Engaging the vascular component of the tumor response

Recent research has shed new light on the critical role of tissue microvasculature in regulating the tumor response to radiation and drugs. In this issue of *Cancer Cell*, Moeller et al. (2005) demonstrate that HIF-1 activation during the course of fractionated radiotherapy initiates pleiotropic adaptive responses in both tumor cells and the microvascular network, radiosensitizing tumor cells but concomitantly conferring tumor radioresistance due to protection of the microvascular endothelium. HIF-1 thus serves as a legitimate target for differential modulation of tissue response to radiation.

Over the past several years, there has been an evolving transformation in our understanding of the role of the microvasculature in normal tissue and tumor responses to radiation and drugs. The classical model of tumor radiotherapy defines depletion of tumor stem cells and undifferentiated progenitor clonogens as obligatory for the curative effect of radiation, while damage to tumor microenvironmental elements is believed to provide modulating, though not mandatory, signals. Recent genetic and pharmacologic studies have, however, revealed that crosstalk between tumor cells and the host-derived tumor microvascular network, both damaged by radiation, constitutes a generic element of the pathogenesis of radiation-induced tissue damage. The studies by Moeller et al. (2004, 2005) provide the first mechanistic insight into tumor cell-microvascular interactions that occur during the course of fractionated radiotherapy. Radiation treatment of human cancer is commonly delivered by fractionated schemes, consisting of a daily small dose, repeated until a potentially curative tumor-specific dose has accumulated. Fractionated radiation has been favored because it affords protection to normal tissues relative to tumors, as normal tissue stem cells appear to be more proficient in radiation damage repair during the interfractional intervals than tumor clonogens.

Prior work of Moeller et al. (2004) provided strong evidence that bursts of reactive oxygen species (ROS), generated by waves of hypoxia/reoxygenation occurring after each radiation exposure, lead to induction of hypoxia-inducible factor-1 (HIF-1) activity. The HIF-1 transcription factor regulates adaptive responses to changes in tissue oxygena-

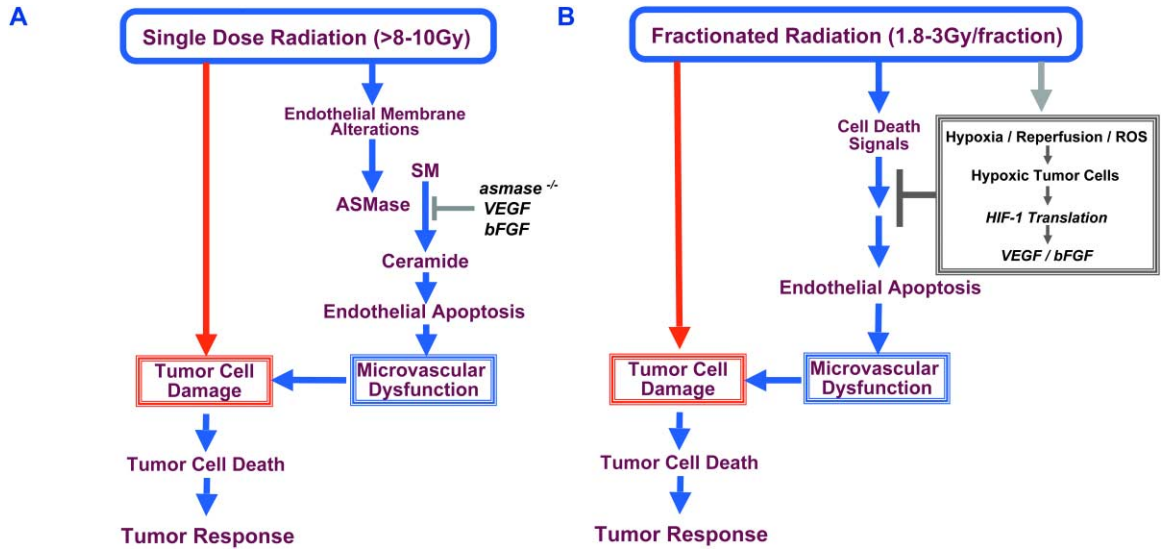

Figure 1. Models of microvascular endothelial engagement in tumor response to single-dose or fractionated radiotherapy

Endothelial damage appears to be induced by both the high treatment doses (>8–10 Gy) of single-dose radiotherapy (**A**) and the low-dose (1.8–3 Gy) exposures of fractionated radiotherapy (**B**). The resulting microvascular dysfunction confers conversion of sublethal radiation lesions in tumor cells into lethal lesions via an as yet unknown mechanism.

