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Modulation of Cell Death in the Tumor Microenvironment

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The microenvironment of solid human tumors is characterized by heterogeneity in oxygenation. Hypoxia arises early in the process of tumor development because rapidly proliferating tumor cells outgrow the capacity of the host vasculature. Formation of solid tumors thus requires coordination of angiogenesis with continued tumor cell proliferation. However, despite such neovascularization, hypoxia is persistent and frequently found in tumors at the time of diagnosis. Tumors with low oxygenation have a poor prognosis, and strong evidence suggests this is because of the effects of hypoxia on malignant progression, angiogenesis, metastasis, and therapy resistance. The presence of viable hypoxic cells is likely a reflection of the development of hypoxia tolerance resulting from modulation of cell death in the microenvironment. This acquired feature has been explained on the basis of clonal selection—the hypoxic microenvironment selects cells capable of surviving in the absence of normal oxygen availability. However, the persistence and frequency of hypoxia in solid tumors raises a second potential explanation. We suggest that stable microregions of hypoxia may play a positive role in tumor growth. Although hypoxia inhibits cell proliferation and in tumor cells will eventually induce cell death, hypoxia also provides angiogenic and metastatic signals. The development of hypoxia tolerance will thus allow prolonged survival in the absence of oxygen and generation of a persistent angiogenic signal. We will discuss the concept of hypoxia tolerance and review mechanisms used by cancer cells to acquire this phenotype. The concept of hypoxia tolerance has important implications for current and future therapeutic approaches. Most therapeutic efforts to combat hypoxia have focused on targeting the presence of hypoxia itself. Our hypothesis predicts that targeting the biological responses to hypoxia and the pathways leading to hypoxia tolerance may also be attractive therapeutic strategies.

The Concept of Hypoxia Tolerance

Evidence implicating hypoxia in the pathogenesis of solid human tumors continues to accumulate. Tumor hypoxia was hypothesized approximately 50 years ago to be important in the radiotherapeutic management of cancer because hypoxic cells are intrinsically more resistant to radiation than aerobic cells.[1] This initial interest in the radiobiological consequences of tumor hypoxia formed the basis of decades of research that, in recent years, has led to a close examination of the biological phenotypes of hypoxic cells. A number of seminal discoveries has led to the realization that hypoxia may be even more important than originally believed, contributing not only to therapy resistance but also to tumor malignancy. A wealth of data has shown that hypoxia can contribute to the malignant phenotype of tumors. Hypoxia has been implicated in promoting metastasis, angiogenesis, and the selection of cells with a more malignant phenotype.[2-8] The importance of hypoxia has been shown clinically when it predicts for worse outcome in the treatment of cancer of the head and neck, uterine cervix, and soft-tissue sarcomas.[9-11]

In nonpathological tissue, a structurally and functionally normal vasculature provides cells with an adequate oxygen and nutrient supply. However, the situation for aggressively growing tumors is much different. Although deregulated cell growth may be sustained by the host vasculature for a short period of time, rapid cell proliferation will eventually lead to excessive demand for oxygen. The establishment of hypoxia is thus believed to occur very early in the development of a tumor, producing a microenvironment that is hypoxic, acidic, and low in nutrients. The response of cells to this environment is critical for the continued growth of the tumor.

If the tumor mass is to maintain its growth, a continuous supply of oxygen and nutrients is essential. Hypoxia can stimulate the formation of new blood vessels through various mechanisms such as increased secretion of vascular endothelial cell growth factor (VEGF).[12] Although angio-

