Antiangiogenic therapy and tumor progression

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Angiogenesis is necessary for tumor growth (a rationale for antiangiogenic therapy), but hypoxia caused by such a therapy will, in theory, drive tumor progression and metastasis. To reconcile conflicting notions, we discuss that, first, although a shift from normoxia (21% O_2) to hypoxia indeed activates cancer cells for aggressive behavior, this may not occur during therapy, because most cancers are not normoxic to start with. Second, only successful antiangiogenic therapy, which is capable of controlling cancer, will select for resistance and progression. After all, in order to occur, therapy-induced tumor progression must be preceded by tumor regression.

The concept of angiogenic therapy of cancer is based on several elegant ideas and insights in tumor biology. First, angiogenesis is absolutely required for tumor growth (Folkman, 1971). Therefore, inhibition of angiogenesis (antiangiogenic therapy) can prevent tumor growth (Folkman, 1971, 2003; Hanahan and Folkman, 1996; Kerbel and Folkman, 2002; Harris, 2002). Second, due to high proliferative activity, tumor-derived endothelial cells can be targeted selectively. Third, due to low mutation rate, endothelial cells will be unlikely to acquire drug resistance associated with an adaptive mutation. In other words, endothelial cells are resistant to resistance (Kerbel, 1997).

However, an endothelial cell is not the final target of antiangiogenic therapy. It is the cancer cell, of course, that is the intended target (Figure 1). By targeting endothelial cells, the therapy causes hypoxia, which in turn targets cancer cells. Yet, cancer cells can become resistant to hypoxia (Graeber et al., 1996; Yu et al., 2002). For example, there is evidence to suggest that when cancer cells are drug resistant, a tumor is still sensitive to antiangiogenic therapy (Klement et al., 2002). In contrast, when cancer cells are hypoxia resistant, a tumor is relatively resistant to antiangiogenic therapy (Yu et al., 2002). By arresting growth and/or causing death of hypoxia-sensitive cells, hypoxia selects for apoptosis-resistant and increasingly malignant cells (Graeber et al., 1996; Kim et al., 1997). In agreement, hypoxia induces benign-to-malignant melanoma progression in vitro (Stackpole et al., 1994). This seems to be a therapeutic problem by itself. But to make matters worse, as we will discuss in the next section, hypoxia activates cancer cells, namely increases their capability to induce angiogenesis, to invade and metastasize.

Activation by hypoxia (reactive resistance)

In the presence of oxygen, the α subunit of hypoxia-inducible factor (HIF) is hydroxylated and binds to VHL, which in turn targets it for degradation (Ivan et al., 2001; Jaakkola et al., 2001; Kaelin, 2002; Safran and Kaelin, 2003). Under hypoxia, HIF-1α is rapidly accumulated, and HIF-1 transactivates hundreds of genes, including angiogenic and autocrine growth factors and receptors, glycolitic enzymes, and extracellular proteases (Semenza, 2000, 2002). In addition, hypoxia inhibits secretion of antiangiogenic factors, such as thrombospondin-1 (TSP-1), thus stimulating angiogenesis (Hanahan and Folkman, 1996; Laderoute et al., 2000), resolving hypoxia (Figure 1). Such a compensatory hypoxic response may hamper antiangiogenic therapy (reactive resistance). Angiogenic factors such as vascular endothelial growth factor (VEGF) and bFGF induce survivin in endothelial cells and significantly reduce the proapoptotic potency of chemotherapy on endothelial cells (Tran et al., 2002). Angiogenic factors, which are produced by cancer cells in response to hypoxia, may act to shield tumor endothelial cells (Tran et al., 2002). By activating hypoxic response in cancer cells, antiangiogenic therapy may promote metastasis and invasion (Bottaro and Liotta, 2003; Pennacchietti et al., 2003; Steeg, 2003). As emphasized, however, antiangiogenic therapy has not been shown to induce metastases in the clinic (Kieran et al., 2003). Yet, current antiangiogenic therapy may not be effective enough to induce hypoxia in tumors: if therapy does not effectively cause remissions, it may not promote late metastases either. There is overwhelming evidence that hypoxia, in theory, must promote metastasis and invasive growth (for reference, see Hockel and Vaupel, 2001; Blagosklonny, 2001; Semenza, 2002). First, hypoxia and HIF-1 expression correlate

