

Overcoming Physiologic Barriers to Cancer Treatment by Molecularly Targeting the Tumor Microenvironment

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Abstract

It is widely recognized that the vasculature of the tumor is inadequate to meet the demands of the growing mass. The malformed vasculature is at least in part responsible for regions of the tumor that are hypoxic, acidotic, and exposed to increased interstitial fluid pressure. These unique aspects of the tumor microenvironment have been shown to act as barriers to conventional chemotherapy or radiation-based therapies. It now seems that while the vasculature initiates these tumor-specific conditions, the cells within the tumor respond to these stresses and add to the unique solid tumor physiology. Gene expression changes have been reported in the tumor for vascular endothelial growth factor, carbonic anhydrase IX, and pyruvate dehydrogenase kinase 1. The activity of these gene products then influences the tumor physiology through alterations in vascular permeability and interstitial fluid pressure, extracellular acidosis, and mitochondrial oxygen consumption and hypoxia, respectively. Novel molecular strategies designed to interfere with the activities of these gene products are being devised as ways to overcome the physiologic barriers in the tumor to standard anticancer therapies. (Mol Cancer Res 2006;4(2):61–70)

Unique Microenvironment of Solid Tumors

The microenvironment of a solid tumor has several characteristics that distinguish it from the corresponding normal tissue. These differences are thought to be due to the interaction between the poorly formed tumor vasculature and the physiologic characteristics of the cells within the tumor (1). The interaction between the tumor cells and tumor vasculature results in three recognized microenvironmental hallmarks of solid tumors: high interstitial fluid pressure (IFP), low oxygen tension or hypoxia, and low extracellular pH

(pHe; ref. 2). The mechanisms responsible for these micro-environmental conditions are diagrammed in Fig. 1. In normal tissues, the vascular system is regulated by a balance of proangiogenic and antiangiogenic molecules, which ensures that an efficient and orderly network of blood vessels is maintained to meet the metabolic demands of the tissue. In addition, a network of lymphatics is available to drain fluid and cellular byproducts from the interstitium. This balance of growth factors in the tumor is perturbed, which leads to the development of a disorganized vasculature with multiple structural and functional abnormalities (1) and a complete lack of lymphatics (3, 4). Functionally, the ability of the tumor vasculature to deliver nutrients and remove waste products is badly compromised. Tumor vessels often have an incomplete endothelial lining and lack a layer of pericytes or an intact basement membrane, making them more permeable than those in normal tissues. The architecture of the vasculature is also highly disorganized (1, 5). Tumor vessels are generally long and highly chaotic, and many vessels have an unusually large diameter. The mean vascular density of tumors is generally lower than in normal tissues, and diffusion distances are greater. However, vessel organization is highly heterogeneous and hotspots may occur where the vascular density is higher than in normal tissues. Finally, the vascular network often contains blind-ends, arteriolar-venous shunts, and plasma channels that are devoid of RBC (6).

One direct result of the fenestrated tumor vasculature and lack of lymphatics is the inappropriate accumulation of vascular contents in the tumor and a resulting elevated IFP (7, 8). The interstitium of a tissue is the space between the cells and the vascular compartment. This space can represent a large fraction of the total tissue volume and it is essential for molecular transport from the blood vessel to the cells and back again. As early as the 1950s, it was recognized that solid tumors often had significantly elevated IFP (9). Normal interstitial pressure is close to atmospheric, and normal transcapillary pressure is slightly higher (1-3 mm Hg) within the vessels to aid in the convective flow of solutes from the vascular space to the interstitial space. Three factors combine to elevate the IFP within the tumor: the diminished function of the tumor blood vessels and lymphatics, the osmotic forces that draw solutes into the tissue, and the contractile characteristics of the tumor stroma (8, 10). All three of these factors are altered in the tumor and combine to elevate IFP to levels of up to 100 mm Hg (11-13), and all three are potential targets of