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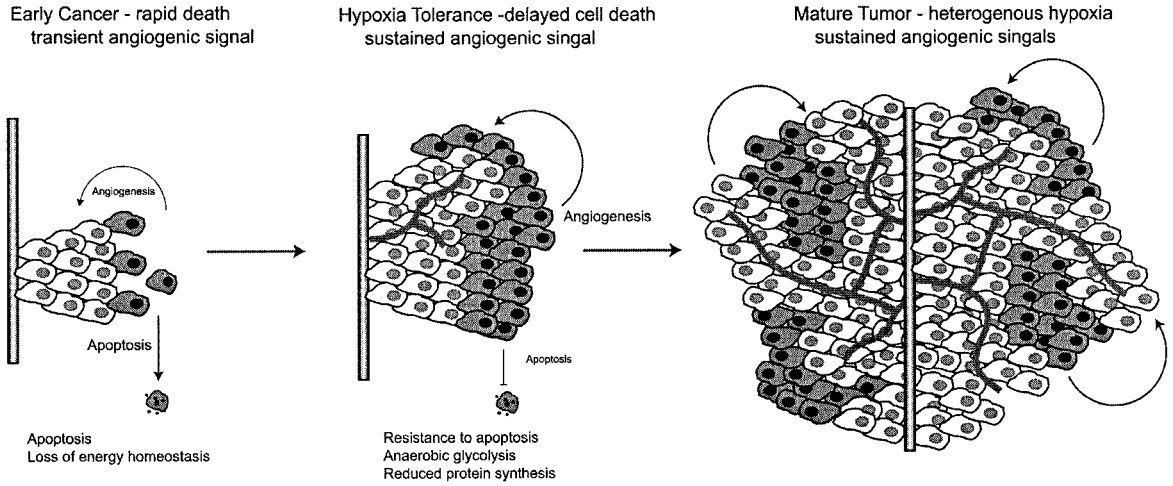

Figure 1. Hypoxia tolerance in the development of cancer. Hypoxia is hypothesized to arise early in the process of tumor development. Deregulation of cell growth results in an excess demand for oxygen and leads to cellular hypoxia. In normal and minimally transformed cells, hypoxia leads to cell death through activation of apoptosis or through loss of ATP. To continue to proliferate, tumors need to induce angiogenesis. During tumor evolution, cells become resistant to hypoxia-induced cell death. We hypothesize that this hypoxia tolerance arises such that a persistent hypoxia-induced angiogenic signal can be produced. The continued presence of viable hypoxic cells allows the coordination between angiogenesis and tumor expansion. This hypothesis implies that heterogeneity in oxygenation is beneficial to overall tumor growth.

genesis clearly occurs in solid tumors, clinical data have shown that hypoxia remains a common feature of tumors.[9,10,13-15] Even in the case of tumors that have developed throughout a period of 30 years or more, hypoxia is persistent. This implies that the vasculature that develops in tumors is inadequate to provide normal levels of oxygenation. Consistent with this idea is the observation that tumor vasculature is often abnormal, characterized by sluggish or intermittent blood flow, leakiness, and structural abnormalities that further contribute to tumor hypoxia.[16,17] We can thus conclude that hypoxia occurs early and remains a common feature of tumors throughout their development.

Cells can survive for only limited periods of time at low oxygen. In normal cells, hypoxia leads to the inhibition of cell growth and eventually to cell death. These same effects are also observed frequently in tumor cells but are generally less severe and/or develop with slower kinetics. This hypoxia tolerance is often explained as a result of a selective pressure in tumors that are forced to develop in an environment characterized by low oxygen availability. However, one could also hypothesize that the persistence of hypoxia in human tumors is a reflection of the fact that hypoxia can act as a net positive factor in tumor growth. Because hypoxia can stimulate angiogenesis, the presence of heterogeneous areas of hypoxia may be beneficial to the overall growth of the tumor. This hypothesis predicts that tumors containing hypoxia tolerant cells will maintain a growth advantage—a prediction supported by many examples from both the laboratory and the clinic.[2-8,18-20] Not only will such cells be able to survive limited exposures to hypoxia and perhaps proliferate again when oxygen becomes available, these cells can deliver a more prolonged angiogenic signal ensuring coordinated angiogenesis and cell growth. Thus, tumor cell growth, hypoxia, and angiogenesis become intrinsically linked. As tumor cells learn to tolerate hypoxia, both tumor cell growth and angiogenesis will be positively affected.

This leads us to an intriguing question. Is tolerance to hypoxia in cancer a common, or even necessary event in tumorigenesis? And if so, what are the implications for current therapies? If we can better understand the molecular mechanisms that control the adaptive responses to hypoxia in tumors, improved therapeutic approaches to the treatment of malignancies may be developed (Fig 1).[27]