Endothelial apoptosis and microvascular dysfunction contribute significantly to tumor cell lethality and tumor cure by the single-dose approach. Radiation induces translocation of endothelial cell ASMase into glycosphingolipid- and cholesterol-enriched plasma membrane rafts, where it hydrolyses sphingomyelin (SM) to generate the proapoptotic second messenger ceramide, initiating transmembrane signaling of apoptosis. Inhibition of this process by ASMase depletion or by proangiogenic growth factors markedly attenuates the lethal response of tumor cells to this mode of radiotherapy.

In contrast, the endothelial cell damage induced by the low-dose exposures of fractionated radiotherapy does not enhance tumor cell death effectively, as the death signaling pathway in endothelium is repressed by concomitant activation of tumor cell HIF-1. ROS generated by waves of hypoxia/reoxygenation occurring after each radiation exposure lead to translation of HIF-1 mRNA transcripts stored in specialized cytosolic stress granules of hypoxic tumor cells. This adaptive response generates VEGF and other proangiogenic factors that attenuate radiation-induced apoptosis in endothelial cells. Genetic inhibition of the HIF-1 response leads to extensive endothelial apoptosis, microvascular dysfunction, enhanced tumor cell death, and tumor growth delay. The mechanism of endothelial damage in this response remains unknown, and a possible involvement of the ASMase pathway has not as yet been assessed. This observation indicates a potential for pharmacologic targeting of HIF-1 to improve the outcome of fractionated radiotherapy via engagement of the endothelial apoptosis component.

tion, engaging more than 60 target genes, all driven by promoters that contain a hypoxia response element (HRE) (Semenza, 2003). Remarkably, HIF-1 upregulation during fractionated radiotherapy does not result from gene transcription secondary to waves of hypoxia. Rather, the reoxygenation-induced ROS appear to initiate translation of preformed HIF-1 mRNA transcripts, stored in specialized cytosolic stress granules. Sequestration of HIF-1 transcripts in these granules is preconditioned by chronic tumor hypoxia, which develops in many tumors when neoangiogenesis lags behind the expansion of the tumor cell compartment. The postradiation translation of HIF-1 results in upregulation of VEGF and other proangiogenic factors that protect tumor endothelium, conferring radioresistance. Moeller et al. (2005) now report that in contradistinction to endothelial cell protection, radiation-induced HIF-1 also regulates gene products that enhance tumor cell radiosensitivity through increased apoptotic potential, proliferation rates, and ATP metabolism. However, p53 is required for HIF-1-mediated tumor cell apoptosis. Since more than half of tumor phenotypes are p53-mutated, the vascular-mediated radioresistance appears dominant in affecting overall tumor sensitivity to fractionated irradiation. In this context, genetic or pharmacologic HIF-1 inactivation resulted in dramatic regression of tumor microvasculature, apparently secondary to deprivation of HIF-1-activated factors, and significant enhancement of tumor growth delay, regardless of whether HIF-1 inhibition occurred before or after completion of radiotherapy.

These unique observations raise further questions regarding the mechanism of endothelial engagement in the response to fractionated radiotherapy. Clearly, an endothelial damage component is required for tumor microvascular regression, since HIF-1 deprivation alone does not elicit endothelial damage or tumor response in control unirradiated tumors. The kinetics of the response to HIF-1 deprivation suggest that irradiated endothelium harbors pro-death signals, quenched by HIF-1-induced growth factors and cytokines. These pro-death signals appear to be sustained in endothelium, since genetic inactivation of HIF-1 after completion of radiotherapy still conferred endothelial cell death and enhanced tumor growth delay. Moeller et al. suggest that HIF-1 may protect the endothelium via VEGF and/or bFGF. However, other tumor models in which endothelial survival is conferred by adjacent tumor cell signals have shown that VEGF is not sufficient for maintaining endothelium. Chin et al. (1999) reported that expression of H-Ras^{V12G} in a doxycycline-inducible H-Ras^{V12G} mouse melanoma model is required for survival of host-derived tumor endothelium. Withdrawal of doxycycline and H-Ras^{V12G} downregulation resulted in marked apoptosis of tumor microvascular endothelium, which could not be rescued by retrovirally enforced VEGF expression. The impact of endothelial protection on the outcome of clinical fractionated radiotherapy protocols thus requires further clarification, as it appears to introduce an element of radioresistance. While the experimental protocol used by Moeller et al. consisted of three fractions of 3 Gy delivered over 36 hr, curative fractionated radiotherapy usually involves 25–40 fractions of 1.8–2 Gy each, delivered over several weeks. Hence, it remains to be established whether HIF-1 induction by waves of reoxygenation-induced ROS can be sustained over such a prolonged series of repeated stimulations, and whether the endothelium might develop resistance to HIF-1-mediated signals when repeated with such frequency.