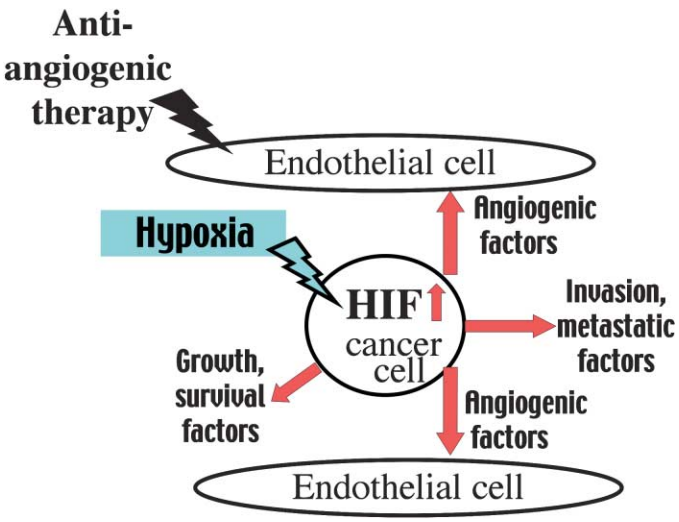

Figure 1. Reactive resistance to antiangiogenic therapy

Inhibition of angiogenesis causes hypoxia, which in turn causes HIF-1 stabilization in cancer (and stromal) cells. HIF-1 transactivates angiogenic factors to stimulate angiogenesis, thus resisting antiangiogenic therapy. In addition, hypoxia stimulates factors of invasion and metastasis. This hypoxic response prevents cancer cell death and may stimulate migration, invasion, and metastasis.

with metastasis and poor prognosis (Hockel et al., 1999; Zagzag et al., 2000; Hockel and Vaupel, 2001). Second, following exposure to acute hypoxia, cancer cells become highly tumorigenic and metastatic (Cairns et al., 2001). Third, hypoxia activates invasive and metastatic characteristics of cancer cells in vitro. Hypoxia and HIF-1 transactivate genes that are critical for invasion and metastasis. In hypoxic cancer and stromal cells, HIF-1 induces autocrine and paracrine growth/survival factors including VEGF, FGF, adrenomedullin, PAI-1, nitric oxide synthase, cathepsin D, matrix metalloproteinase 2, urokinase plasminogen activator receptor (uPAR), fibronectin 1, keratins, vimentin, transforming growth factor α, and autocrine motility factor, which are proteins that play established roles in the pathophysiology of invasion (Garayoa et al., 2000; Rofstad et al., 2002; Semenza, 2003). For example, neutralizing antibodies against uPAR block tumor cell invasion induced by hypoxia (Krishnamachary et al., 2003). Among recent examples, it has been shown that hypoxia stimulates tumor invasion by activating hepatocyte growth factor (HGF), resulting in c-met activation, elevated motility, and invasion (Pennacchietti et al., 2003), and HIF transcriptionally induces the chemokine receptor CXCR4, which governs cancer cell metastasis (Staller et al., 2003). It is well established that shifting cells from normoxia (21% O_2) to hypoxia activates pathways of invasion and metastasis. This suggests that antiangiogenic therapy will activate cancer cells for invasion, metastasis, and aggressive behavior. However, the problem with this premise is that normoxia, as defined above, does not exist. No cell (except for aerobic microorganisms) lives in air.

From near-hypoxia to deep hypoxia

Normal conditions in vivo are near-hypoxic or hypoxic. This detail may change our view on fundamental processes such as cell senescence, for instance. While fibroblasts undergo cell senescence in "normoxic" (21%) conditions, they are immortal under conditions of 3% oxygen (Parrinello et al., 2003). And certain normal cells do not grow in vitro at all.