Mechanisms of Hypoxia Tolerance

The formation and continued proliferation of solid tumors requires persistent angiogenesis. Reminiscent of many biological processes, angiogenesis is the result of subtle and often complex interactions balancing pro- and antiangiogenic molecules. This equilibrium is upset in various diseases, including cancer. Angiogenesis is virtually nonexistent in healthy adult tissue with the exception of a few physiological processes such as wound healing and the female menstrual cycle.[21,22] Stable regions of tumor hypoxia may upset this balance and provide the requisite proangiogenic signal. Viable hypoxic cells initiate angiogenesis principally through HIF-1 dependent upregulation of VEGF, although it also facilitates this process through a variety of other mechanisms.[23] We propose that development of hypoxia tolerance through modulation of cell death in the tumor microenvironment may be a common pathway that allows the generation of a persistent angiogenic signal. Whereas in normal cells hypoxia will lead to cell death and thus removal of the proangiogenic signal, hypoxia-tolerant cells maintain their survival and delivery of the signal. Two principle mechanisms have emerged that can explain how cell death in the tumor microenvironment is altered. The first is through suppression of intrinsic cell death pathways normally initiated by hypoxia and the second is through adaptation to the hypoxic environment through decreased energy use and increased energy production.

Hypoxia Tolerance

Suppression of cell death pathways. Hypoxia imposes a stress response that can lead to cell death. In many cell types, hypoxia promotes apoptosis through the induction of genes such as *p53*,[4] *bik*,[24] *bnip3*,[25,26] and others. However, the cellular decision of life or death is the result of the net balance between proapoptotic and antiapoptotic (survival) signals. It is thought that the very existence of cancer implies suppressed apoptosis and deregulated dependence on survival signals.[27] Suppression of proapoptotic signals often occurs through mutations in apoptosis-triggering tumor suppressor genes such as p53. Similarly, antiapoptotic (survival)–signaling pathways are often constitutively upregulated through activation of oncogenes such as ras or loss of tumor suppressors such as PTEN. Deregulated susceptibility to apoptosis may in itself lead to increased resistance to death induced by environmental stress such as hypoxia. The suppression of apoptosis in cancer cells also may contribute to genomic instability by failure to eliminate damaged cells. This provides cancer cells with an inherent adaptability to stress conditions such as hypoxia and hence substantial responsiveness to the selection pressure that it evokes. Thus, the presence of hypoxic areas in tumors may contribute to malignancy by promoting the clonal expansion of cell variants with a survival advantage in this microenvironment.

The relevance of hypoxia-induced selection pressure has been shown experimentally in several models. Graeber and colleagues[4] showed that a small number of transformed cells lacking the apoptosis stimulating tumor suppressor p53 would overtake similar cells expressing p53 when treated with hypoxia in vitro. Likewise, Kim et al[5] showed that the exposure of cell cultures to hypoxia greatly accelerated the selection for subpopulations of cells with diminished apoptotic potential. In vivo, hypoxic and apoptotic areas coincided in transplanted tumors expressing wild-type p53 but not in p53-deficient tumors.[4] Furthermore, the conversion of well-vascularized solid tumors to hypoxic ascites tumors favors the selection of cell variants with mutant p53.[28] The selection of apoptosis-resistant cells by hypoxia can also occur through p53-independent pathways. An example of this was shown in human colorectal cancer cells that acquired sustained resistance to apoptosis after hypoxia exposure in spite of active p53.[29]

Increased Energy Production. As described earlier, one of the means by which cells can become hypoxia tolerant is through selection of cells with mutations in genes that predispose them to hypoxia-induced cell death. However, tumor cells may also become hypoxia tolerant through adaptation of normal physiologic responses. The lack of oxygen necessitates a switch from oxidative phosphorylation to anaerobic glycolysis for adenosine triphosphate (ATP) production, and this switch has been shown to coincide with the oxygen gradient around blood vessels.[31] Increased glycolysis during hypoxia is facilitated by increasing the activity and expression of proteins in the glycolytic pathway such as glucose transporters (GLUTs),[32] phosphoglycerate kinase-1 (PGK-1),[33] and pyruvate kinase M (PK-

M).[34] In fact, the hypoxia-induced transcription factor HIF-1 mediates transcriptional activation of the entire glycolytic pathway from glucose uptake to lactate production.[8] Transcription of HIF-1–responsive genes is stimulated through binding of HIF-1 to a hypoxia response element (HRE) in the gene promoter. The HIF-1 transcription factor itself is regulated by a post-translational mechanism. HIF-1 is a heterodimer consisting of the 2 subunits, HIF-1α and HIF-1β, which are both ubiquitously expressed. HIF-1β protein is stable, whereas HIF-1α is targeted for ubiquitination by the von Hippel-Lindau tumor suppressor protein (VHL) and rapidly degraded by the proteasome under well-oxygenated conditions.[35] VHL recognizes hydroxylized prolyl residues in the HIF-1α protein, which remain unhydroxylated during hypoxic conditions.[36,37] Thus, HIF-1α is stabilized during hypoxia and can dimerize with its partner HIF-1β to induce transcription of HRE-responsive genes.

