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

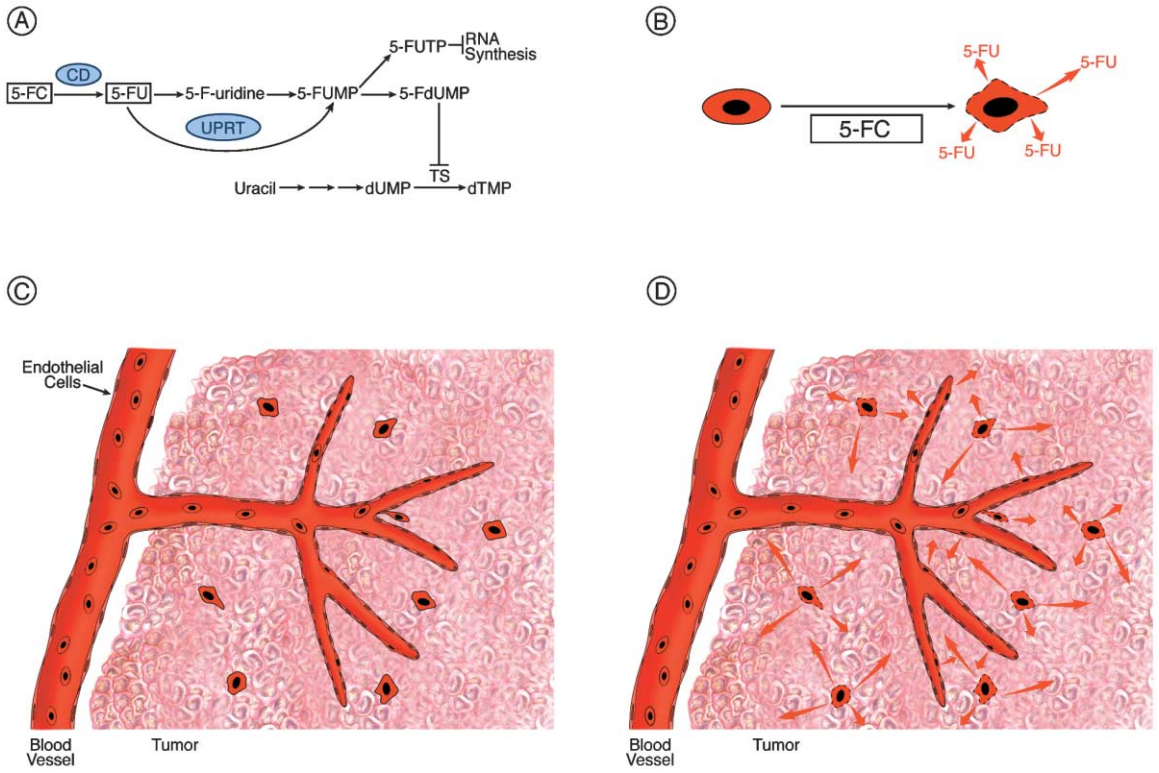

Figure 1. Antitumor therapy with genetically modified embryonic endothelial progenitor cells

A: Pathway of conversion of 5-FC into 5-FU and its active metabolites (Chung-Faye et al., 2001). The CD and UPRT enzymes are highlighted.

B: Upon treatment with the prodrug 5-FC, genetically modified embryonic endothelial progenitor cells undergo apoptosis.

C: Genetically modified embryonic endothelial progenitor cells become incorporated in tumor blood vessels and stroma after intravenous administration (Vajkoczy et al., 2003; Hatzopoulos et al., 1998).

D: Treatment with the prodrug 5-FC triggers a local bystander effect in lung metastases of tumor mouse models (Wei et al., 2004).

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reported in this issue of *Cancer Cell* (Wei et al., 2004).

Could a cell system serve as a therapeutic Trojan horse? In theory, genetically modified cells could even offer certain advantages as gene therapy vectors relative to viruses. However, rigorous criteria will certainly be applied for developing a suitable cell-based vehicle. They include simple harvesting procedures, tolerance to ex vivo expansion, a high level of permissiveness to gene transfer, adequate tissue tropism upon systemic introduction, and low risk of untoward cell proliferation or differentiation in patients. Usually, transplanted foreign cells are rejected by an immune response elicited in the host by antigens present on the cellular graft. As nonimmunogenic universal donor cells (which can avoid graft rejection and/or graft versus host disease) are not available, emphasis had until recently been placed on various adult human cell types such as fibroblasts, myoblasts, and endothelial cells, mostly because they can be easily obtained from biopsy-derived specimens and expanded in tissue culture.

In searching for suitable cells that might be candidates for development as gene therapy vectors, one does not have to look very far. The field of stem cell biology has taken tremendous strides within the past five to ten years. Stem cells with totipotency and self-renewal are currently being heralded as having great promise—as yet largely unmet—in regenerative and therapeutic medicine (Korbling and Estrov, 2003). Along these lines, great interest has been focused on the development of multipotent progenitor cells capable of participating in the formation of the endothelium (Rafii and Lyden, 2003). While a possible hematogenic contribution to the vasculature has long been recognized (Kennedy and Weissman, 1971), the origin, character, and share of circulating endothelial cells during tumor angiogenesis remain poorly defined to date.