The paradigm of endothelial involvement in fractionated radiotherapy, described by Moeller et al., is evidently distinct from the mechanism of endothelial involvement in curative single-dose radiation (Figure 1). The clinical use of single-dose radiotherapy has increased recently due to the emergence of image-guided, high-precision targeting of human tumors. This approach maximally avoids surrounding normal tissues, enabling application of large tumoricidal radiation doses with minimal normal tissue toxicity. Studies by Garcia-Barros et al. (2003) revealed that exposure of mouse MCA129 fibrosarcoma and B16 melanoma to single doses of 15–20 Gy is followed by a rapid wave of endothelial apoptosis at 1–6 hr as the earliest anatomical evidence of tissue damage. Tumor cells appeared intact for 2–3 days, subsequently succumbing to treatment and leading to different rates of local tumor cure or tumor growth delay, depending on dose and tumor type. The mechanism of endothelial apoptosis in this response has been extensively explored (Kolesnick and Fuks, 2003) and shown to be mediated via the acid sphingomyelinase (ASMase) pathway. ASMase hydrolyzes sphingomyelin to generate the proapoptotic second messenger ceramide. Radiation induces rapid translocation of a secretory form of ASMase from cytosol into glycosphingolipid- and cholesterol-enriched rafts in the outer leaflet of the plasma membrane (Gulbins and Kolesnick, 2003), where ceramide is rapidly generated, coordinating transmembrane signaling of apoptosis. Endothelial cells are 20-fold enriched in secretory ASMase compared with any other cell in the body, and are particularly sensitive to radiation-induced apoptosis in vitro and in vivo via the ASMase pathway.

The studies by Garcia-Barros et al. (2003) showed that the early-phase microvascular endothelial apoptosis is mandatory for tumor cure, as MCA129 fibrosarcoma and B16 melanoma grown in apoptosis-resistant *asmase*$^{-/-}$ or *Bax*$^{-/-}$ mice were completely resistant to 15–20 Gy single-dose irradiation. These observations indicated that radiation-induced lesions in tumor cells were by themselves not lethal, and that their conversion to lethal damage is tightly coupled to the endothelial apoptotic response. The mechanism of the endothelial-tumor linkage is still unknown. It might involve leakage of a circulating factor, a bystander effect secondary to endothelial damage, or transient local ischemia/reperfusion produced by the acute microvascular dysfunction and its rapid reversal, perhaps by recruitment of circulating marrow-derived endothelial progenitor cells (Garcia-Barros et al., 2003). Of great interest are preliminary observations that human tumor specimens irradiated ex vivo within 15 min of surgical resection show the same rapid wave of endothelial apoptosis and dose-response profile for apoptosis as in the animal studies, except for grade IV glioblastoma, which exhibits apoptosis resistance (Fuks and Kolesnick, unpublished data). The endothelial responses in mouse and human tumor specimens both display an apparent threshold at 8–10 Gy and a maximal response at 20–25 Gy. The endothelial-stem cell linked mechanism would, therefore, not be activated by fractionated radiation schemes using <8 Gy/fraction, as employed by Moeller et al. This endothelial-stem cell linkage mechanism was also shown to mediate normal tissue damage after single-dose exposure of the intestines and lung (Kolesnick

and Fuks, 2003; Paris et al., 2001), suggesting that this represents a generic response mechanism for mammalian tissue damage by large single-dose irradiation. The possibility that a similar crosstalk between microvasculature and tumor clonogens occurs during fractionated radiotherapy when the HIF-1-mediated endothelial protection is removed, such as reported by Moeller et al., represents a testable hypothesis.

In principle, the studies of Moeller et al. support the notion that fractionated radiotherapy, like single-dose radiation, engages a vascular component of the tumor response. In the case of fractionated radiotherapy, however, this response is largely attenuated by adaptive signals generated by HIF-1 activation. Hence, Moeller et al. suggest that HIF-1 may represent a valid target for radiosensitization via derepression of endothelial cell death. However, they caution that HIF-1 inactivation, if it is to be therapeutically efficacious, should be scheduled to optimize tumor cell radiosensitization. In contrast, the endothelial death signal produced by large-dose exposure (>8–10 Gy) may precede or be of sufficient magnitude to overcome HIF-1 anti-death protection. These provocative studies should open up new avenues for basic research into mechanisms of endothelial cell damage and the role of the microvascular response in therapy, potentially providing new pharmacologic targets for improving radiation and other anticancer treatments.