Evidence showing the significance of the HIF-1 pathway for tumor cell viability has accumulated over the last few years. HIF-1α– or β–deficient Chinese hamster ovary cells have been shown to be sensitive to hypoglycaemia and altered redox status by inhibition of cytochrome oxidase,[38] and mouse embryo fibroblasts lacking HIF-1α grow more slowly under hypoxia than wild-type cells.[39] Using genetically matched cell lines derived from wild-type and HIF-1α knockout mice, a proteomic analysis showed that HIF-1 was strictly required for the upregulation of key enzymes in the glycolytic pathway.[39] The decreased glycolytic capacity of HIF-deficient cells resulted in dramatically lowered free ATP levels. These findings are in support of a significant role for HIF-1 in the protective adaptation to the tumor microenvironment. This notion has been confirmed in vivo because HIF-1–deficient transformed cells are less tumorigenic than wild-type cells and that the resulting xenografts show slower growth. The slower tumor growth in these studies could not be attributed to differences in tumor vascularization, and the authors therefore concluded that it was because of impaired upregulation of glycolysis as observed in vitro.[39,40]

Constitutively upregulated HIF and enhanced glycolosis even under aerobic conditions is a common characteristic of many tumors, suggesting that this pathway may become deregulated as a consequence of tumor progression.[41] The observed reduced growth rates of HIF-deficient tumors provide an explanation for why this may be beneficial for tumor development. Presumably, a constitutively activated glycolytic pathway can contribute to hypoxia tolerance by allowing tumor cells to maintain their energy homeostasis during periods of low-oxygen availability.

Decreased Energy Consumption

Another strategy for increasing survival under conditions of limiting oxygen and energy is to decrease ATP consumption. A well-characterized consequence of hypoxic stress is a pronounced repression in the rate of oxygen consumption and energy turnover.[42,43] The main ATP-demand pathways in hypoxic cells are the Na^+/K^+ ATPase pump, protein synthesis, and degradation and gluconeogenesis.[44,45] It has been estimated that under severe hypoxia, the ATP demand for protein synthesis drops to about 7% of that of normoxic cells. This drop correlates with a substantial and rapid drop in the rate of protein synthesis.[46] The rapid kinetics of this response and the fact that it precedes ATP depletion argues for a tightly regulated mechanism that is activated by low oxygen availability.[47] The molecular pathways responsible for the downregulation of protein synthesis during hypoxia are not yet completely understood. However, recent data suggest the involvement of eukaryotic initiation factors (eIFs) that modulate translation initiation.[48] The eIFs facilitate the correct assembly of the messenger RNA (mRNA) template, the ribosomal 40S subunit and the aminoacylated initiator transfer RNA (Met-tRNA). A rate-limiting factor in this process is eIF2, which binds Met-tRNA in its energized eIF2-guanosine triphosphate form, and thereby recruits Met-tRNA to the ribosome. The activity of eIF2 is tightly regulated through phosphorylation of the eIF2α subunit, which inhibits the exchange of guanosine triphosphate for guanosine diphosphate bound to eIF2, and thereby the binding and recruitment of Met-tRNA. Translation has previously been shown to be downregulated through phosphorylation of eIF2α in response to stress such as virus infection, unfolded proteins, serum starvation, or amino acid deprivation. Recently it was shown in several transformed cell lines that hypoxia induces a rapid phosphorylation of the eIF2α subunit, which results in reduced protein synthesis.[49] The phosphorylation of eIF2α appears to be mediated by the endoplasmic reticulum kinase

PERK, which previously has been known to be activated in response to unfolded proteins in the endoplasmic reticulum. This rapid inhibition in protein translation in response to hypoxia strongly suggests that this pathway is critical for cells to survive during hypoxic exposure. It will be interesting to test if disruption of this response will sensitize cells to hypoxia-induced cell death.

