Intratumoral hypoxia, radiation resistance, and HIF-1

Failure to achieve complete remission after radiation therapy is a predictor of patient mortality, and hypoxic cancer cells are more likely to survive radiation therapy. Recent studies have shown that radiation-induced endothelial cell death results in secondary tumor cell killing. In this issue of *Cancer Cell*, Moeller et al. (2004) now provide evidence that radiation induces HIF-1-mediated expression of VEGF and bFGF in tumor cells, which promotes endothelial cell survival.

A major paradigm shift in cancer biology has involved the realization that cancers are not just clonal populations of cells gone awry, but rather are complex multicellular tissues in which individual cells respond to intercellular signals, the microenvironment, and the therapies directed against them. A driving force for this shift has been the emergence of angiogenesis as a critical factor in cancer progression, from the initial development of a vascularized primary tumor, which is no longer limited by the diffusion of O_2 from host vessels, to the establishment of metastases, which define the terminal stage of the disease.

The importance of the tumor microenvironment in determining clinical outcome was highlighted by Gray et al. (1953), who proposed that hypoxic cancer cells are resistant to radiation therapy. The effect of hypoxia was interpreted as reflecting the requirement for O_2 as a source of radiation-induced radicals that mediate tumor cell killing. The more general principle, which was not appreciated at the time, is that hypoxia is an important selective force in the progression of many cancers (reviewed in Semenza, 2003).

The discovery of radiation resistance of hypoxic cells was followed decades later by the demonstration that hypoxia is the principal physiologic stimulus for angiogenesis and, subsequently, by the discovery of hypoxia-inducible factor 1 (HIF-1) as the major transcriptional regulator of hypoxia-induced angiogenesis through transactivation of genes encoding multiple angiogenic growth factors including vascular endothelial growth factor (VEGF) (reviewed in Semenza, 2003). An important connection between radiation and angiogenesis was made by the demonstration that the tumor vasculature represents an important target of radiation therapy and a major determinant of the clinical response (Garcia-Barros et al., 2003).

Clinical and preclinical studies have also implicated HIF-1 in radiation resistance. In patients with oropharyngeal cancer, overexpression of HIF-1α in tumor biopsy is associated with an increased risk of failure to achieve complete remission after radiation therapy (Aebersold et al., 2001). Conversely, tumor xenografts of HIF-1α null transformed mouse embryo fibroblasts manifest increased radiation sensitivity (Unruh et al., 2003).

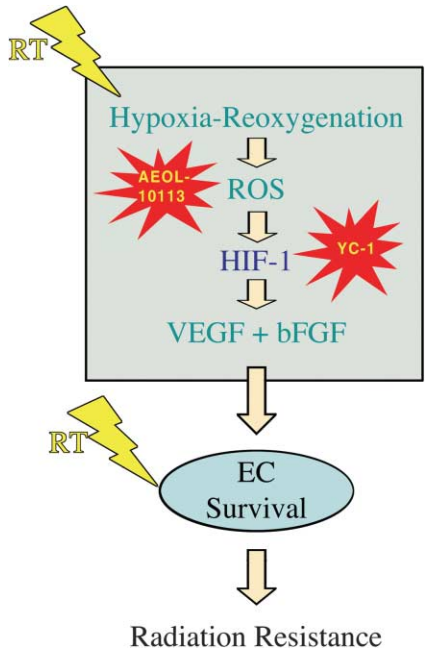

Figure 1. Radiation induces HIF-1 activity and production of survival factors

Radiation therapy (RT) results in the reoxygenation of hypoxic tumor cells and the production of reactive oxygen species (ROS) that induce the activity of hypoxia-inducible factor 1 (HIF-1), which directly activates transcription of the *VEGF* gene encoding vascular endothelial growth factor. Expression of basic fibroblast growth factor (bFGF) is also induced in a HIF-1-dependent manner by unknown mechanisms. The secretion of VEGF and bFGF by tumor cells promotes endothelial cell (EC) survival. Maintenance of a functional vasculature increases the survival of tumor cells. Thus, to the extent that radiation-induced EC death contributes to secondary tumor cell killing, activation of the HIF-1 response pathway may contribute to radiation resistance. The response can be blocked by the administration of either AEOL-10113, a superoxide dismutase mimetic, or YC-1, a small molecule inhibitor of HIF-1.

An elegant study by Moeller et al. (2004) in this issue of *Cancer Cell* further establishes the connection between HIF-1, tumor vasculature, and radiation resistance. The authors demonstrate that irradiation of tumor xenografts induces HIF-1 activity, leading to the expression of VEGF and basic fibroblast growth factor (bFGF), which act to prevent radiation-induced endothelial cell (EC) death (Figure 1). Remarkably, the induction of HIF-1 does not start until 12 hr and peaks at 48 hr after radiation. Moeller et al. (2004) provide evidence that radiation-induced reoxygenation of hypoxic tumor cells results in the production of reactive oxygen species (ROS) that induce HIF-1 activity, as determined by the expression of green fluorescent protein (GFP) driven by a promoter containing a hypoxia response element (HRE).

