

Hypoxia and Metastasis

□□ *Commentary on Rofstad et al., p. 1971*

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In this issue of *Clinical Cancer Research*, Rofstad et al. (1) report on the significance of chronic (diffusion-limited) and acute (fluctuating or perfusion-limited) hypoxia in D-12 and R-18 human melanoma xenografts on the development of metastatic disease. That tumors may contain different subpopulations of hypoxic cells has been known for many years. Early work showed that hypoxia can affect the response to the primary tumor to treatment with either radiation or certain drugs. In particular, the resistance of hypoxic cells to radiation has been used as an assay of the fraction of viable hypoxic cells in a tumor, hence the term “radiobiologically hypoxic cells.” Such cells are expected to be at oxygen levels below 5 to 10 mm Hg, although there is evidence that this level may vary depending on various factors, including the level of cysteine and other nonprotein sulfhydryls in the cells (2). The original concept of hypoxia in tumors, put forward by Thomlinson and Gray almost 50 years ago, was of hypoxic cells residing at the limits of oxygen diffusion from functional blood vessels and being on the edge of regions of necrosis in the tumor. Such cells were believed to be exposed to prolonged or chronic hypoxia, and a variety of studies in tumor models and in spheroids established that tumor cells could remain viable in such environments for hours to a few days (3, 4). Later, it was recognized that fluctuations in blood flow (perfusion limitations) may play an equally important role in the development of hypoxia in tumors, such that cells in tumors may be exposed to short-term hypoxia termed acute or fluctuating hypoxia. Recent studies of blood flow and oxygen levels in tumors have established that blood flow fluctuations and resulting oxygen levels in the tissue can have cycles from minutes to a few hours (5–7).

Thus, it has been established that most human tumors develop a pathophysiologic microenvironment during growth, characterized by an irregular microvascular network and regions of chronically and transiently hypoxic cells (8), although it is likely that there is no discrete temporal boundary between these two states in the tumor, rather it is a continuum. Furthermore, various studies have established

that there is substantial heterogeneity in the proportion of hypoxic cells in individual tumors, presumably due to the stochastic development of their vasculature (9). The development of drugs that are reductively metabolized to compounds that accumulate to form immunodetectable covalent adducts in hypoxic cells has provided markers of hypoxic cells that can be visualized in tumor sections (10). One such drug, pimonidazole, was used in the study by Rofstad and colleagues to identify chronically hypoxic cells. The proportion of acutely hypoxic cells in the tumor was then defined as the difference between the fraction of pimonidazole-labeled cells and the fraction of radiobiologically hypoxic cells because all cells that are hypoxic at the time of the irradiation are detected in this latter assay. The pimonidazole was injected 4 h before sacrifice of the animals for tumor excision and its half-life in mouse plasma (or tumor) is ~30 min. Because the drug must be metabolized for adducts to be detected, the effective temporal boundary in this study between acute and chronic hypoxia is likely to be in the range of 1 to 2 h of hypoxia, although it may be recognized that many of the cells exposed to acute hypoxia may fluctuate through multiple hypoxic cycles.

In the last 15 years, both experimental and clinical studies have shown that hypoxia also plays an important role in tumor progression, affecting both metastatic spread and selection of cells with more aggressive phenotypes (11–13). These findings have led to a large effort to understand how cells respond to hypoxic exposure and, in particular, to define what changes in gene regulation and expression are induced by such exposure (14–16). These studies have shown that 1% to 2% of expressed genes can be affected by hypoxia but that many of these changes are cell type specific, so that although a general hypoxic gene signature that correlates with poorer treatment outcome has been defined (17), relevant signatures may vary from one cell type to another. Furthermore, nearly all of the experimental studies to date have exposed the cells to conditions of prolonged (~24 h) hypoxia; thus, they have not clearly addressed whether cells may respond differently to acute fluctuating hypoxia versus chronic hypoxia. Similarly, many of the clinical studies have used an oxygen electrode (Eppendorf polarographic oxygen electrode) to assess the level of hypoxia in the tumors and this technique does not discriminate between acute and chronic hypoxia.

Thus, a question has arisen about the relative importance of acute versus chronic hypoxia in promoting metastasis and tumor progression. It is this question that is addressed in the article by Rofstad et al. in this issue of *Clinical Cancer Research*. The authors studied metastases in two human melanoma xenografts (D-12 and R-18) that they have previously shown can be affected by hypoxia (18, 19). They estimated the fraction of chronically and acutely hypoxic