Another rate-limiting eukaryotic initiation factor is eIF4E, which recognizes and binds the m^7G cap-structure of the 5′ mRNA. eIF4E facilitates the bridging of the mRNA to the 40S subunit through participation in the scaffolding eIF4F protein complex. eIF4E is mainly regulated through a set of binding proteins (4E-BPs) that bind eIF4E in their hypophosphorylated form and thereby inhibit its participation in the eIF4F complex. The 4E-BPs have previously been shown to be phosphorylated in response to stimuli such as hormones or growth factors, and dephosphorylated in response to stress such as heat shock, virus infection, or serum starvation, leading to stimulation and repression of translation, respectively. We have recently found that hypoxia disrupts the eIF4F complex through multiple mechanisms including dephosphorylation of the 4E-BPs.[50] Furthermore, we have also shown that the eIF4E translocates to the cell nucleus and thus becomes unavailable for translation during hypoxic conditions.[50]

The regulation of eIF2α and eIF4E in response to hypoxia appears to be significantly weaker in human normal fibroblasts than in human tumor cell lines (Wouters, unpublished data, 2002). This finding supports the idea that regulatory responsiveness to hypoxia is an adapted feature of tumor cells that is beneficial for their survival.

Therapy Implications

The fact that hypoxia negatively impacts on therapy is well established. Hypoxic cells are radiation and chemoresistant for a variety of reasons,[51] and thus effective therapy requires strategies to overcome this resistance. Many attempts have been made, most of these focused on trying to restore normal oxygenation (or mimic it) to the tumor. The concept that tumor cells become hypoxic tolerant, and furthermore that the presence of microregions of hypoxia may be advantageous to overall tumor growth, has its own therapeutic implications. New approaches aimed specifically at exploiting or altering the mechanisms that lead to hypoxia tolerance may provide better efficacy in future therapies

Restore Oxygenation

In the past 40 years, numerous attempts have been made to improve radiotherapy by restoring (or mimicking with hypoxic radiosensitisers) the oxygen supply to tumor cells. This includes such treatments as hyperbaric oxygen breathing, transfusions to improve the haemoglobin level, and electron affinic radiosensitisers. Although early studies showed mixed results, a large meta-analysis of all oxygen-modifying head and neck cancer trials did show a significant improvement in local control and disease-specific survival.[52,53] This is likely a reflection of the fact that hypoxic cells are radiation resistant, and even modest improvements in oxygenation should improve outcome. More recently, the use of recombinant erythropoietin (EPO) has become subject of study. EPO is a HIF-1–regulated haematopoietic growth factor produced by the kidneys in response to hypoxia. It stimulates the erythrocyte production in the bone marrow. Several trials have been conducted to investigate the effect of recombinant human EPO in patients with low hemoglobin (Hb) levels. A relationship between Hb levels and the response to radiation therapy has been shown for carcinomas of the uterine cervix,[54-56] head and neck cancers,[57-61] lung cancer,[62-64] bladder cancer,[65-68] and prostate carcinoma.[69] In these studies, patients with low Hb levels achieved lower local control rates and survival.

The combination of accelerated radiotherapy with carbogen and nicotinamide, known as the ARCON protocol, is also being evaluated in the clinic. Carbogen (95% O_2 + 5% CO_2) reduces diffusion limited hypoxia and nicotinamide can antagonize vasculature shutdown.[14,70-74] A significant effect on both locoregional control and disease specific survival for stage T_3-T_4 SCC laryngeal tumors has been reported.[75,76] In a recent publication an overall local control rate of 80% was reported for T_3 and T_4 larynx carcinomas,[77] offering possibilities for organ preservation. A phase II trial for bladder cancer also showed better results using the ARCON protocol, compared with historical data.[78]

Despite some success of these approaches, the concept of improving tumor oxygenation ignores

Table 1. Targeting Treatment at Hypoxia

Hypoxia tolerance	Restore cell death pathways
	Target survival pathways
	Block adaptation to ATP preservation
Viable hypoxic cells	Restore oxygenation
	Bioreductive drugs
	Gene therapy with anaerobic bacteria
Biological response	Block angiogenesis
	Block metastasis

in part the biological effects of hypoxia that may be important in malignancy and treatment response outside of therapy resistance. In other words intrinsic resistance to therapy is only one of the mechanisms by which hypoxia impacts on prognosis. Our increased understanding of the biology of hypoxic cells has led to new ideas for treating hypoxic tumors (Table 1).

