Progression of Lung Cancer: Role of Hypoxia and the Metabolic Tumor Microenvironment

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Contents

Abstract

Hypoxia and nutrient deprivation are frequently present in the microenvironment of solid tumors, like lung cancer. Poor perfusion due to aberrant tumor vessels and large diffusion distances, as well as high consumption (e.g., of glucose), are the underlying causes. In addition, lactate accumulates, creating an acidic tumor microenvironment. The cancer-promoting role of hypoxia and the underlying molecular mechanisms are quite well characterized: activation of angiogenesis via upregulation of vascular endothelial growth factor (VEGF), induction of apoptosis resistance, selection of resistant clones under severe hypoxia, and others. In contrast, the impact of nutrient deprivation and lactate accumulation on cancer progression and cancer cell metabolism are less well understood. In the present chapter, we summarize recent clinical and preclinical data on hypoxia

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and nutrient deprivation in cancer with special emphasis on lung cancer. The contribution of cofactors, like anemia, and the consequences for carcinogenesis and cell metabolism are discussed.

18.1 Lung Cancer

Lung cancer remains the leading cause of cancer deaths worldwide [1]. Lung cancer is often advanced at diagnosis, and 5-year survival among lung cancer patients is poor [1]. In advanced-stage lung cancer, platinum-based chemotherapy is the backbone of treatment [2, 3]. However, chemotherapy resistance, primary or acquired, is frequent [2, 3].

Histologic classification divides lung cancer into two major categories, non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC). NSCLCs include three major subtypes, adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma. Further sub-differentiation is performed according to oncogenic driver mutations, guiding targeted therapy, e.g., activating mutations of the epidermal growth factor receptor (EGFR) or chromosomal rearrangements, leading to echinoderm microtubule-associated protein-like 4 (EML)-anaplastic lymphoma kinase (ALK) fusion proteins [3].

18.2 The Metabolic Cancer Microenvironment

Nutrient and oxygen (O_2) deprivation is frequent in solid cancers, like lung cancer [4, 5]. Although angiogenesis is activated early in cancer growth, the newly formed vascular network is frequently aberrant, with leaky vessels and irregular blood flow [6]. In addition, cancers "outgrow" their supply by continuing proliferation and consumption of glucose and O_2 [6]. Cancer cells, therefore, face the challenge of limited and unreliable supply of O_2 and nutrients [7]. Hypoxia, nutrient limitation, and lactate accumulation all put environmental pressure on cancer cells. In the present chapter, recent clinical and preclinical data on hypoxia and nutrient deprivation in cancer with special emphasis on lung cancer are summarized, and the consequences of these microenvironmental factors for carcinogenesis and cell metabolism are discussed.

18.2.1 Necrosis: Crisis at the Border of Supply

With increasing distance from blood vessels, O_2 and glucose concentrations rapidly decline, often associated with the development of necrosis [4, 5, 8]. In lung cancer, necrosis resulting from nutrient and O_2 limitation is frequently present, especially in squamous cell carcinoma, but also in giant-cell carcinoma and small-cell lung cancer, and to a lesser extent also in adenocarcinoma [9]. In contrast, preinvasive lesions, like squamous dysplasia/carcinoma in situ (CIS) or atypical adenomatous

hyperplasia, do not contain necrosis [9]. Likewise, in bronchioloalveolar carcinoma, a form of adenocarcinoma in situ, necrosis is absent [9]. Since the limit of O_2 diffusion and the viable zone around microvessels have been shown to overlap in early studies, necrosis has been attributed primarily to critical hypoxia (anoxia), which does not permit cell survival (for review see [6]).

18.2.2 Hypoxia

18.2.2.1 Cause and Incidence of Hypoxia in Cancer

Hypoxia is caused by poor perfusion ("perfusion-limited hypoxia") and by the diffusion limit for O_2 ("diffusion-limited hypoxia") in solid cancers [6, 10]. Perfusion-limited hypoxia results from fluctuations in tumor microvessel oxygenation and/or perfusion, e.g. if vessels are temporarily closed [11]. Also reverse flow can occur. This fluctuating flow can result in transient hypoxia. If closed vessels are reperfused after re-opening, this may result in hypoxia-reperfusion injury of affected tumor tissue and microvascular endothelial cells [6]. In contrast, diffusion-limited hypoxia occurs in tumor areas located near the diffusion limit for O_2, which has been shown to be less than 200 μm [12]. Hypoxia is typically present in the vicinity of a necrosis; however, from a macroscopic point of view, hypoxic areas are heterogeneously distributed within a tumor [10]. Tumor hypoxia can be further enhanced by reduced O_2 transport in the blood, as in anemia (found in roughly 30% of patients at diagnosis) [10], or reduced blood oxygenation due to lung diseases [13] (Fig. 18.1).