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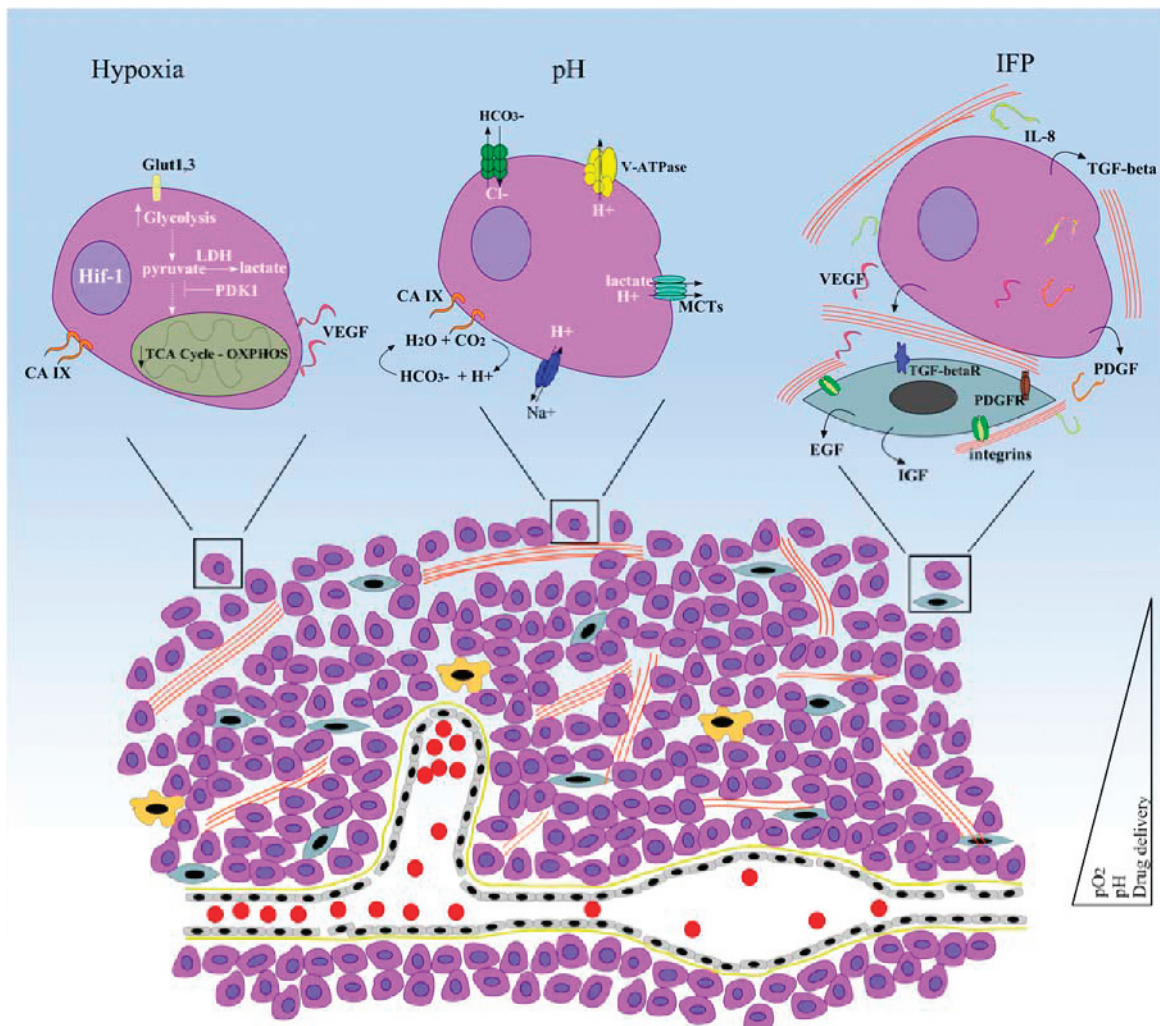


FIGURE 1. Microenvironmental conditions of the tumor present barriers to therapy. Chaotic tumor vasculature contains unstable endothelium, leaky vessels, dead-ends, and instability in RBC flux. Poor oxygen delivery by the defective vasculature and oxygen consumption by the tumor cells result in hypoxic areas. Oxygen deprivation and activation of HIF-1 mediates adaptation of tumor cells to hypoxia by increasing glucose import and utilization in the cytoplasm (glycolysis and anaerobic conversion to lactate) while down-regulating the major source of oxygen consumption, the mitochondria. Induction of VEGF contributes to angiogenesis, whereas induction of carbonic anhydrase IX and XII contributes to the acidification of the ECM. Proton pumps at the plasma membrane and monocarboxylate transporters (MCTs) maintain the pH within normal levels, with a concomitant acidosis of the ECM (pHe). Increased numbers of activated fibroblasts and macrophages contribute to the formation of highly contractile ECM, rich in collagen fibers, raising the IFP. The release of cytokines and angiogenic factors by cancer and stroma cells creates a complex network of interactions regulating the plasticity of the connective tissue and the perfusion of the tumor. The genetic, biochemical, and physiologic factors that regulate these barriers to therapy represent targets for novel molecularly targeted agents.

molecularly directed therapy. However, unlike oxygen tension (pO_2) and pHe, which are highly heterogeneous, tumor IFP is relatively uniform throughout the center of the tumor, dropping to normal, slightly negative values at the extreme periphery (14). Consequently, transcapillary fluid flow and convective transport of therapeutic molecules in tumors is greatly impaired (15).