Exploit Hypoxia

The concept that hypoxia tolerance in tumors is a selected phenotype that supports angiogenesis provides us with new problems and possibilities for therapy. Instead of attempting to rid tumors of hypoxia, which may be impossible because of hypoxia tolerance and the nature of their vasculature, we can instead attempt to exploit hypoxia for therapeutic advantage. Stable regions of hypoxia in human tumors provide the possibility of directing therapy specifically against this unique feature. Current attempts to exploit this feature of tumors include both pharmaceutical and gene-therapy approaches.

Bioreductive Drugs

Bioreductive drugs are compounds that are reduced by enzymes to their toxic, active metabolites. They are designed such that this metabolism occurs only or preferentially in the absence of oxygen. The use of these drugs in combination with traditional therapies has the potential to greatly improve treatment outcome by increasing cytotoxicity to the hypoxic tumor fraction. In theory, such an approach can be superior to an alternative therapy that would fully reoxygenate the tumor.[80] Tirapazamine is the leading compound in this class of agents and has shown promising results in a number of clinical trials when used in combination with cisplatin and/or radiotherapy. A wide variety of cell lines are sensitive to tirapazamine, regardless of their p53 status, and require 50 to 150 times higher dose for the same toxicity under aerobic conditions.[81,82] For a more detailed discussion of bioreductive agent therapies, see the accompanying article by Stratford et al in this issue.

Gene Therapy With Bacterial Vectors

The aim of gene therapy is to transfer genetic material to the tumor cell or its microenvironment in quantities sufficient to obtain a therapeutic level of expression. However, strategies devised to date have limited efficiency, most notably because of deficiencies in the delivery systems used. A recent approach to this problem uses the concept of targeting anaerobic bacteria to the hypoxic/necrotic areas of solid tumors. Currently, both *Clostridium spp* and attenuated *Salmonella typhimurium* auxotrophs are being investigated as systems to deliver antitumor compounds specifically to the tumor site.[83,84] The latter strain grows under aerobic and anaerobic conditions, with selectivity for tumors reported as a consequence of its auxotrophic nature. The specificity of clostridia for tumors resides in its obligate requirement for anaerobic conditions. Intravenously injected spores of a nonpathogenic clostridial species have been shown to localize to, and germinate in, the hypoxic/necrotic regions of solid tumors. Although growth alone in the tumor is not sufficient for therapeutic efficacy, the possibility now exists to engineer *Clostridium spp* to produce a variety of therapeutic proteins with anticancer properties. Clostridia can thus be used as highly selective in situ cell factories able to produce and secrete antitumor therapeutics specifically at the tumor site. Moreover, it has been shown that the immune response does not hinder repeated administration of clostridial spores, that colonization can be improved using vascular targeting, and that gene expression can be stopped at any time using suitable antibiotics. We and others showed that it is possible to express therapeutic proteins, not only in vitro but also in vivo after administration of the recombinant clostridia to tumor-bearing animals.[85,86]

Target Biological Responses to Hypoxia

We have proposed that one of the principle reasons that tumors contain hypoxic cells is the fact that these cells can provide a prolonged angio-

genic signal. One can thus envisage a situation in which non-proliferating or even non-clonogenic hypoxic cells contribute in a positive way to tumor growth. The concept that hypoxia tolerance is critically important for tumor growth suggests that interfering with either the mechanisms that lead to hypoxia tolerance or the biological consequences of the resulting hypoxic areas in tumors (angiogenesis) may become new effective means of treatment. Therapeutic interventions to counteract these biological responses are possible at different levels.

Block Angiogenesis

Perhaps the most important consequence of tumor hypoxia is the induction of angiogenesis. Progressive growth of solid tumors is largely dependent on this process[87] and thus antiangiogenic therapy may relieve much of the impact of hypoxia on prognosis. The complex process of angiogenesis offers potential therapeutic targets at different levels.[88]

VEGF is perhaps the most important factor in tumors for promoting compensatory angiogenesis in circumstances of oxygen shortage.[89] It promotes endothelial cell migration, modulates proteases needed for basement degradation, and stimulates plasminogen expression. Hypoxic upregulation of VEGF occurs as a result of both increased transcription mediated by HIF-1 and increased mRNA stability dependent on the 3′ untranslated region.[90,91] VEGF receptors have been shown to be upregulated in surrounding endothelial cells.[92] Therefore, VEGF, its receptors (flt-1 and flk-1) and the signal transduction pathway present realistic therapeutic targets. Several therapeutic strategies aimed at targeting the process of angiogenesis, including those aimed at interfering with VEGF signaling pathways, are currently under active investigation both in the laboratory and the clinic. This topic is the subject of another article in the current issue (Siemann and Shi, in this issue).