How do ROS generated during reoxygenation induce HIF-1 activity? The half-life and transcriptional activity of HIF-1α are negatively regulated by O_2-dependent prolyl and asparaginyl hydroxylation, respectively (reviewed in Semenza, 2003). During the hydroxylation reactions, Fe (II) in the active site of the hydroxylases is oxidized to Fe (III). The hydroxylases utilize ascorbate as a cofactor to reduce Fe (III) back to Fe (II). Thus, hydroxylase activity may be inhibited as a result of ROS-mediated depletion of ascorbate.

Moeller et al. (2004) demonstrate that administration of the free radical scavenger AEOL-10113 blocks the induction of HIF-1 activity and results in dramatic regression of the tumor vasculature and a significant enhancement of tumor growth delay. Ascorbate administration has been shown to inhibit HIF-1α expression in cancer cells ex vivo (Knowles et al., 2003). Could ascorbate administration to patients potentiate the effects of radiation by blocking induction of HIF-1 and VEGF?

The authors also show that treatment of tumor-bearing mice with YC-1, which is a small-molecule inhibitor of HIF-1 (Yeo et al., 2003), impairs tumor growth after a dose of radiation (10 Gy) that alone had

little effect on tumor growth. The results of treatment with AEOL-10113 or YC-1 after radiation therapy have potentially important clinical implications. Further studies are required to determine whether this sort of sequential therapy at higher doses can have synergistic effects leading to tumor ablation.

Finally, the authors demonstrate that reoxygenation of cultured cells following hypoxia results in a dramatic increase in GFP activity compared to hypoxia alone. They provide evidence that this is related to the hypoxia-induced formation of stress granules, which sequester mRNAs transcribed by HIF-1 and prevent their translation until reoxygenation occurs. This result is in contrast to many studies that have demonstrated increased activity of HRE-driven luciferase reporter genes and increased secretion of endogenous VEGF protein by tumor cell lines exposed to continuous hypoxia. The presence of an internal ribosomal entry site, which allows cap-independent translation, is a feature of the mRNAs encoding HIF-1α, VEGF, and possibly other hypoxia-inducible gene products (Stein et al., 1998). Thus, there may be two categories of hypoxia-inducible gene products, those that are translated during hypoxia and those that are not translated until reoxygenation.

The combination of mechanistic insight into fundamental aspects of cancer biology and the potential therapeutic implications makes this a remarkable paper. One factor that may limit the extent to which these results are applicable to human cancers is that the data were derived from tumor xenografts. Recent studies suggest that the vasculature of tumor xenografts differs significantly from the vasculature of spontaneously arising tumors (Ruzinova et al., 2003). Subcutaneous injection of cancer cells into nude mice results in the rapid growth of a tumor, the vascularization of which is dependent upon the recruitment of bone marrow-derived endothelial progenitor cells. In contrast, the vasculature of slower-growing spontaneous tumors may occur primarily via the process of angiogenesis, in which new capillaries bud from existing host vessels and grow into the tumor. Thus, it will be important to replicate the findings of Moeller et al. (2004) in a spontaneous tumor model.

The results reported by Moeller et al. (2004) represent an important advance linking intratumoral hypoxia, HIF-1, EC survival, and radiation resistance. Like all good studies, their work generates many provocative questions. Further studies will determine the extent to which these intriguing observations are relevant to cancer patients and their treatment.

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Engineered embryonic endothelial progenitor cells as therapeutic Trojan horses

While the hematogenic contribution of circulating endothelial cells to tumor angiogenesis is not entirely understood, one can exploit this phenomenon as a therapeutic strategy. In this issue of *Cancer Cell*, Wei et al. (2004) show that murine embryonic endothelial progenitor cells preferentially home to sites of lung metastases, evade immunological rejection, and can exert a bystander antitumor effect when modified to contain a suicide gene construct that activates a prodrug. Treatment with the prodrug led to improved survival in syngeneic and nonsyngeneic tumor-bearing mouse models. The conceptual advance put forward by this study might result in translational applications.

The Trojan horse was as a clever instrument of war used by the Greeks to deceive the Trojans. Epic accounts have it that the Greeks built a giant hollow wooden horse—loaded with armed soldiers—which was delivered as a gift offering of peace. The strategy worked when the soldiers quietly climbed down from the horse and opened the city gates, thus providing for access to and defeat of Troy after a decade of siege by the Greek army. In spite of its overuse (a Google search reveals over 1.1×10^6 hits), this ancient military analogy can perhaps be best used to describe an antitumor approach