Tumor Hypoxia: Causative Factors, Compensatory Mechanisms, and Cellular Response

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ABSTRACT

Hypoxia is a characteristic feature of locally advanced solid tumors resulting from an imbalance between oxygen (O2) supply and consumption. Major causative factors of tumor hypoxia are abnormal structure and function of the microvessels supplying the tumor, increased diffusion distances between the nutritive blood vessels and the tumor cells, and reduced O2 transport capacity of the blood due to the presence of disease- or treatment-related anemia. Tumor hypoxia is a therapeutic concern since it can reduce the effectiveness of radiotherapy, some O2-dependent cytotoxic agents, and photodynamic therapy. Tumor hypoxia can also negatively impact therapeutic outcome by inducing changes in the proteome and genome of neoplastic cells that further survival and malignant progression by enabling the cells to overcome nutritive deprivation or to escape their hostile environment. The selection and clonal expansion of these favorably altered cells further aggravate tumor hypoxia and support a vicious circle of increasing hypoxia and malignant progression while concurrently promoting the development of more treatment-resistant disease. This pattern of malignant progression, coupled with the demonstration of a relationship between falling hemoglobin level and worsening tumor oxygenation, highlights the need for effective treatment of anemia as one approach for correcting anemic hypoxia in tumors, and in so doing, possibly improving therapeutic response. The Oncologist 2004;9(suppl 5):4-9

INTRODUCTION

The role of hypoxia in increasing tumor resistance to radiation therapy was demonstrated at the beginning of the last century [1, 2]. It was not until 1953 that the crucial role of oxygen (O2) in radiation response was fully acknowledged [3]. More recently, tumor hypoxia has been shown to decrease the efficacy of certain cytotoxic drugs including cyclophosphamide, carboptatin (Paraplatin®; Bristol-Myers Squibb; Princeton, NJ), carmustine (BiCNU®; Bristol-Myers Squibb), and melphalan (Alkeran®; Celgene Corporation; Warren, NJ) [4, 5]. Moreover, investigations conducted over the past two decades have demonstrated that tumor hypoxia,
in addition to diminishing therapeutic efficacy, plays a pivotal role in malignant progression. Advances in the area of tumor hypoxia and malignant progression were made possible primarily by the introduction of a computerized polarographic needle electrode system (oxygen tension [pO\textsubscript{2}] histography) in the late 1980s [6, 7]. This system allowed rapid and reliable collection of pO\textsubscript{2} values, enabling the identification and characterization of tumor hypoxia and assessment of its clinical relevance [8]. With this technology, hypoxic tissue areas (areas with pO\textsubscript{2} values ≤2.5 mmHg) have been shown to be a characteristic feature of locally advanced solid tumors and to occur across a wide range of human malignancies [8]. These areas tend to be heterogeneously distributed within the tumor mass; locations include sites adjacent to tumor areas with pO\textsubscript{2} values comparable with those found in neighboring cancer-free tissue [9].

**CAUSES OF TUMOR HYPOXIA**

Tumor hypoxia results from an imbalance between the cellular O\textsubscript{2} consumption rate and the O\textsubscript{2} supply to the cells [10]. Hypoxia can be caused by a number of factors, most of which are perfusion-, diffusion-, or anemia-related [8, 10, 11]:

- Perfusion-related (acute) hypoxia is caused by inadequate blood flow in tissues. Tumor microvasculatures frequently have severe structural and functional abnormalities, such as a disorganized vascular network, dilations, an elongated and tortuous shape, an incomplete endothelial lining, a lack of physiological/pharmacological receptors, an absence of flow regulation, and intermittent stasis. Perfusion-related O\textsubscript{2} delivery leads to ischemic hypoxia, which is often transient.

- Diffusion-related (chronic) hypoxia is caused by an increase in diffusion distances with tumor expansion. This results in an inadequate O\textsubscript{2} supply for cells distant (>70 μm) from the nutritive blood vessels. Diffusion-related hypoxia may also be caused by deterioration of diffusion “geometry,” for example, concurrent versus countercurrent blood flow within the tumor microvessel network.

- Anemic hypoxia is caused by reduced O\textsubscript{2} transport capacity of the blood subsequent to tumor-associated or therapy-induced anemia. Experimental studies have shown that the O\textsubscript{2} supply to tumors is greatly reduced and hypoxia is intensified at hemoglobin levels below 10-12 g/dl, especially when low O\textsubscript{2} transport capacity coincides with a low perfusion rate (Fig. 1) [8, 9].

Conditions similar to those seen in anemic hypoxia can be caused by carboxyhemoglobin formation in heavy smokers, since carbon monoxide-blocked hemoglobin can no longer transport O\textsubscript{2} (toxic hypoxia). Additionally, tumor microvessels may be perfused (at least transiently) by plasma only, which leads to a rapid induction of hypoxia because only a few cells at the arterial end are adequately oxygenated under these conditions [8, 10].