The reduced blood flow and the leaky tumor vasculature elevate IFP in two major ways (8). First, the breaks in the endothelium of the tumor vessels result in a leakage of fluid and osmotic proteins from the blood into the interstitial space. This results in reduced hydrostatic pressure in the vessel and increased colloid osmotic pressure within the interstitium. The lack of a lymphatic system exacerbates this problem because

there is no mechanism to draw the excess osmoles back into the vascular compartment. Thus, the functional characteristics of the tumor vessel, combined with a hydrostatic/osmolar imbalance in the tumor, shift the Starling equilibrium (net hydrostatic force across the capillary wall) and passively elevate tumor IFP (10). In addition to the hydrostatic and osmolar forces that develop as a consequence of the architecture of the tumor vessels, there is an active process in the tumor stroma that also regulates IFP through the contractile characteristics of the extracellular matrix (ECM). *In vitro* experiments show that normal fibroblasts grown in a collagen gel will take on contractile characteristics and actively compress the gel (16). The process is mediated by stimulation through platelet-derived growth factor (PDGF; ref. 17) and transforming growth factor- β

receptors (18) and through the interactions of β_1 integrin and collagen (19). These cellular responses are inherent in the normal fibroblasts and the ECM and therefore represent logical targets for drug therapy designed to reduce IFP.

Impaired blood flow through the tumor also leads to reduced oxygen delivery, which is a major factor contributing to regions of tumor hypoxia. The delivery of oxygen to the tumor cannot meet the demand in the tumor cells, so large regions of the tumor exist in a chronic state of supply-demand mismatch. Normal tissue pO_2 ranges between 10 and 80 mm Hg depending on the tissue type, whereas tumors contain regions where the pO_2 is <5 mm Hg (20, 21). These chronically hypoxic regions arise because a large proportion of tumor cells lie beyond the distance that oxygen can diffuse from the nearest vessel (usually 100–200 μm ; ref. 22). The diffusion distance is determined primarily by the rate of oxygen consumption of the tumor cells, a variable that varies widely both within and between tumors (23). Mathematical models suggest that variations in oxygen consumption may have a large effect on the hypoxic fraction of solid tumors (24). Interestingly, recent experimental data suggest that the physiologic regulation of oxygen consumption within the tumor cells is as important as the reduced oxygen delivery in determining tumor hypoxia. In addition, fluctuations in tumor RBC flux can produce temporal changes in pO_2 and intermittent episodes of hypoxia even in cells relatively close to vessels (25). Therefore, within individual tumors, tissue oxygenation is highly heterogeneous both spatially and temporally (23).

Direct measurement of the pH within a solid tumor with electrode-based techniques determined that the tumor compartment often has a relatively acidic pH when compared with the corresponding normal tissue (26). The overall net accumulation of acid within the tumor was originally thought to be simply the result of lactate accumulation due to the high rate of aerobic and anaerobic glycolysis within the tumor cells. However, new data generated from genetically modified model tumors determined that even tumors that are impaired in their ability to execute glycolysis and generate lactate are still able to generate an acidic environment (27, 28). Using ^{31}P magnetic resonance spectroscopy to measure the characteristic pH-dependent chemical shift of intracellular and extracellular probes within the tumor (29), it was determined that the acidity within the tumor is a combination of the pHe and the internal, intracellular pH (pHi). Cells within the tumor are capable of maintaining a reasonably neutral cytosolic pH in the face of external acidosis (30). The pH gradient across the plasma membrane is maintained by the activity of a variety of ion pumps, including the monocarboxylate H^+ cotransporter, the vacuolar H^+ ATPase, the Na^+/H^+ exchanger, and the Na^+ -dependent $\text{Cl}^-/\text{bicarbonate}$ exchanger (31). The spatiotemporal pH gradients within the tumor are due to a complex interaction between the metabolic state of the tumor cells and the ion-pumping characteristics of the cells and can vary significantly in spontaneous tumors (32). Interestingly, these patterns do not necessarily coincide with the regions of the tumor that are hypoxic (33). However, regardless of the source of H^+ ions, the impaired clearance of the products of metabolism by the tumor vasculature significantly contributes to the low pH levels observed in solid tumors (34).

Microenvironmental Barriers to Conventional Therapy

The microenvironmental conditions discussed above present unique barriers to specific cancer therapy modalities. For example, hypoxia has long been known to inhibit effective radiation killing *in vitro* (35). Oxygen is a potent radiosensitizer, with well-oxygenated cells requiring one-third of the dose of anoxic cells to achieve a given level of cell killing (36). This relative resistance of hypoxic cells is thought to explain the clinical correlation between tumor oxygenation and patient response to radiotherapy. A large number of clinical trials have established the relationship between tumor hypoxia and poor clinical outcome for patients treated with radiotherapy for cervical carcinoma (37), soft tissue sarcoma (38), and head and neck cancer (39).

