Chapter 3
Hypoxia in Tumors: Pathogenesis-Related Classification, Characterization of Hypoxia Subtypes, and Associated Biological and Clinical Implications

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Abstract Hypoxia is a hallmark of tumors leading to (mal-)adaptive processes, development of aggressive phenotypes and treatment resistance. Based on underlying mechanisms and their duration, two main types of hypoxia have been identified, coexisting with complex spatial and temporal heterogeneities. Chronic hypoxia is mainly caused by diffusion limitations due to enlarged diffusion distances and adverse diffusion geometries (e.g., concurrent vs. countercurrent microvessels, Krogh- vs. Hill-type diffusion geometry) and, to a lesser extent, by hypoxemia (e.g., in anemic patients, HbCO formation in heavy smokers), and a compromised perfusion or flow stop (e.g., due to disturbed Starling forces or intratumor solid stress). Acute hypoxia mainly results from transient disruptions in perfusion (e.g., vascular occlusion by cell aggregates), fluctuating red blood cell fluxes or short-term contractions of the interstitial matrix. In each of these hypoxia subtypes oxygen supply is critically reduced, but perfusion-dependent nutrient supply, waste removal, delivery of anticancer or diagnostic agents, and repair competence can be impaired or may not be affected. This detailed differentiation of tumor hypoxia may impact on our understanding of tumor biology and may aid in the development of novel treatment strategies, tumor detection by imaging and tumor targeting, and is thus of great clinical relevance.

Keywords Tumor hypoxia • Acute hypoxia • Chronic hypoxia • Hypoxia subtypes • Hypoxia classification

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Dedicated to the memory of Dr. Britton Chance on the occasion of his 100th birthday (July 24, 2013), and remembering many exciting discussions on the inadequate and heterogeneous oxygenation of breast cancer.

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1 Introduction

Approximately 50–60 % of human tumors contain hypoxic regions which show complex spatial and temporal heterogeneities [1–3]. Tumor hypoxia is known to trigger (mal-)adaptive processes, increased tumor aggressiveness and resistance to O_2-dependent therapies (e.g., standard radiotherapy, some forms of chemotherapy, photodynamic therapy, immunotherapy and hormonal therapy), all together leading to a poor clinical prognosis [4–6]. Based on underlying mechanisms and their duration, two main types have been identified: chronic and acute hypoxia. This traditional classification is based on empirical observations and generally overlooks the multiple pathogenetic processes involved.

Chronic hypoxia is mainly caused by diffusion limitations [7], whereas acute hypoxia has been thought to preferentially result from temporary flow stops [8, 9]. In each of these hypoxia subtypes, oxygen supply is critically reduced, but perfusion-dependent delivery of diagnostic and therapeutic agents, supply of nutrients and removal of waste products, and repair competence can vary or may be unaffected [10]. Thus, detailed differentiation of tumor hypoxia may impact on our understanding of tumor biology and may aid in the development of novel treatment strategies (e.g., modulation of fractionation schedules), in tumor detection by imaging and tumor targeting, and is thus of upmost clinical importance far beyond an academic discussion. In this chapter, an updated review of the pathogenesis of hypoxia subtypes and their biological/clinical implications is therefore presented. Eventually, this updated classification of tumor hypoxia may also result in a better understanding of mismatches between perfusion and hypoxia in tumor imaging.

2 Chronic Hypoxia

According to a recent quantification of hypoxia in vital tumor tissue of xenografted squamous cell carcinomas of the head and neck, chronic hypoxia is the dominating subtype (mean = 77 % of total hypoxia [range: 65–86 %], total hypoxia covering 45 % of vital tumor tissue [range: 27–57 %]), with pronounced heterogeneity between individual tumors and between tumor lines [11].

Causes, the estimated time frame, major biological consequences, the therapeutic relevance and the prognostic power of chronic hypoxia are listed in Table 3.1. By definition, a reduced or abolished oxygen supply is inherent in each of these different pathogenetic mechanisms leading to chronic hypoxia. Perfusion- (convection-) dependent nutrient supply, waste removal, delivery of anticancer agents (e.g., chemotherapeutic drugs, antibodies or immune cells) or diagnostic agents for tumor imaging can be impaired or may not be affected, depending on the causative mechanism (Table 3.2).

Table 3.1 Causes and major consequences of chronic hypoxia (selection, updated from [10])

Synonyms used	Continuous h., diffusion-limited h., sustained h., long-term h.
Causes (pathogenesis)	1. Diffusion-limited hypoxia due to – Enlarged diffusion distances – Adverse diffusion geometries (concurrent vs. countercurrent tumor microvessels, Krogh- vs. Hill-type diffusion geometry) – Extreme longitudinal intravascular oxygen gradients – Shunt perfusion 2. Hypoxemic hypoxia – Tumor-associated anemia – Therapy-induced anemia – Small liver tumors supplied by portal vein – HbCO-formation in heavy smokers 3. Compromised perfusion of microvessels – Disturbed Starling forces due to high interstitial fluid pressure (transmural coupling) – Solid-phase stress by non-fluid components (compression) – Intratumor thrombosis
Time frame	Hours→weeks (under experimental conditions)
Major biological consequences	Slowing of proliferation rate, regressive changes (apoptosis, necrosis), HIF-destabilization
Therapeutic relevance	Resistance to O_2-dependent therapies (RX, O_2-dependent CX, photodynamic therapy, immunotherapy, hormonal therapy)
Prognostic power	Strong adverse and independent prognostic factor (e.g., for overall survival in cervix cancer, for local control in head and neck cancer)