**OXYGENATION STATUS OF TUMORS**

Much of the current knowledge on the oxygenation status in solid tumors is based on the findings of studies of cervical cancer conducted on conscious women before treatment. In those studies, approximately 60% of locally advanced squamous cell carcinomas of the uterine cervix showed hypoxic and/or anoxic areas [8, 10]. Overall, mean and median pO\textsubscript{2} values were substantially lower in tumor tissue than in normal tissue. Mean and median pO\textsubscript{2} values in locally advanced cervical cancer were 16 mmHg and 10 mmHg, respectively, compared with respective values of 40 mmHg and 42 mmHg in the normal cervix of nulliparous patients (Fig. 2) [8]. Furthermore, the studies showed that the oxygenation status and extent of hypoxia were independent of clinical size, tumor stage, histopathologic type (squamous cell carcinoma versus adenocarcinoma), and grade of malignancy. Also, the oxygenation patterns were found to be independent of patient age, parity, menopausal status, and smoking habits [9, 10]. Particularly noteworthy was the finding that, in the cervical cancers, the median pO\textsubscript{2} value tended to increase when hemoglobin levels were raised to between 10 g/dl and 14 g/dl [12]. Similar increases in pO\textsubscript{2} relative to increasing hemoglobin levels were recently demonstrated in patients with breast cancer (Fig. 3) [13].

**REGULATORY AND COMPENSATORY MECHANISMS DURING ANEMIA**

In normal tissues, adequate oxygenation is maintained if hemoglobin levels are kept above 8 g/dl [14]. Between

![Figure 1. Hypoxic tissue fraction (pO\textsubscript{2}< 1 mmHg) as a function of hemoglobin level and tumor blood flow (TBF). Computed data for human breast cancer xenotransplanted into nude rats. Reprinted from Vaupel et al. [9] with permission.](image-url)
hemoglobin levels of 8 g/dl and 4 g/dl, oxygenation of normal tissues, although somewhat reduced, mostly remains adequate due to a physiological compensation for O2 deficiencies, primarily through an increase in blood flow (Table 1, Fig. 4). In contrast, locally advanced tumors cannot adequately counteract the reduction in O2 supply and thus are not able to avoid the development of hypoxia (Fig. 3).

Becker et al. recently demonstrated the relationship between falling hemoglobin level and decreasing tumor oxygenation in normal and tumor tissue in 133 patients with squamous cell carcinomas of the head and neck [15]. In that study, pretreatment polarographic pO2 measurements of the tumors were obtained for all patients. In addition, pO2 measurements of sternocleidomastoid muscles were made in 66 patients. Patients were categorized by hemoglobin level as being severely anemic (<11.0 g/dl), mildly anemic (females 11.0-11.9 g/dl, males 11.0-12.9 g/dl), or normal (females ≥12.0 g/dl, males ≥13.0 g/dl). The investigators found significant (p < 0.0001) differences in oxygenation between the primary tumors (average median pO2 = 12 mmHg, range 0-58 mm Hg) and the sternocleidomastoid muscles (average median pO2 = 38 mmHg, range 21-68 mm Hg). For the

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**Table 1.** Primary compensatory mechanisms in tissue hypoxia (normal versus malignant) [8]

<table>
<thead>
<tr>
<th>Condition</th>
<th>In normal tissue</th>
<th>In tumors</th>
</tr>
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<tbody>
<tr>
<td>Perfusion-related (ischemic) hypoxia</td>
<td>O2 utilization ↑</td>
<td>Absent</td>
</tr>
<tr>
<td>Diffusion-related hypoxia</td>
<td>(Blood flow ↑)</td>
<td>Absent</td>
</tr>
<tr>
<td>Anemic hypoxia</td>
<td>Blood flow ↑</td>
<td>Absent</td>
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66 patients in whom both tumor and sternocleidomastoid muscle pO$_2$ measurements were made, the median pO$_2$ was substantially higher in muscle tissue than in tumor tissue for each patient.

In the analysis of tumor oxygenation by hemoglobin category, no significant difference in oxygenation was found between mildly anemic and normal patients, but severely anemic patients had a median pO$_2$ value significantly below that found in patients with mild anemia and in patients with no anemia (5 mmHg versus 13 mmHg and 15 mmHg, respectively; $p = 0.0001$ for both). In contrast, no significant differences in muscle pO$_2$ were detected among patients in the three hemoglobin categories (Fig. 5).

According to Becker et al., these findings suggest that tumor tissue is more sensitive to a low hemoglobin level than resting skeletal muscle, presumably because the inadequate vascular structure in malignant tissue is unable to compensate for the deficiency in O$_2$ transport by decreasing vascular resistance and thus increasing blood flow [15]. They, thus, concluded that correcting the hemoglobin level may provide one possible mechanism for significantly increasing tumor oxygenation and thereby improving treatment outcome. Similar findings were published recently by Vaupel et al. for breast cancer [13].