Typically, experiments testing the effects of hypoxia tend to compare cells cultured under hypoxia/anoxia (meaning oxygen levels between 0% and 3% or following chemical anoxia as produced by DFX and cobalt) versus so-called normoxic conditions (invariably 21% O_2). Yet, "normoxia" defined as 21% oxygen (160 mm Hg, pO_2) is at least 4 times higher than the physiological in vivo normoxia. In venous blood, there is an average 40 mm Hg O_2, which is a result of equilibrium with oxygen in tissues. And while some tissues have higher than average oxygen levels, in other tissues (and especially solid tumors), oxygen levels are lower than the average. In fact, certain tissues, including cartilage, bone marrow, and placenta, require hypoxic conditions for their proper function; proliferation of hematopoietic and placental cells is stimulated by HIF-1 induction, a consequence of tissue hypoxia (Adelman et al., 1999, 2000; Schipani et al., 2001). Hypoxia is a state that causes a compensatory response such as the accumulation of HIF-1. In fact, HIF-1 increases exponentially with decreases in oxygen such that it is half maximal at O_2 concentrations between 1.5 and 2% and maximal at 0.5% O_2 (Jiang et al., 1996). In vivo, even normal cells exist at near-hypoxia. A case in point is the HIF-1-mediated production of erythropoietin occurring as the result of even a slight decrease in blood hemoglobin levels (Semenza and Wang, 1992).

The bottom line is that 21% O_2 is not physiological, especially for tumors where oxygen levels of around 1% (5–10 mm Hg) are an accepted borderline between well and poorly oxygenated tumors (Hockel et al., 1999; Hockel and Vaupel, 2001). Thus, one could say that even normally oxygenated (>10 mm Hg) tumors are mostly hypoxic. With this in mind, the overexpression of HIF-1 in tumors is likely to mean that tumors almost always live under hypoxia.

To start with, the metastatic and invasive properties of cancer cells are activated by physiological hypoxia. Instead on inflicting hypoxia, an effective antiangiogenic therapy will shift hypoxia (the normal oxygen state of tumors) to a deeper hypoxia. A response to a shift from hypoxia to anoxia (severe oxygen depletion) is HIF-1 independent and differs from hypoxic response (Ameri et al., 2003). In addition, severe oxygen depletion is associated with lack of glucose and other nutrients, starving cells to death. This is a classic goal of antiangiogenic therapy (Folkman, 2003). Paradoxically, antiangiogenic therapy might also increase the efficiency of the tumor vasculature, increasing the delivery of oxygen and drugs (Hansen-Algenstaedt et al., 2000; Jain, 2001). By eliminating excess endothelial cells, the resulting tumor vessels would be more conductive to the delivery nutrients, oxygen, and drugs. This explains potentiation of radiation and chemotherapy by antiangiogenic therapy (Pan et al., 2003). If the goal is to deprive the tumor of its blood supply, therapy must continue until the vasculature no longer functions (Jain, 2001).

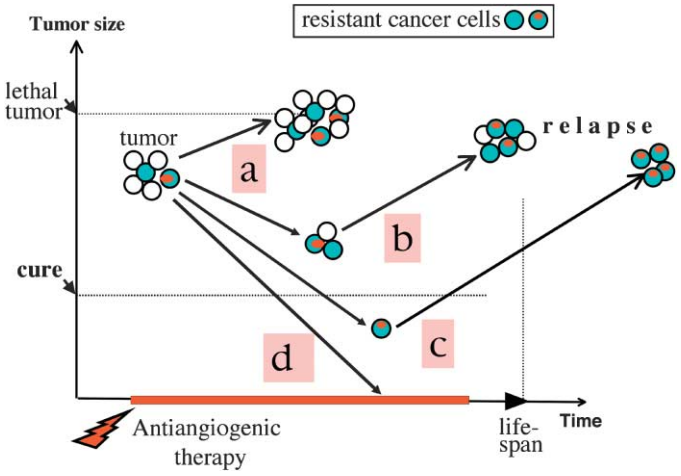

Figure 2. Antingiogenic therapy from tumor regression to resistance (tumor progression)