Although the mechanisms are not as well defined, tumor hypoxia also causes resistance to several types of chemotherapy. Oxygen concentration can have a direct effect on the effectiveness of drugs, such as mephalan, bleomycin, and etoposide, which require molecular oxygen for maximal efficiency (40). Extreme hypoxia is also known to decrease the rate of cell division and ultimately cause cell cycle arrest, which can decrease the effectiveness of drugs that are more active against proliferating cells (41). Finally, hypoxia has also been shown to select for apoptosis-resistant cells in model tumors (42).

In addition to hypoxia, other features of the tumor microenvironment can reduce the effectiveness of chemotherapy in several different ways. For systemic delivery of drugs, heterogeneous tumor perfusion and long diffusion distances can result in greatly reduced drug concentrations in tumor tissue (43, 44). Specifically, the high IFP in tumors can present a barrier to efficient drug delivery by decreasing transcapillary fluid flow and convective transport of compounds from the bloodstream into the tumor interstitium. This is especially important for large molecules, such as antibodies and other proteins, as they rely more heavily on convection as opposed to simple diffusion for transport (15). It was in 1987 that Jain proposed that this elevated IFP was a barrier to efficient delivery of blood-borne chemotherapies to the tumor cells (7).

Many studies in human and rodent tumors have observed a relationship between decreased drug uptake and elevated IFP. Several clinical studies have also shown that elevated IFP is a poor prognostic factor for patient outcome. Patients with lymphoma or melanoma have been shown to have better response to their chemotherapy if they have IFP that drops during treatment (45). Interestingly, even cervical cancer patients treated with radiotherapy can be predicted to have poor outcome if their tumors have elevated IFP (12). It is now apparent that therapeutic interventions that are designed to reduce IFP could be added to augment the conventional treatments of cancer.

Low pHe and the resulting pH gradient across the plasma membrane of tumor cells also present a barrier to drug delivery for many chemotherapeutic agents (46). Large lipophilic molecules pass through biological membranes most efficiently in an uncharged state. This is the case for both plasma membranes and intracellular membranes. Many commonly used chemotherapeutic agents are either weak bases (pKa 5.5–6.8) or weak acids (pKa 7.8–8.8). In an acidic extracellular environment, the weak bases, such as doxorubicin, mitoxantrone, or

vinblastine, are more likely to exist in a charged state, and this inhibits their transport across the plasma membrane, significantly reducing their cellular uptake *in vitro* (47). Interestingly, low pH also impairs the effectiveness of paclitaxel and topotecan despite their chemical structures that do not predict pH-dependent ionizations (48). There is also evidence that these compounds can be sequestered in acidic endosomes within tumor cells, impairing their penetration through tumor tissue (49). Experiments using tissue culture models and experimental tumors have shown that the effectiveness of these agents is significantly reduced in acidic extracellular environments.

Modifying Tumor Oxygenation

A variety of strategies designed to improve tumor oxygenation have been investigated and are listed in Table 1. The original ideas were based on increased oxygen delivery, and although there is evidence for a small improvement in outcome, no method has been widely accepted into clinical practice (1, 50). Increasing oxygen carrying capacity in the blood through transfusion or erythropoietin administration have had mixed results (51-53). The most promising approach still under investigation employs carbogen breathing in combination with nicotinamide infusion during radiotherapy (54). This approach aims to reduce both chronic, diffusion-limited hypoxia through the use of carbogen gas breathing and acute, perfusion-limited hypoxia through the use of the vasoactive drug nicotinamide to render tumors more radiosensitive. In early clinical testing, increased tumor control rates have been observed in phase II trials in bladder cancer (55) and head and neck cancer (54), and phase III trials are currently ongoing.

Blood vessel normalization in response to anti-vascular endothelial growth factor (VEGF) therapies may cause significant changes to tumor physiology, including increased tumor oxygenation (56). Although initially counterintuitive, it has been shown that during the early phases of anti-VEGF therapy tumor blood vessels acquire a more normal structure and organization, which results in increased perfusion, drug delivery, and oxygenation (56). Likewise, in an orthotopic model of glioblastoma, treatment with an antibody against

VEGF receptor 2 (DC-101) resulted in transient increase in tumor oxygenation for several days post-treatment (57). This window of reoxygenation coincided with an increase in tumor radiosensitivity. Similarly, treatment of animals bearing model tumors with thalidomide has also been shown to transiently increase tumor oxygenation presumably through interference with basic fibroblast growth factor and VEGF signaling and with vessel normalization (58). Measurements of tumor oxygenation in patients undergoing antiangiogenic therapy are required to determine the magnitude and kinetics of these effects in human cancer. Such information should allow radiation and chemotherapy protocols to be sequenced appropriately to take advantage of the alterations to the tumor microenvironment.