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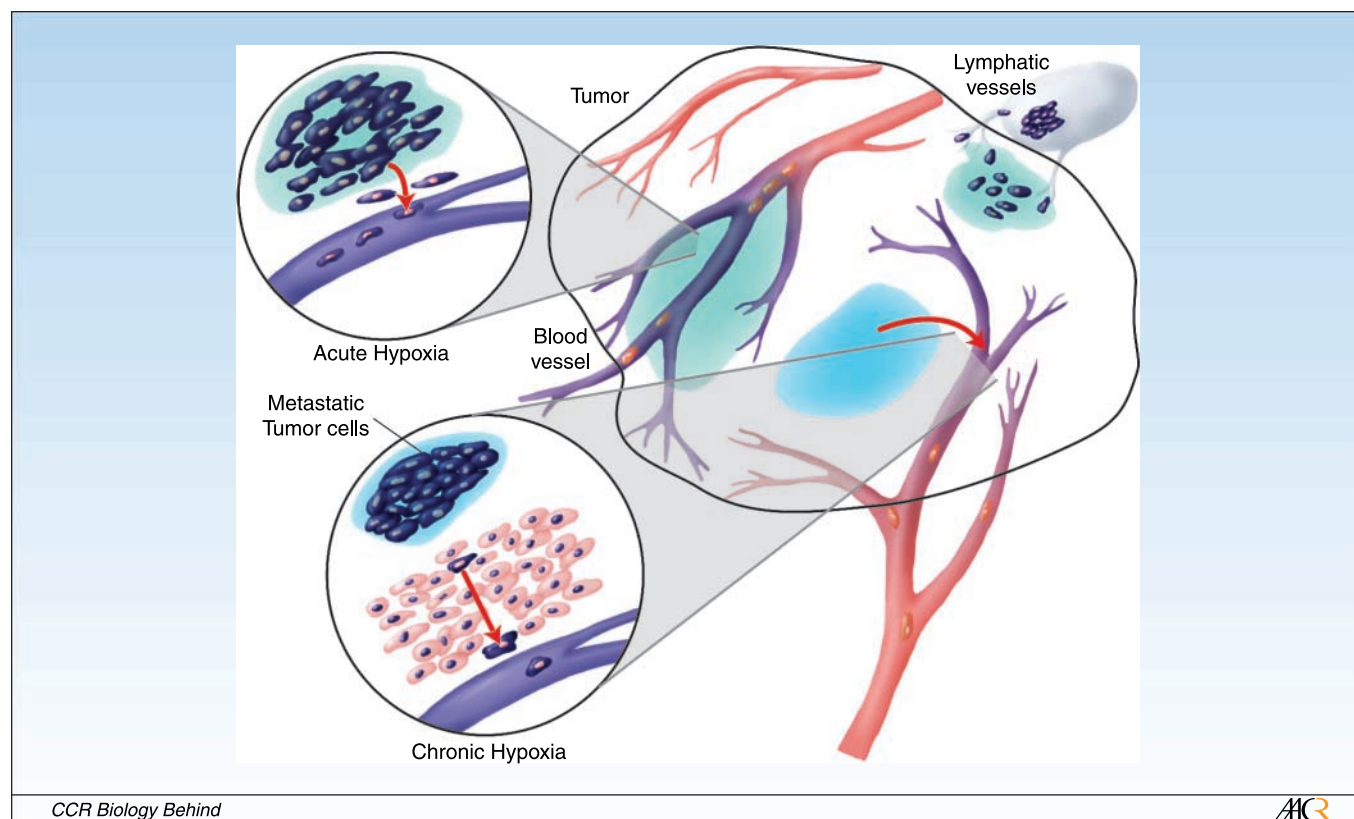


Fig. 1. Schematic illustration showing areas of acute and chronic hypoxia in the tumor. Acutely hypoxic regions are nearer the blood vessels. Tumor cells exposed to acute hypoxia have easier access to blood circulatory system and may therefore have greater likelihood of forming distant metastasis than cells in areas of chronic hypoxia that occur at a distance from the blood vessels. Lymphatics tend to occur only in the periphery of tumors; thus, regions of acute hypoxia in the tumor periphery may promote invasion/migration of the cells into lymphatics leading to increased lymph node metastasis.

cells in each tumor as described above and monitored the development of lung (D-12) or lymph node (R-18) metastases in each mouse. The results showed that the level of acute hypoxia and the level of chronic hypoxia were not correlated with each other in the individual tumors but that both were higher, on average, in tumors in mice that developed spontaneous lung or lymph node metastases. Furthermore, the difference in the level of acute hypoxia in tumors in mice with metastases versus those without metastases was greater than that for chronic hypoxia, suggesting a greater importance of acute hypoxia in driving the development of metastases.

The mechanisms that result in the increased metastatic potential of tumor cells exposed to hypoxia have not been fully defined but recent studies have shown a role for increased resistance to apoptosis and increased invasive capacity (20, 21). These findings are consistent with observations by Rofstad and colleagues that have linked increased levels of urokinase-type plasminogen receptor and interleukin-8 to hypoxia and metastasis in the R-18 and D-12 tumors, respectively (18, 19). Because acute hypoxia results from pronounced heterogeneity of the tumor blood flow distribution, whereas chronic hypoxia occurs at the limits of oxygen diffusion (100-200 μm) away from blood vessels, it is easy to suppose that cells exposed to acute hypoxia may have easier access to functional blood or lymphatic vessels (see Fig. 1). Furthermore, such cells probably also have a higher energy status than chronically hypoxic cells (4, 22, 23) and are also less likely to be exposed to other adverse

conditions associated with the tumor microenvironment (e.g., acid pH); hence, they may be better able to survive through the complex process of tumor metastasis (24). However, it remains unclear whether exposure to acute (fluctuating) hypoxia triggers the same changes in gene expression as are observed following exposure to chronic hypoxia.

The current study emphasizes the significance of fluctuating hypoxia rather than diffusion-limited hypoxia in terms of investigating hypoxia-related prognostic variables and treatment strategies. Further work to extend the findings to a wider range of tumor types is needed to determine its applicability beyond melanoma, although the results are consistent with studies in other tumor types in which deliberate exposure of tumor-bearing animals to cyclic hypoxia increases the development of metastasis (25, 26). In the context of the development of new drugs targeting hypoxic cells and metastasis, one can speculate that the finding that cells exposed to acute hypoxia may be more important in metastasis development could be good news because drugs should have easier access to the acutely hypoxic cells than chronically hypoxic cells that are at a distance from the vasculature. However, this gain may be mitigated by reduced toxicity if the drugs are not delivered effectively to the cells due to poor blood flow and/or are designed to be toxic only under severely hypoxic conditions. Overall, the findings argue that, in studies of the molecular basis of the effects of hypoxia on tumor progression and in related studies of drug development, more emphasis needs to be placed on the effects of fluctuating hypoxia.

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