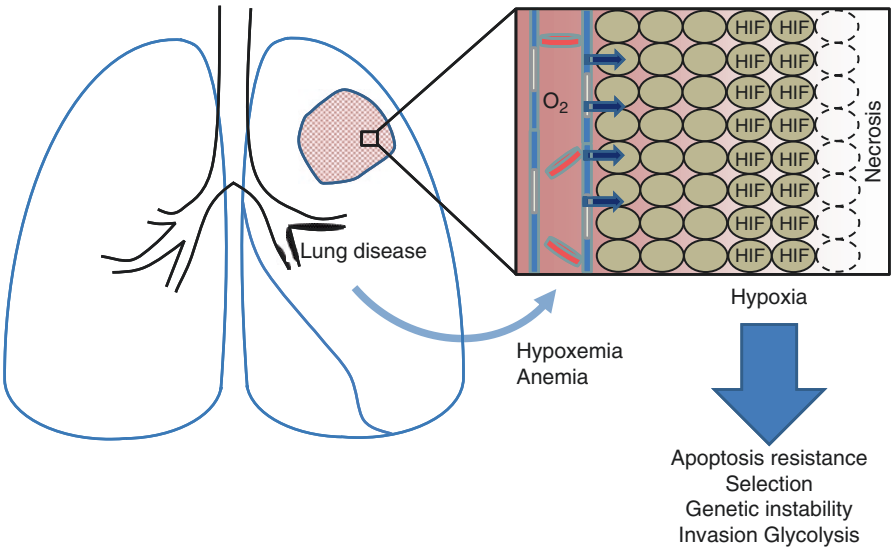

Fig. 18.1 Causes and consequences of hypoxia in lung cancer. The oxygen (O_2) supply of cancer cells is limited by diffusion, by irregular blood flow, and by an abnormal vascular architecture. Furthermore, anemia and lung diseases may reduce O_2 availability. *HIF* hypoxia-inducible factor

Direct O_2 measurements in cancers revealed that the mean O_2 levels are lower in cancers compared to corresponding normal tissues [8, 10]. The normal O_2 concentration is 80–100 mmHg in the blood and between approximately 25 and 70 mmHg in different normal tissues [8, 10]. Direct intraoperative O_2 measurements using polarographic electrodes in two studies on NSCLC patients revealed median tumor pO_2 values of 16.6 mmHg and 13.5 mmHg, respectively [14, 15]. The median tumor pO_2 was consistently lower than values from adjacent normal lung tissues [14].

Additional information on lung cancer oxygenation was obtained from studies using hypoxic radiotracers, e.g., [^{18}F]fluoromisonidazole ([^{18}F]FMISO) or [^{18}F]fluoroazomycin arabinoside, which allow the detection of hypoxia in vivo. [^{18}F]FMISO enters the cells by diffusion and is reduced by nitroreductase enzymes to form reduction products that bind to intracellular macromolecules when the oxygen tension is less than 10 mmHg and is then trapped intracellularly, allowing the determination of the hypoxic fraction in tumors in vivo (for review see [16]). In NSCLC, the mean hypoxic fraction (defined as proportion of pixels with elevated [^{18}F]FMISO signal, i.e., a tumor-to-blood ratio of >1.2 or >1.4.) was found to be variable, ranging from 1.3 to 94.7%. The median values were 48% and 58% in two different studies, respectively (for review see [16]). Overall, the oxygenation status in lung cancer varies from hypoxic to nearly normal; in general, hypoxia appears to be less pronounced than in, e.g., head and neck squamous cell carcinoma [15].