Table 3.2 Subtypes of chronic hypoxia according to causative mechanisms and associated transport capacities via blood flow/convection and extravascular diffusion

Causes	Diffusion limitations	Hypoxemia	Interstitial hypertension or solid stress
Blood flow	Maintained	Maintained	Reduced/abolished[b]
Nutrient supply (e.g., glucose, glutamine), waste removal	Reduced/abolished[a]	Maintained	Reduced/abolished[b]
Delivery of growth factors etc.	Reduced/abolished[a]	Maintained	Reduced/abolished[b]
Delivery of anti-cancer agents	Reduced/abolished[a]	Maintained	Reduced/abolished[b]
Delivery of imaging agents	Reduced/abolished[a]	Maintained	Reduced/abolished[b]

[a]Dependent on distance away from tumor microvessel
[b]Pressure-dependent

3 Acute Hypoxia

Pathophysiologically speaking, acute hypoxia can be divided into two further subgroups: ischemic and hypoxemic hypoxia, the latter being characterized by plasma flow only (Table 3.3). In fluctuating or intermittent hypoxia caused by spontaneous fluctuations of red blood cell fluxes, hypoxia levels during the temporary

Table 3.3 Causes and major consequences of acute hypoxia (selection, updated from [10])

Synonyms used	Transient h., short-term h., perfusion-limited h., cyclic h., fluctuating h., intermittent h.
Causes (pathogenesis)	1. Temporary flow stop in microvessels – Due to tumor or blood cell aggregates, fibrin plugs – Ischemic hypoxia due to vascular remodeling 2. Transient hypoxemia – Temporal plasma flow in microvessels – Fluctuating red blood cell fluxes
Time frame	Minutes→hour (not well defined; spontaneous hypoxia cycles show spatial and temporal irregularities)
Major biological consequences	Leads to HIF-activation, cyclic reoxygenation episodes (ROS formation), increases genomic instability promoting tumor cell survival, selection of aggressive and apoptosis-resistant cell clones
Therapeutic relevance	Acquired treatment resistance via changes in the transcriptome, gene expression, proteome and genome
Prognostic power	Adverse prognostic factor

Table 3.4 Subtypes of acute hypoxia according to causative mechanisms and associated transport capacities via blood flow/convection and extravascular diffusion

Causes	Flow stop	Transient hypoxemia
Perfusion	Abolished	Maintained
Nutrient supply, waste removal	Abolished	Maintained
Delivery of growth factors	Abolished	Maintained
Delivery of anti-cancer agents	Abolished	Maintained
Delivery of imaging agents	Abolished	Maintained

drop of intravascular hematocrit are often not reached (e.g., [12]). In these cases, effects preferentially triggered by the formation of reactive oxygen species have to be considered. Ischemic hypoxia is preferentially caused by transient flow stops or critically reduced perfusion due to physical obstructions, such as by aggregates of tumor cells, blood cells or fibrin clots in the vessel lumen. In analogy with Table 3.2, relevant supply conditions are listed for subtypes of acute hypoxia in Table 3.4.

4 Hypoxia-Associated Biological and Clinical Implications

Major biological and clinical implications of chronic and acute hypoxia are listed in Tables 3.1, 3.2, 3.3, and 3.4. Unfortunately, there is no clear consensus on the biological and clinical consequences of the different hypoxia subtypes [13]. For example, there is opposing in vivo experimental evidence that chronic hypoxia can lead to both regressive changes and tumor progression depending on the cell line used, whereas for acute hypoxia there is a general trend towards development of an invasive phenotype. This is most likely due to changes in the transcriptome, gene expression,

proteome and metabolome (metabolic reprogramming). In addition, experimental evidence has demonstrated that in vitro acute hypoxia leads to genomic instability due to reduced DNA damage repair or due to the generation of reactive oxygen species during hypoxia-reoxygenation episodes (for reviews see [14, 15]). A number of in vivo experimental studies have verified that acute hypoxia can cause accelerated tumor growth and metastasis. Based on so far inconsistent data, there seems to be a general trend towards development of a more aggressive phenotype upon acute hypoxia than after chronic hypoxia. Responses following acute hypoxia most probably are driven by a transient stabilization of HIF-1α, possibly the most important factor for hypoxia-induced signaling, whereas the expression and activity of HIF-2α is often less prominent in cancer cells but can be strong in macrophages [16]. HIF-2α may not stimulate the glycolytic pathway [17].

5 Conclusions

Biological and therapeutic consequences seem to be different for "static," chronic hypoxia and "dynamic," acute hypoxia. Thus, a distinction between and quantification of these subtypes may be mandatory. Acute and chronic hypoxia have been convincingly described for the experimental/preclinical setting. Direct evidence in clinical (radio-)oncology has so far not been provided, mainly due to the lack of valid detection and quantification methods. Thus translation of experimental findings into the clinical practice urgently needs more advanced technologies (e.g., imaging techniques, use of valid modeling) before modifications in currently used radiotherapy regimens are implemented [13].

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