Zvi Fuks[1] and Richard Kolesnick[2,*]

[1]Department of Radiation Oncology
[2]Program of Molecular Pharmacology and Chemistry
Memorial Sloan-Kettering Cancer Center
1275 York Avenue
New York, New York 10021
*E-mail: r-kolesnick@ski.mskcc.org

Selected reading

Chin, L., Tam, A., Pomerantz, J., Wong, M., Holash, J., Bardeesy, N., Shen, Q., O'Hagan, R., Pantginis, J., Zhou, H., et al. (1999). Nature *400*, 468–472.

Garcia-Barros, M., Paris, F., Cordon-Cardo, C., Lyden, D., Rafii, S., Haimovitz-Friedman, A., Fuks, Z., and Kolesnick, R. (2003). Science *300*, 1155–1159.

Gulbins, E., and Kolesnick, R. (2003). Oncogene *22*, 7070–7077.

Kolesnick, R., and Fuks, Z. (2003). Oncogene *22*, 5897–5906.

Moeller, B.J., Cao, Y., Li, C.Y., and Dewhirst, M.W. (2004). Cancer Cell *5*, 429–441.

Moeller, B.J., Dreher, M.R., Rabbani, Z.N., Schroeder, T., Cao, Y., Li, C.Y., and Dewhirst, M.W. (2005). Cancer Cell, this issue.

Paris, F., Fuks, Z., Kang, A., Capodieci, P., Juan, G., Ehleiter, D., Haimovitz-Friedman, A., Cordon-Cardo, C., and Kolesnick, R. (2001). Science *293*, 293–297.

Semenza, G.L. (2003). Nat. Rev. Cancer *3*, 721–732.

DOI: 10.1016/j.ccr.2005.07.014

A common pathway for genetic events leading to pheochromocytoma

Mutations in *VHL*, *RET*, *NF1*, *SDHB*, *SDHC*, and *SDHD* can give rise to pheochromocytoma/paraganglioma. These different genetic lesions may all act by decreasing the activity of a 2-oxoglutarate-dependent oxygenase, SM-20/EglN3/PHD3, resulting in reduced apoptosis of neural crest cells during development.

Hereditary tumor syndromes have given numerous insights into cancer biology. Often it is straightforward to see a link between the genetic defect and tumor predisposition. This is the case if the gene product impinges on pathways involved in cell proliferation or cell death. In other examples, maintenance of the genome is compromised, so that the likelihood of developing mutations that lead to an increase in proliferation or death is increased. But the link between the genetic defect and tumor predisposition is not always clear. Arguably, these cases are most likely to lead to truly novel insights into tumor development. A paper from Bill Kaelin's group in this issue of *Cancer Cell* suggests that apparently unlinked genes implicated in paraganglioma act in a single common pathway (Lee et al., 2005). The authors provide evidence that all the genetic defects act by decreasing the likelihood of apoptosis of neural crest cells at the time during development when levels of nerve growth factor (NGF) become limiting. The study gives particular insight into the interesting issue of how mutations affecting succinate dehydrogenase components could be tumorigenic.

Paragangliomas are tumors of the autonomic nervous system. Non-chromaffin paragangliomas are rare, are described as "chemodectomas," and are located in the head and neck. Chromaffin paragangliomas are much commoner, have endocrine activity, and are referred to as "pheochromocytomas." These are usually located in the adrenal medulla but sometimes occur in the pre- and paravertebral thoracoabdominal regions. Familial paraganglioma syndromes can

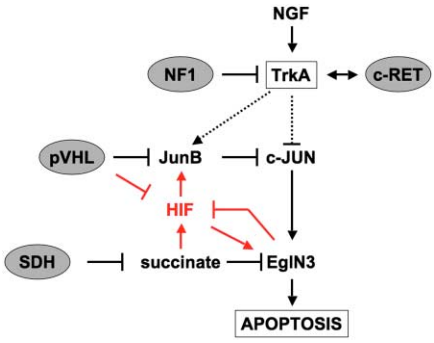

Figure 1. As NGF levels become limiting during development, mutations affecting *NF1*, *RET*, *VHL*, and SDH subunits all decrease apoptosis mediated by SM-20/EglN3/PHD3. Adapted from Lee et al. (2005) to show interactions with HIF.