cells may be a secondary event resulting from the demise of the endothelial cells on which they depend. This is analogous to the endothelial-dependent growth of tumor cells, which depend for their survival on new blood vessels formed by proliferating endothelial cells (angiogenesis) (*3*).

Paris and colleagues established in previous work that (i) systemic administration of basic fibroblast growth factor (bFGF) enhances murine epithelial stem cell survival and decreases mouse mortality after whole-body irradiation, and (ii) irradiation of microvascular endothelial cells generates ceramide, a proapoptotic lipid that facilitates endothelial cell death (*4*). In their new experiments, the authors show that systemic administration of bFGF, an endothelial cell mitogen and survival factor, overrides the apoptotic signal from ceramide, protecting gut endothelial cells and epithelial stem cells from the effects of whole-body irradiation. The bFGF did not protect bone marrow, however, and animals subsequently died from destruction of bone marrow stem cells. Microvascular endothelial cells express the receptor for bFGF, whereas epithelial stem cells in the intestinal crypts do not (*2*), suggesting that bFGF protects the gut mucosa from radiation damage through its effects on endothelial cells. In the authors' most compelling experiment, mice lacking the gene for acid sphingomyelinase—an enzyme required for ceramide production that is highly expressed in endothelium—are protected from the radiation-induced destruction of the gut mucosa. When wild-type mice that are able to generate ceramide were irradiated, the death of endothelial cells in the microvasculature preceded that of epithelial stem cells in the crypts.

These findings are consistent with a two-compartment model for the irradiation-induced death of intestinal cells: endothelial cells in the gut microvasculature die first, followed by epithelial stem cells that depend on endothelial cell support. This two-compartment model is reminiscent of the way in which tumor growth is inhibited by endothelial cell blockers (antiangiogenic therapy) (*5*). Tumor cells grow as a perivascular cuff around a blood vessel (see the figure). They stimulate endothelial cell proliferation and the growth of new blood vessels by releasing endothelial mitogens and chemotactic factors, such as bFGF and vascular endothelial growth factor. Endothelial cells, in turn, protect tumor cells by releasing at least 20 growth and survival factors including heparin-binding epithelial growth factor and interleukin 6 (*6*). If mice bearing tumors are treated with antiangiogenic therapy—which targets proliferating endothelial cells in newly forming blood vessels—endothelial cell apoptosis precedes tumor cell apoptosis by 3 to 5 days, suggesting that tumor cells are dependent on endothelial cells for survival (*7*). A similar two-compartment model has also been proposed for growth of normal tissue, which seems to depend on the prior expansion of endothelial cells and angiogenesis. Expansion of the endothelial cell population in the microvasculature of prostate (*8, 9*), fat (*10*), and regenerating




**Endothelial cells take center stage.** Microvascular endothelial cells that line capillary blood vessels are situated very close to normal tissue cells such as epithelial cells in the gut mucosa. This close apposition enables endothelial cells and epithelial cells to communicate with each other by release of growth factors and hormones. Epithelial cells are also able to derive oxygen and nutrients from blood vessels. (**A**) Islet cells of the pancreas are sandwiched between two capillary vessels (fat cells and most muscle cells are similarly arranged). (**B**) Liver cord cells are arranged around a central capillary. (**C**) Epithelial stem cells of the gut mucosa reside in the crypts of Lieberkühn (box) and are separated from the microvasculature by a very short distance (~100 μm), which enables oxygen to diffuse from the blood vessels into the crypts. (**D**) In contrast, tumor cells form multiple layers around a capillary blood vessel such that the most remote tumor cells are oxygen-deprived (hypoxic or anoxic). [Modified from (*3*)]

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liver (*11*) is required before these tissues can grow, expand, or regenerate.

In the Paris *et al.* work, a single large dose of radiation caused endothelial cell apoptosis in the intestinal mucosa. If a similar effect is seen with fractionated radiotherapy (a more clinically relevant treatment), this could have profound implications for cancer therapy. For example, if the microvascular endothelial cell is the principal target of radiotherapy and damage to the epithelial stem cell is a secondary event, this relationship may also hold for endothelial cells and the tumor cells they support. This scenario would explain the synergistic effects obtained when radiotherapy is combined with antiangiogenic therapy (*12, 13*). Even if the endothelial cell response is only a component of the tumor response to radiation, attacking both compartments is a logical therapeutic strategy.