By suppressing proliferation and survival of angiogenesis-dependent cancer cells, antiangiogenic therapy causes therapeutic response (remission) but simultaneously selects for resistant cancer cells.
A: Ineffective therapy causes neither remissions nor resistance.
B: The therapy suppresses most angiogenesis-dependent cancer cells but spares less sensitive cells. In relapse, a tumor is moderately resistant.
C: Effective therapy suppresses most cancer cells and only a few most resistant cells survive. Therefore, remission may be deep and curative. Once relapsed, tumor is hypoxia resistant and aggressive.
D: The most effective therapy suppresses all cancer cells.

Tumor regression and progression: Two sides of one coin

Almost by definition, an effective therapy must select for cells resistant to therapy. By killing sensitive cells and sparing mutant cells that are resistant, therapy selects for resistance. These resistant cells proliferate, forming a resistant tumor in relapse (Figure 2, relapse). Only when therapy is not effective, thus allowing even most sensitive cells to proliferate, is there no

selection for resistance (Figure 2A). So selection for resistance is a hallmark of an effective therapy and a consequence of tumor regression. Most importantly, despite selective pressure for resistance, cancer may never relapse. First, there may be no cells that are sufficiently resistant to tolerate very effective therapy. This (curative) outcome will be likely, when a population of cancer cells is small and a mutation rate is low (Blagosklonny, 2002). There are several examples of effective therapies. The Bcr-Abl inhibitor Gleevec selects for resistance, exactly because it is selective and effective against Bcr-Abl-expressing leukemia. By killing all leukemia cells that depend on Bcr-Abl, it selects for cells with mutant or amplified Bcr-Abl and other mechanisms of resistance (Druker, 2002; Sawyers, 2002). As another example, tissue-selective therapies of prostate and thyroid cancers with antiandrogens (Reese, 2000) and radioactive iodine (Pelikan et al., 1997), respectively, are very selective and effective, causing clinical responses and remissions. These effective therapies eventually select for drug resistance, associated with tumor progression. Once relapsed, hormone-independent prostate cancer and anaplastic thyroid cancers are most aggressive, therapy resistant, and lethal cancers (Zietman et al., 1996; Pelikan et al., 1997). Similarly, antibiotics select for antibiotic-resistant bacteria. Despite this obstacle, antibiotics are very useful in therapy of bacterial infections. In fact, antibiotics select for resistant bacteria because they kill nonresistant bacteria. In all cases, selection for resistance cannot occur without therapeutic response or at least temporal disease regression.

In this light, it is not surprising that an antiangiogenic therapy, if effective, will select for resistance. The question is what exactly will be selected. Overexpression of HIF may help cells to survive low oxygen and starvation. However, HIF-1 simultaneously induces and inhibits apoptosis and proliferation (Carmeliet et al., 1998; Harris, 2002). For example, HIF-1 induces proapototic BNIP and inhibits cell proliferation by activating CDK inhibitors (Sowter et al., 2001; Goda et al., 2003). HIF-2 can mediate apoptosis in low glucose (Brusselmans et al., 2001). On the other hand, HIF-1 renders pancreatic cancer cells resistant to apoptosis induced by hypoxia and nutrient deprivation (Akakura et al., 2001). Cells, lacking HIF-1, may grow faster than parental cells (Carmeliet et al., 1998). On the other hand, loss of HIF-1 retards solid tumor growth because of defective angiogenesis (Ryan et al., 1998; Carmeliet et al., 1998). Therefore, to provide a selective advantage, there must be a shift in the balance such that HIF-1 neither induces apoptosis nor inhibits growth. In other words, overexpresion of HIF-1 requires the absence of growth inhibitory and proapoptotic pathways downstream of HIF-1. In aggressive cancers, overexpression of HIF-1 is accompanied by loss of p53, insensitivity to p21, and ability to proliferate in hypoxia (Salnikow et al., 2000). It could be predicted that selection for cells that can proliferate in the presence of a constantly elevated HIF-1 can yield cells lacking restrictions of the cell cycle and apoptotic machinery. These are hallmarks of malignant cells.