One interesting aspect of antiangiogenic therapies possibly related to the concept of hypoxia tolerance is that there are examples of gradual loss of response, especially when drugs are administered as monotherapies. Several recent reports[107,108] support this notion and provide a possible explanation. Kerbel and colleagues[108] showed that the genetic background of a tumor cell (in particular the presence/absence of p53) may be an important determinant of response to antiangiogenic therapy. They concluded that loss of p53 may allow tumor cells to survive the temporary inhibition of angiogenesis as a consequence of their reduced apoptotic potential during hypoxia. Antiangiogenic therapy is expected to result in increased tumor hypoxia, and thus tumors that are better able to survive hypoxia would be expected to maintain a growth advantage. As discussed earlier, the reduced reliance on vascular supply through modulation of cell death during hypoxia can occur through many mechanisms including changes in the HIF-1 pathway.[109]

Block Tolerance

The recent suggestion that even antiangiogenic strategies have reduced efficacy against tumors that have developed mechanisms of increased hypoxia tolerance suggests it may be necessary to target an even earlier step in this system. One obvious strategy is to interfere with the mechanisms that tumor cells have used to modulate their sensitivity to cell death in response to hypoxia. Some approaches are based on the knowledge that several genes, when mutated, contribute not only to tumor progression but also to survival under hypoxic conditions. Gain-of-function mutations in key oncogenes and/or loss-of-function mutations in tumor suppressor genes can prevent commitment of cells to apoptosis. Perhaps the best-characterized survival signaling pathway is mediated by PI3-kinase (PI3K-PTEN-AKT-FRAP/MTOR pathway). This pathway has been implicated in both the response of cells to hypoxia and to angiogenesis.[110] Pharmacologic agents that inhibit PI3K (Wortmannin or LY294002) or its downstream effectors FRAP/MTOR (rapamycin) have been shown to have some therapeutic efficacy.[111] Altering the malignant phenotype by blocking dominant negative oncogenes that are implicated in the hypoxic response (such as myc and ras) at transcriptional or translational levels is also an attractive target being evaluated in several clinical trials. Methods involve the use of antisense oligonucleotides,[112] ribozymes,[113] and intracellular single-chain antibodies.[114] Antibodies to HER2/neu or the epidermal growth factor receptor, both of which can provide survival and angiogenic signals, are also attractive candidates as contributors to hypoxic cell survival. These antibodies are being tested

and have shown some significant efficacy against angiogenesis in vivo.[115,116] Based on the fact that p53 induces apoptosis in response to hypoxia, perhaps the most attractive method to restore cell death is to restore p53 function. Many investigations using gene therapy with p53 are already underway.[117,118] Other attractive approaches involve the conversion of anti-apoptotic or proliferative signals into signals that trigger apoptosis.[119,120]

Another important mechanism of hypoxia tolerance arises through the increased ability to regulate ATP supply during hypoxic exposure. As discussed earlier, hypoxic tumor cells are able to decrease their requirements for ATP by shutting down overall protein synthesis while at the same time inducing a transition from oxidative phosphorylation to anaerobic glycolysis for the generation of ATP. Interfering with either of these 2 processes would be expected to selectively target hypoxia tolerant cells. The process of translation initiation is facilitated by the eukaryotic initiation factors, and several of these factors are controlled through upstream signaling pathways. Thus, it may be possible to interfere with this response indirectly with agents that influence these upstream pathways. For example, translation initiation is increased in response to PI3 kinase signaling through FRAP/mTOR. Deregulation of inhibited translation initiation during hypoxia would be expected to result in increased ATP consumption and thus make tumor cells less able to survive prolonged periods of hypoxia.

Similarly, it may be possible to interfere with ATP generation in hypoxic tumor cells. Tumors in general have increased rates of glycolysis, a fact that may be related to hypoxia tolerance. Targeting of the enzymes or substrates involved in this well understood pathway would thus also be expected to specifically target hypoxic cells or at least reduce the tolerance of tumor cells to hypoxia. Recent results showing reduced tumor growth in transformed cells that are unable to induce anaerobic glycolysis as a result of loss of HIF-1 support this idea.[39]

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