Modeling of tumor oxygen levels suggests that decreasing tumor oxygen consumption may be more effective than increasing oxygen delivery as a means to reducing tumor hypoxia (24). These models predict that decreasing oxygen consumption by 30% would have an effect equivalent to increasing oxygen delivery by 4-fold. The use of the general mitochondrial inhibitor *m*-iodobenzylguanidine to increase model tumor oxygenation and response to radiation has been reported (59, 60). Similar approaches using the Crabtree effect have shown increases in tumor oxygenation following bolus glucose administration and the resulting decrease in mitochondrial function (61). Unfortunately, the molecular regulators of oxygen consumption in tumor cells are poorly defined, and it is therefore difficult to devise strategies to block mitochondrial activity and oxygen consumption specifically in the tumor, without unwanted side effects.

Considering the importance of targeting oxygen delivery as well as oxygen consumption, mild hyperthermia has emerged as a possible approach to improving tumor oxygenation before radiotherapy. Although the utility of cytotoxic hyperthermia as a primary therapy modality for solid tumors has not been shown convincingly, the possibility of using mild hyperthermia to alter tumor physiology still exists (62). Although cytotoxic hyperthermia (>42-43°C) impairs blood flow and increases the extent of hypoxia, mild hyperthermia (41-42°C) has been shown to improve oxygenation of experimental and human tumors in a

Table 1. Strategies to Increase Efficacy of Therapy by Modifying Tumor Oxygenation

Strategy	Therapeutic Approach	Mechanism of Action	References	
Increasing oxygenation to overcome resistance to radiation and chemotherapy	Hyperoxic gas inhalation	Carbogen and nicotinamide	Increased blood oxygenation Increased perfusion	Kaanders et al. (50)
	VEGF blockade	Bevacizumab DC-101 Thalidomide	Vascular normalization and increased perfusion	Winkler et al. (57) Ansiaux et al. (58)
	Mild hyperthermia	Heating to 41-42°C	Increased perfusion Decreased oxygen consumption	Dewhirst et al. (62)
	Respiratory inhibition	Hyperglycemia ± <i>m</i> -iodobenzylguanidine	Decreased oxygen consumption	Snyder et al. (61)
	Ras inhibition	Farnesyl transferase inhibitors	Unknown	Brunner et al. (65) Delmas et al. (66) Shi et al. (67)
Decreasing oxygenation to enhance the activity of hypoxia directed therapies	HIF-1/pyruvate dehydrogenase kinase 1 inhibition	HIF-1 inhibitors Pyruvate dehydrogenase kinase 1 inhibitors	Increased oxygen consumption	Papandreou, in press

Table 2. Strategies to Improve Drug Uptake by Modifying IFP

Molecular Target	Drug	Mechanism of Action	References
VEGF	Bevacizumab	Vascular normalization	Wildiers et al. (111) Willett et al. (75)
VEGF receptor 2	DC-101	Vascular normalization	Winkler et al. (57)
PDGF receptor β	Glivec	Decreased stromal cell contraction	Peitras et al. (77-79)
Transforming growth factor- β	Transforming growth factor- β decoy receptor	ECM remodeling	Lammerts et al. (82)
Unknown	Prostaglandin E ₁	Vascular normalization	
Hyaluronan	Hyaluronidase	Decreased stromal cell contraction	Salnikov et al. (83)
Collagen	Collagenase	ECM remodeling	Eikenes et al. (84)
Nontargeted	Taxol	ECM remodeling	Eikenes et al. (85)
		Induction of apoptosis and decompression of tumor vessels	Taghian et al. (87)

large number of studies (63). Experimental evidence suggests that this increase is due to both an increase in perfusion and a decrease in oxygen consumption that can persist for up to 24 hours following treatment (64). Although further investigation of these effects in human tumors is required, it seems that mild hyperthermia given before radiation may be an effective means of overcoming hypoxic radioresistance.

A more targeted approach to modifying tumor oxygenation has emerged through the study of farnesyl transferase inhibitors to block Ras signaling. Multiple groups have reported that treatment of xenografted tumors with farnesyl transferase inhibitors can increase tissue oxygenation as measured by a decrease in hypoxic marker binding (65, 66). Importantly, this effect is greatest in tumors bearing oncogenic Ras mutations, and it results in increased sensitivity to radiotherapy (67). The mechanism responsible for this effect is not known but may involve changes in the tumor vasculature or tumor cell oxygen consumption.

An alternative approach to the problem of hypoxia in solid tumors is to exploit this unique situation using hypoxia-dependent cytotoxic therapies (68). Several such therapies are currently in development and include bioreductive drugs, such as tirapazamine and AQ4N, which are converted to their cytotoxic forms under hypoxic conditions (69); cytotoxic gene therapy using hypoxia-responsive promoters (70); and obligate anaerobic bacterial vectors that colonize the necrotic regions of tumors for gene-directed enzyme prodrug therapy (71). Because these therapeutic strategies are predicted to be effective only against hypoxic tumors, it is possible that their efficacy could be enhanced by making tumors more hypoxic during their administration.