From remission to cure

Thus, response to therapy (inhibition of growth and/or survival of cancer cells) and selection for resistance will occur simultaneously (Figure 2). By inhibiting vessel-dependent cancer cells, antiangiogenic therapy might be expected to favor cells that overexpress HIF-1, and lack apoptotic machinery and cell cycle control. Following an inadequate therapy (Figure 2A), the tumor will not become resistant. But then therapeutic benefits will be also minimal. Following an effective therapy, only resistant cancer cells will remain. Once relapsed, the tumor will be resistant (Figure 2C). If only a few cancer cells survive, the relapsed tumor (if it relapses) will be the most resistant. But there may be no cells that are sufficiently resistant to tolerate most effective therapy (Figure 2D).

So what limits antiangiogenic therapy: low efficacy of the therapy (Figure 2A) or therapy-induced tumor progression (Figures 2B and 2C)? There is no indication that antiangiogenic therapy promotes metastasis and progression (Kieran et al., 2003), but it does not cause remissions either. Therefore, current antiangiogenic therapy is limited mainly by the shortage of selective drugs and their rational combinations that can "starve the tumor to death." There are several strategies to increase efficacy of antiangiogenic therapy. Potent VEGF blockade can cause regression of established tumors and metastases (Huang et al., 2003). Also, metronomic chemotherapy (continuous low-dose chemotherapy) can be successfully combined with inhibitors of VEGF or VEGF receptor signaling (Klement et al., 2000; 2002). A combination of antiendothelial drugs and anti-HIF drugs is an attractive therapeutic modality. By themselves, inhibitors of HIF-1 are a mechanism-based antiangiogenic therapy, because it is a HIF-mediated response that drives angiogenesis in the first place. Inhibitors of HIF-1 are currently under development (Rapisarda et al., 2002; Harris, 2002; Mabjeesh et al., 2003; Knowles et al., 2003; Semenza, 2003; Giaccia et al., 2003). For example, geldanamycin, which targets HIF-1 for degradation (Mabjeesh et al., 2002), synergizes with the antiangiogenic stress imposed by Id1 loss to lead to a complete remission of aggressive tumors in mice (de Candia et al., 2003). One can expect that anti-HIF agents will prevent both a compensatory activation of cancer cells and selection for cells with an overexpressed HIF-1. In the absence of HIF-1, lack of oxygen and nutrients will result in acidosis and tissue necrosis; no tumor cell can be resistant to tissue necrosis. As an additional strategy to exploit hypoxia, antiangiogenic therapy could be combined with inactive prodrugs, which are activated by hypoxia (Dachs et al., 1997; Brown and Giaccia, 1998). For example, under hypoxic conditions, tirapazamine is reduced to a radical that leads to DNA double-strand breaks, single-strand breaks, and base damage (Peters and Brown, 2002). And finally, instead of inhibition of HIF-1, its overexpression can be exploited by designing HIF-1-dependent drugs (Sutphin et al., 2004). Given this variety of potential therapeutic approaches, we can imagine effective antiangiogenic therapies of the future. Although hypoxia activates cancer cells for aggressive behavior, most cancer cells are inherently activated by physiological hypoxia; and antiangiogenic therapy may not galvanize them further. Effective therapy will, however, select for resistant and aggressive cancer cells during therapy-induced tumor regression. Despite this, following effective therapy, the disease may not necessarily have a chance to relapse (Figure 2C).

Acknowledgments

I apologize to those authors whose primary references were not cited due to space limitations.

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