**CELLULAR RESPONSES TO HYPOXIC STATES**

Hypoxia can influence tumor cells in one of two ways, either by acting as a stressor that impairs growth or causes cell death (slowing of proliferation, apoptosis, or necrosis) or by serving as a factor that ultimately results in malignant progression and increased resistance to radiation therapy and other cancer treatments [8, 11]. To a large extent, the increases in malignant progression and treatment resistance are manifestations of hypoxia-induced proteomic and genomic changes within the tumor cells.

**Proteomic Changes**

Evidence from recent studies suggests that sustained (>6-8 hours) or fluctuating hypoxic stress (pO$_2 \leq$7 mmHg) can lead to alterations (stimulation or inhibition) of gene expression, as well as posttranscriptional and posttranslational modulations that result in changes in the tumor cell proteome (Fig. 6) [9-11, 16].

These hypoxia-induced proteomic changes may, in turn, lead to growth stasis or impairment through molecularly mediated cell-cycle arrest, differentiation, programmed cell death (apoptosis), or necrosis [17-21]. Hypoxia-induced cell-cycle arrest at the G1/S check point may be triggered by a hypoxia-inducible factor one alpha (HIF-1α)-mediated activation of the cyclin-dependent kinase inhibitors p21 and p27 [22]. This response seems to be independent of p53, even though p53 accumulates under hypoxia [23]. Instead, an increased level of p53 under hypoxic conditions may lead to the alternative activation of apoptosis with Apaf-1 and caspase-9 as downstream effectors [24]. However, hypoxia may also induce p53-independent apoptosis pathways involving genes of the BCL-2 family and others. Experimental studies have suggested that hypoxia may act as a morphogen to induce terminal differentiation of cells, and hypoxia is known to result in necrotic cell death. Overall, the effects of hypoxia-related proteomic changes leading to tumor cell growth stasis and death may explain the delayed recurrences, dormant micrometastases, and growth retardation observed in large tumor masses [11, 25-27].

Alternatively, hypoxia-induced proteomic changes may promote tumor propagation by enabling cells to adapt to nutritive deprivation, or by facilitating proliferation, local invasion, and metastatic spread, thereby permitting the cells to escape their hostile environment. One of the major promoters of tumor cell adaptation to hypoxic stress is the transcription factor HIF-1, which accumulates in response to declining cellular O$_2$ levels [10, 11]. HIF-1 activates a battery of more than 30 genes, many of which express protein products involved in O$_2$ delivery (e.g., erythropoietin), angiogenesis (e.g., vascular endothelial growth factor [VEGF]), energy preservation (e.g., glucose transporters and glycolytic enzymes), and other processes essential to tumor cell survival, propagation, and spread (Fig. 7) [10, 16]. Angiogenesis is an especially important factor in tumor progression since a tumor usually cannot grow beyond ~1 mm in diameter without an adequate blood supply [28]. A more detailed discussion of HIF-1 and angiogenesis, including their impact on tumor progression, is provided by Vaupel [29] elsewhere in this supplement.
Genomic Changes

Tumor cell survival and proliferation or, alternatively, growth impairment, stasis, and cell death are not solely dependent on proteomic changes. Mutations in oncogenes and/or tumor suppressor genes are generally thought to be of crucial importance for the development of tumor aggressiveness. Hypoxia (\(pO_2 \leq 0.7 \text{ mmHg}\)) promotes genomic instability, thereby increasing the number of mutations (genetic variants). Hypoxia concomitantly exerts a strong selection pressure [10, 11, 16]. Tumor cell variants with adaptations favorable to survival under hypoxic conditions (e.g., lower capacity for cell-cycle arrest or apoptosis, greater angiogenic potential) may have growth advantages over nonadapted cells in the hypoxic microenvironment and expand through clonal selection. The expansion of cell clones with favorable proteomic and genomic adaptive changes can, in turn, exacerbate tumor hypoxia, thereby establishing a vicious circle of increasing hypoxia and subsequent malignant progression. At the clinical level, the consequences of this vicious circle are translated into more local recurrences, locoregional spread, and distant tumor metastases, and greater resistance to radiation therapy and certain forms of chemotherapy [10].

CONCLUSION AND SUMMARY

Tumor cell responses to hypoxic stress include adaptive proteomic changes allowing the cells to overcome nutritive deprivation or to escape from their hostile environment by proliferation, invasion, or metastatic spread. The survival and propagation advantages of tumor cells can be further enhanced by genomic changes such as loss of apoptotic potential. These new cell variants have advantages over less adapted cells in a hypoxic microenvironment and expand through clonal selection, often becoming the dominant cell type. These variants further intensify hypoxia, establishing a vicious circle of hypoxia, malignant progression, and treatment resistance. In the clinical setting, a relationship has been demonstrated between falling hemoglobin level and decreasing tumor oxygenation. Thus, efforts should be made to prevent or correct disease- and treatment-related anemia in cancer patients in order to reduce tumor hypoxia and thereby possibly improve therapeutic outcome.
REFERENCES


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