One approach to achieving increased hypoxia in a tumor-specific manner is implied by the recent finding that oxygen consumption in tumor cells is down-regulated by hypoxia-inducible factor-1 (HIF-1) activity.² It was recently reported that tumors express high levels of pyruvate dehydrogenase kinase 1 (72). HIF-1 increases the expression of pyruvate dehydrogenase kinase 1 *in vitro*, which acts to inhibit pyruvate dehydrogenase and prevent the utilization of pyruvate in the mitochondrial tricarboxylic acid cycle.³ This results in decreases in mitochondrial oxygen consumption of at least 50%. These findings suggest that inhibiting HIF or pyruvate dehydrogenase kinase 1 in solid tumors would cause an increase in oxygen consump-

tion, thereby increasing the extent of hypoxia and improving the effectiveness of hypoxic toxins listed above. Because HIF is predominantly expressed in tumors and not in normal tissues, inhibiting its activity should produce tumor-specific increase in hypoxia.

Lowering Tumor IFP

Interventions that have been shown to reduce IFP and increase drug delivery are listed in Table 2 and can be divided into two categories: drugs designed to normalize vasculature and reduce vessel leakiness and drugs designed to reduce ECM contractility (8). To date, the most successful compound that has shown efficacy through preclinical tests and randomized phase III studies is bevacizumab or anti-VEGF antibody (73). VEGF is a biologically potent molecule, and its high level in the tumor suggests that its removal can have several significant effects. In addition to its role in endothelial cell survival and blood vessel growth, VEGF (also known as vascular permeability factor) is responsible for much of the leaky characteristic of the tumor vessels (74). Clinical studies have shown that bevacizumab can lead to decreased IFP (75) and increased survival in patients with advanced colorectal cancer treated in combination with 5-fluorouracil and leucovorin (76). This mechanism is thought to be through vascular normalization that results in reduced protein leakage into the interstitium and better laminar flow through the vessels (56).

In addition to the targeting of the vascular component of the tumor, the targeting of the stromal fibroblast-ECM interaction has also shown success in preclinical models. *In vitro* studies implicated PDGF in modulating this stromal contraction (17), so PDGF antagonists have been investigated as modulators of tumor IFP. The small-molecule PDGF receptor inhibitor imatinib has been shown to lower IFP (77) and increase drug delivery and efficacy when given with epothilone B (78), taxol, 5-fluorouracil (79), or radiolabeled antibodies (80). Interestingly, the PDGF inhibitors have to be given before the chemotherapy to see the interaction, suggesting a physiologic response to the first drug that results in the potentiation of the second, not just an effect of combining two therapeutic agents together.

Less well-characterized mechanisms for reducing IFP are also under development. Blockade of transforming growth factor- β receptors has been shown to lower IFP through vessel normalization (81) and reduction in ECM deposition (82), augmenting chemotherapy in model tumors (81). Addition of prostaglandin E₁ to 5-fluorouracil-based chemotherapy has also

² I. Papandreou, Cell Metabolism, in press.

³ Kim et al., Cell Metabolism, in press.

increased treatment efficacy in rodent tumors consistent with reductions in IFP (83). Finally, the ECM itself has been a direct target through the delivery of enzymes designed to digest either the haluronic acid (84) or the collagen (85). Both these agents have shown efficacy at reducing IFP and increasing delivery of therapeutic agents to tumor cells *in vivo*.

Traditional chemotherapies have also been shown to be able to normalize IFP in tumors. Preclinical studies suggested that paclitaxel was able to reduce IFP through the induction of cell death and the decompression of the tumor vessels (86). A recent report has confirmed these findings in patients and found that treatment of breast cancer patients with paclitaxel leads to a reduction in the tumor IFP, whereas treatment with doxorubicin did not (87). The decreased IFP was accompanied with a corresponding increase in tissue oxygenation. Therefore, patients treated by paclitaxel would benefit from this treatment in multiple ways: by direct action of the drug itself, by reducing IFP and increasing penetration of future chemotherapy, and by increased oxygen delivery to potentiate radiation therapy. The timing of the therapeutic regimen is therefore important if one agent is designed to elicit a physiologic change in the tumor that will increase efficacy of subsequent treatments.

Finally, several groups have identified gene expression changes that occur in response to elevated hydrostatic pressure in astrocytes (88) and chondrosarcomas (89). Gene induction has been identified in immediate-early genes, ECM-encoding genes, and matrix remodeling proteases. Decreasing IFP in the tumor may therefore have additional, unidentified benefits related to inhibiting these stress-responsive gene expression profiles.