Since the first isolation of adult endothelial progenitor cells (Asahara et al., 1997), many individual steps have been put in place to allow the use of circulating adult endothelial cells for therapeutic purposes. In previous work, Hatzopoulos and colleagues have developed a different approach: they isolated (Hatzopoulos et al., 1998) and characterized (Vajkoczy et al., 2003) a population of endothelial progenitor cells from mouse embryos. On an independent line of research, Chung-Faye et al. (2001) established a bifunctional fusion gene construct (cytosine deaminase: uracil phosphoribosyltransferase) to catalyze the stepwise conversions of the prodrug 5-fluorocytosine (5-FC) into the cytotoxic drug 5-fluorouracil (5-FU), and of 5-FU to its active metabolites (Figure 1A). Together, these studies form the rationale for using genetically modified embryonic endothelial progenitor cells to deliver a therapeutic gene.

To develop a cellular vehicle able to reach angiogenic and metastatic sites after systemic administration, Wei et al. (2004) set out to exploit the natural tropism of circulating embryonic endothelial progenitor cells. In a proof-of-concept

study, the authors tested whether embryonic endothelial progenitor cells containing a suicide gene construct would have antitumor effects when administered systemically. The embryonic endothelial progenitor cells used by the authors have been isolated at the onset of angiogenesis (E7.5) of mouse development (Hatzopoulos et al., 1998), and have been characterized as presenting a Tie-2 positive, c-kit positive, Sca-1 positive, and Flk-1 negative/low phenotype (Vajkoczy et al., 2003). Because such embryonic endothelial progenitor cells do not express MHC I proteins, they appear to be "stealth" to natural killer cytolysis, and thus can serve as gene therapy vehicles in both syngeneic and nonsyngeneic mouse tumor models. Moreover, given their apparent self-renewal ability, embryonic endothelial progenitor cells are a convenient reagent for ex vivo expansion and genetic modification. Satisfyingly, the authors demonstrate successful treatment of lung metastases through a complex multistep process. First, ex vivo expanded embryonic endothelial progenitor cells are transfected with the suicide construct to convert 5-FC into 5-FU and its active metabolites (Figure 1B). Second, the engineered embryonic endothelial progenitor cells were intravenously administered into tumor-bearing mice. Third, the cells preferentially localized to lung metastases, where they integrated in tumor blood vessels and extravasated into the tumor stromal microenvironment (Figure 1C). Fourth, upon treatment with 5-FC, protein expression of the suicide construct in the tumors exerted a bystander cytotoxic effect (Figure 1D) that affected metastatic sites and slightly prolonged the lifespan of treated tumor-bearing mice compared to controls.

On the basis of the information from rodent systems presented by Wei et al., a few points remain to be addressed before this methodology can be translated into clinical applications. For example, is there potential for long-term eliciting of neutralizing antibodies secondary to exposure to intracellular proteins, such as the suicide gene product? Is there a possibility that this approach may select for vascularized metastases, since the less vascularized (and therefore more hypoxic) metastases will be preferentially affected? Are there ways to increase the relatively modest effect observed in rodent tumor models? Also, without a plasmid drug selection in vivo, is there a risk that mutational events might eliminate the suicide genetic elements? This reservation is an important one, because mice that received engineered embryonic endothelial progenitor cells but no treatment with 5-FC did actually dies sooner than controls. And, of course, is it feasible—not only scientifically but also from a regulatory policy viewpoint—to isolate and use comparable human embryonic endothelial progenitor cells?

Finally, as the authors point out, homing of embryonic endothelial progenitor cells to lung metastases seems to rely on a pulmonary first-pass effect. While intra-arterial injections may circumvent this problem and allow better access of the embryonic endothelial progenitor cells to metastatic sites downstream from the lung, this would not be convenient. While the evidence presented in Wei et al. suggests that the embryonic endothelial progenitor cells are recruited by hypoxic metastases, it is still unclear whether VEGF is merely a marker of hypoxia or really serves as a functional attractant. Indeed, the same group of investigators have shown that E- and P-selectin and their corresponding ligands can mediate the homing of embryonic endothelial progenitor cells (Vajkoczy et al., 2003), and there is a compelling body of evidence to suggest that the mechanisms of embryonic endothelial progenitor cell homing may vary under different conditions that would change the expression of endothelial cell surface molecules. Thus, other genetic solutions such as the addition of targeting systems might lead to further improvements. Homing of tumor cells to site-specific metastases and leukocytes to sites of inflammation indicates that tissues carry unique marker molecules that are selectively expressed in different vascular beds (Arap et al., 2002; Fidler, 2003). The inner surfaces of blood vessels are covered with diverse populations of endothelial cells. Phenotypes of these cells can vary between different organs, between different parts of the vasculature in a given organ, and even between neighboring endothelial cells of the same organ and the same type of blood vessel. The endothelium is subjected to differential physiological signals, including soluble factors (such as growth factors, interleukins, and chemokines), contact interactions (such as cell-cell and cell-matrix), and other physical and chemical variables (such as pH, pO_2, sheer or stretch stresses, and temperature). In addition, there are many pathological processes (such as inflammatory conditions and malignant tumors) that dramatically influence the endothelial phenotype and may possibly affect the homing of embryonic endothelial progenitor cells. Identification of vascular bed-specific ligand-receptor pairs and knowledge of their tissue distribution and tropism may greatly ameliorate the distribution and therapeutic profile of suicide embryonic endothelial progenitor cells.

In summary, because embryonic endothelial progenitor cells home to sites of lung metastases and evade immunological rejection, they might have potential utility as vehicles for gene therapy if properly engineered. If an improved system can be adapted for use in patients and survive the scientific and regulatory scrutiny required for translation into clinical applications, the promise of therapeutic Trojan horse cells may ultimately be realized. If so, the work of Wei et al. will be considered an important study toward that milestone.

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