A poorly understood feature of radiotherapy treatment is that some tumors are very radiosensitive in vivo (for example, Hodgkin's lymphoma) and others are very radioresistant (for example, glioblastoma), whereas in vitro these tumors have similar or overlapping radiosensitivities (*14*). In vitro, tumor cells are the only target and ionizing radiation directly damages their DNA, inducing them to undergo apoptosis. However, in vivo, there are a multitude of supporting cells (including endothelial cells) that may be more sensitive to ionizing radiation than tumor cells, which then die not because of DNA damage but because they require endothelial cell support. The Paris *et al.* report prepares the stage for studying the effects of radiation on microvascular endothelial cells recruited to the tumor bed during angiogenesis. It may be possible to modify the radiosensitivity of a tumor by increasing or decreasing circulating endothelial inhibitors or stimulators, thereby making the tumor microvasculature more radiosensitive. If further evidence supports the idea that the microvascular endothelial cell is the principal target of ionizing radiation, as indicated by the provocative results of Paris *et al.*, then treating tumors first with angiogenesis inhibitors may sensitize the tumors to ionizing radiation, allowing lower radiation doses to be used.

**References**


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PERSPECTIVES: IMMUNOLOGY

# Tampering with the Immune System

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The immune system must maintain a delicate balance between the positive signals that activate lymphocytes and the negative signals that dampen inappropriate immune responses. If this balance is upset, the immune system either does not respond to pathogens or responds inappropriately, resulting in autoimmune disease. Antigens, cytokines, and death-inducing members of the tumor necrosis factor (TNF) family (*1, 2*) all contribute to homeostatic regulation of the immune system. Several recent reports, including one by Lu and Lemke (*3*) on page 306 of this issue, now implicate the Tyro-3 family of receptor tyrosine kinases as important players in immune regulation. The Tyro-3 family (Tyro-3, Axl, and Mer) may fine-tune the immune response by modulating the activity of macrophages and other antigen-presenting cells (APCs) that present antigen to T and B lymphocytes (*3–5*).

The Tyro-3 receptor tyrosine kinases have an extracellular region composed of two immunoglobulin-like domains and two fibronectin-like domains, and an intracellular kinase domain that contains a distinctive Lys-Trp-Ile-Ala-Ile-Glu-Ser motif [reviewed in (*6*)]. These receptors are overexpressed by many tumors and can transform (immortalize) cultured cells in vitro, suggesting that they provide positive growth-promoting signals to cells. They activate Src family kinases and signaling pathways downstream of Grb2 (an adapter protein in the Ras pathway), promoting cell proliferation and protecting against programmed cell death (apoptosis). The ligands for these receptors, protein S (an anticoagulant) and Gas6, are expressed by many cell types and share similarities with sex hormone–binding globulin. Although the Tyro-3 receptors and their ligands have been well characterized, the signaling pathways that they activate are poorly understood.

In previous work, Lu and Lemke (*7*) reported that triple mutant mice lacking the Tyro-3, Axl, and Mer receptors (TAM) contained large numbers of apoptotic cells in many of their tissues, resulting in reproductive, neural, and immune abnormalities. They now report that these TAM-deficient mice also bear the hallmark features of autoimmunity: increased numbers of activated lymphocytes, lymphocytic invasion of multiple organs, increased serum autoantibodies, and deposition of immune complexes in tissues (*3*). The presence of anti-phospholipid antibodies and cerebral hemorrhages in TAM-deficient animals is reminiscent of the human antiphospholipid syndrome characterized by an increased risk of thrombosis, stroke, and miscarriage. This human autoimmune thrombolytic disorder is associated with a decrease in serum protein S, which is presumed to be a secondary event related to the hypercoagulation deficit. The Lu and Lemke study, however, raises the intriguing possibility that a decrease in serum protein S may contribute directly to the autoimmune process through a decrease in Tyro-3 receptor activation (*3*).

How do the Tyro-3, Axl, and Mer receptors contribute to regulation of the immune system? Tyro-3 receptors are not expressed by quiescent lymphocytes, but are expressed by many other cell types including APCs (see the figure). Both macrophages and CD11b-positive APCs from TAM-deficient mice become hyperactivated when stimulated with bacterial lipopolysaccharide (LPS) in vitro (*3*). Macrophages freshly isolated from TAM-deficient animals have increased expression of major histocompatibility complex class II molecules and produce elevated amounts of interleukin-12, suggesting that

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