Manipulation of Tumor Acidosis

The acidotic pHe of the tumor and the biophysical characteristics of certain chemotherapies predict a model in which alteration of the pHe can lead to therapeutic gain (90). Several effective strategies are listed in Table 3. Most directly, investigators have shown that acutely altered pHe in model tumors can lead to increased response to conventional therapies. Increasing acidosis leads to an increased uptake of weak acids and their effectiveness, whereas increased alkalosis leads to increased uptake of weak bases and their effectiveness. Tumor pHe can be transiently decreased by bolus administration of glucose with or without the mitochondrial inhibitor *m*-iodobenzylguanidine through an increase in the production of lactate (91). This transient drop in

pHe was shown to be successful in augmenting chlorambucil or melphalan efficacy in model tumors (92, 93). Interestingly, the effect seemed to be most dramatic in the cells at a greater distance from the blood vessels, which were also radio-resistant due to hypoxia. Conversely, tumor alkalosis can be induced by bolus administration of sodium bicarbonate. Pretreating animals with bicarbonate before administering mitoxantrone or doxorubicin has been shown to increase the response of model CH3 or MCF7 breast cancers, respectively, without significantly increasing the pHe of normal muscle (90, 94). The chemical structure of camptothecin has even been modified to take advantage of this pH-dependent partitioning across the plasma membrane to increase its antiproliferative effect (95, 96). Although these approaches were successful in treating animal tumors, it is not clear if these acute approaches will yield long-term effectiveness without systemic acid base side effects during multicycle chemotherapy in humans. The converse strategy in which the pHi is lowered to reduce the gradient across the plasma membrane to facilitate drug delivery has not been so successful (97).

Additional pharmacologic approaches have been proposed to target ion pump-derived sources of acid production within the tumor. Intracellular molecules are exposed to membrane-bound organelles that contain additional intraorganellar pH gradients. Acidic, lysosomal vesicles normally exist within the cell for the turnover of cytosolic and membrane-bound proteins. These acidic compartments can act as sinks for the sequestration of weak bases that have passed through the plasma membrane (49). Luciani et al. have shown that pretreatment of cells with vacuolar ATPase-inhibiting drugs, such as omeprazole, leads to sensitization of cells to cisplatin, 5-fluorouracil, or vinblastine *in vitro* and tumors to cisplatin *in vivo* (98). The beneficial effects are thought to be due to two mechanisms. First, inhibition of intracellular vacuole acidification results in less intracellular sequestration of the drugs. Second, inhibition of plasma membrane activity of the vacuolar ATPase leads to reduced pHe in the tumor and to increased uptake and kill *in vivo*. Interestingly, dosing of omeprazole was only effective at sensitizing the tumor if given before the cisplatin, suggesting that a metabolic response was necessary to achieve sensitization (98).

Recent understanding of the mechanisms involved in generating tumor-specific pHe leads to additional targeted approaches in treating it. A rational approach would be to explicitly engineer a drug to pharmacologically address the

Table 3. Strategies to Improve Effectiveness of Chemotherapy by Modifying pH

Treatment	Mechanism	Chemotherapeutic Drug Combination	References
Glucose ± <i>m</i> -iodobenzylguanidine	Decreased pHe via the Crabtree effect and respiratory inhibition	Chlorambucil Camptothecin	Kozin et al. (93)
Sodium bicarbonate	Increased pHe	Mitoxantrone Doxorubicin	Raghunand et al. (90) Raghunand et al. (94)
Omeprazole	Decreased membrane pH gradients via inhibition of vacuolar ATPase H ⁺ -pump	Cisplatin 5-Fluorouracil Vinblastine	Luciani et al. (98)
Carbonic anhydrase IX and XII inhibitors	Increased pHe via inhibition of tumor specific carbonic anhydrase activity	Not yet tested	Cecchi et al. (102)

tumor-specific causes of low pHe. It has been reported that the carbonic anhydrase isoforms XII and especially IX are hypoxia-responsive genes (99). The tumor expression of these isoforms has been seen *in vivo* to be architecturally distant from the blood vessels, implying regions of hypoxia (100). This molecule would therefore represent a tumor-specific regulator of extracellular acid accumulation, especially significant in the regions far from the vessels already suffering from poor perfusion and hypoxia (101). Specific inhibition of carbonic anhydrase IX would therefore affect tumor pHe with limited side effects on normal tissue acid base regulation. Cecchi et al. have derivatized the carbonic anhydrase inhibitor sulfonamide and identified variant molecules with specific ability to inhibit the hypoxia-inducible carbonic anhydrase IX isoform (102). These drugs show efficacy at inhibiting hypoxia-induced acidosis *in vitro* (102) and can now be tested to determine their effect on regulating pHe in tumors and modulating sensitivity to pH sensitive drugs, such as mitoxantrone.

Finally, reducing pHe in tumors may have additional physiologically based benefits as well. Gene expression changes have been identified that are a response to the acidotic environment of the tumor that may contribute to tumor progression. These induced genes include the acid-responsive angiogenic stimulator interleukin-8 (103), the bone remodeling parathyroid hormone/parathyroid hormone receptor pathway (104), the breast cancer resistance protein (96), and tumor necrosis factor- α (105). Additional mechanisms of gene regulation in response to acidity include induction of the Sp1 transcription factor (106) and specific increases in mRNA stability (107). The effect of lowered pH may also influence gene expression by HIF-1 (108), which has already been implicated in tumor progression. Finally, the acidic microenvironment may be inhibitory to the immune system and contribute to a block in antitumor immunity (109).

Molecular Targeting of the Tumor Microenvironment—Present

As our understanding of the molecular basis responsible for these microenvironment changes has improved, so has our ability to devise target-based therapeutic interventions. As we discuss below, investigators have identified several unique gene expression profiles that point to specific genes that are responsible for the physiologic changes within the solid tumor. These differences between the tumor and the normal tissue represent a possible way to treat the cancer without causing unwanted side effects. To date, the most impressive example of this type of targeting tumor gene expression changes is bevacizumab or anti-VEGF antibody therapy (73).

Tumors almost universally have regions of hypoxia, and hypoxia is the major regulator of tumor-specific expression of VEGF (110). VEGF is therefore a unique byproduct of the tumor microenvironment that is not usually expressed in normal tissues. The molecular targeting of VEGF with specific antibodies is not then a direct attack on the tumor itself but on the signaling cascade that is initiated by the changes that occur in the tumor microenvironment. Much of the success of bevacizumab in the treatment of patients with cancer seems to come not from a direct antiangiogenic effect as was first

hypothesized but from its ability to modify the tumor physiology (75). Bevacizumab has been shown to reduce IFP and increase drug uptake (111), potentially increasing tumor oxygenation, and may also raise pHe due to increased vascular function (112). Identifying new candidate molecules that fit this category of “tumor-specific modifiers of physiologic barriers” would offer exciting new targets for molecular intervention.

Molecular Targeting the Tumor Microenvironment—Future

Microarray analysis has identified many gene expression changes in the solid tumor, some of which are due to the tumorigenic process and some that are due to environmental cues (113-115). Although both types of changes could represent novel targets for molecular therapy, it is the second group that seems to more directly influence the tumor microenvironment. Genes have been identified that are responsive to pH, pressure changes, and hypoxia. Certainly the best studied of these genes are those that are responsive to hypoxia, many of which are regulated by the HIF-1 transcription factor (116).

The underlying mechanisms responsible for determining tumor oxygenation, pH, and IFP are often interrelated. It can be difficult to identify a molecular intervention that will address only one aspect of the biology. The example of bevacizumab shows that by diminishing VEGF activity there are not only changes in IFP but also glucose delivery, potentially oxygenation, and possibly even pHe. Blocking VEGF has been so successful that it raises the possibility that inhibiting the ability of HIF-1 to induce VEGF may also be effective. The benefit that is often touted for choosing HIF-1 as a molecular tumor target is its unique activation within the hypoxic tumor (117). There should be little HIF-1 present in normal tissue to yield unwanted side effects of the therapy (118). Several small-molecule inhibitors of HIF have been identified (119-122) and are now being tested for antitumor activity in preclinical trials.

HIF-1 as a Regulator of Tumor Physiology

Several aspects of tumor physiology seem to be directly responsive to the low oxygen environment within the tumor through the activity of the HIF-1 transcription factor. HIF-1 is therefore characterized as responsible for adaptive changes in the hypoxic regions within the tumor (123, 124). There are dozens of genes that HIF-1 can transactivate, including VEGF, glycolytic enzymes, ion channels, protease regulators, and mitochondrial regulators. The possibility of HIF-1 blockade therefore represents an interesting strategy for modifying the tumor physiology (115). HIF-1 is part of the loop responsible for the physiologic condition within the tumor. The tumor vasculature leads to hypoxia, this leads to HIF-1 activation, and additional physiologic changes in response to HIF-1 gene expression changes. Several examples of specific HIF-1 target genes fit well into this model of explaining the changes observed in the tumor microenvironment, including elevated glycolytic enzymes, lactate production, and carbonic anhydrase expression (125).

It is unclear if inhibiting HIF-1 will alleviate the micro-environmental aspects of the tumor or exacerbate the conditions already present. Any physiologic changes should be experimentally identified. The types of changes that occur would then suggest the types of combined therapies that should be added to the HIF-1 blockade. We have found that either genetic or pharmacologic HIF-1 blockade leads to increased hypoxia *in vitro*. If this is the case *in vivo*, then HIF inhibitors would be more effective in combination with hypoxic cytotoxins than with radiation therapy. The key to effective use of this knowledge is to recognize the changes that are going on within the tumor in response to the given agent. By measuring and recognizing the changes that are elicited in the tumor microenvironment, it will be possible to rationally decide what combination of drugs will be most effective.

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