18.2.2.2 Role of Hypoxia in Cancer Progression and Therapy Resistance

Hypoxia exerts multiple effects in cancers, listed in Table 18.1. Importantly, it activates angiogenesis, enhances invasion and metastasis, and leads to radio- and chemotherapy resistance. Radio- and chemotherapy resistance caused by hypoxia has

Table 18.1 Effects of hypoxia in tumor biology

Effect	Mechanism
Selection of hypoxia-resistant clones	Cell death under severe hypoxia or hypoxia reoxygenation and selection of genotypes favoring survival
Suppression of apoptosis	Changes in gene expression, e.g., downregulation of proapoptotic Bid and Bax
Activation of autophagy	Changes in gene expression
Activation of glycolysis	HIF-induced overexpression of GLUT1, HK2, ENO1, and other glycolytic genes
Activation of angiogenesis	HIF-induced overexpression of VEGF, FLT1, ANG1, ANG2, and TIE2
Increased invasion and metastasis	HIF-induced overexpression of c-Met, CXCR4, RIOK3, and LOX
Attraction of tumor-associated macrophages	Increased expression of monocytic chemotactic proteins
Loss of genomic stability	Increased generation of reactive oxygen species
Decreased DNA repair	Downregulation of DNA repair pathways

HIF hypoxia-inducible factor. For reference see [17, 20]

different underlying mechanisms, but induction of apoptosis resistance is the best characterized one. The present view is that apoptosis resistance is either caused by selection of apoptosis-resistant clones or by suppression of apoptosis by alterations in gene expression [4, 17]. The latter seems to occur at rather mild hypoxia, while cell death and thus selection occur under severe hypoxia [4]. Apoptosis resistance under mild hypoxia (1% O_2) was shown to be reversible after 24–48 hours of reoxygenation and was associated with downregulation of Bcl-2-associated X protein (Bax) in NSCLC cell lines [18].

Patients with reduced blood oxygenation due to airway obstruction, e.g., patients with chronic obstructive pulmonary disease (COPD), a common smoking-related disorder, are at elevated risk of developing lung cancer [19]. When mice were subjected to intermittent hypobaric hypoxia (10% O_2) after lung cancer initiation with two different chemical carcinogens, a significantly increased tumor volume, but no increase in tumor frequency, was found [13]. Tumors from hypoxic mice showed increased proliferation and angiogenesis, and the pro-angiogenic growth factors VEGF and FGF were enhanced both in the lungs and tumors of hypoxic mice [13]. However, the exact role of lung diseases, such as COPD in lung carcinogenesis, is yet to be elucidated.

18.2.2.3 Hypoxia-Inducible Transcription Factors

Many of the responses to hypoxia are mediated by the transcription factors hypoxia-inducible factor 1α (HIF1α) and HIF2α, which dimerize with HIF1β and bind to hypoxia-response elements to induce expression of many genes [6]. HIF1α and HIF2α are constitutively expressed. Under normoxic conditions, they are constantly degraded by prolyl hydroxylases, which require O_2 as cofactor. Under hypoxia, HIF1α and HIF2α are stabilized. HIF1α and HIF2α can also be stabilized in a hypoxia-independent manner by growth factor-activated signaling cascades [6, 20].

HIFs play a role in the progression of lung cancer, but also other lung diseases, like pulmonary arterial hypertension and acute lung injury (for review see [21]). Both SCLC and NSCLC exhibit high levels of HIF1α and HIF2α, both of which are associated with poor prognosis [21]. A small molecule inhibitor of HIF1α, PX-478, was effective against tumor growth in an orthotopic mouse model of human lung cancer; however, silencing HIF1α in A549 lung adenocarcinoma cells impaired tumor vascularization and increased the necrotic area when grown as subcutaneous tumors, but did not reduce tumor cell proliferation and only slightly reduced tumor growth [21]. In contrast, reduction of HIF1α levels markedly impaired metastasis in murine models of human lung and mammary cancer [22].

18.2.3 Glucose Deprivation

18.2.3.1 Cause and Incidence of Glucose Deprivation in Cancer

Similar to oxygen, glucose levels decrease in underperfused tumor areas [5] (Fig. 18.2). In normal individuals, the average plasma glucose concentration is approximately 5.5 mM (100 mg/dL), ranging from approximately 3.2 mM (60 mg/dL) to approximately 7.8 mM (140 mg/dL) after meals [23]. In lung cancer,

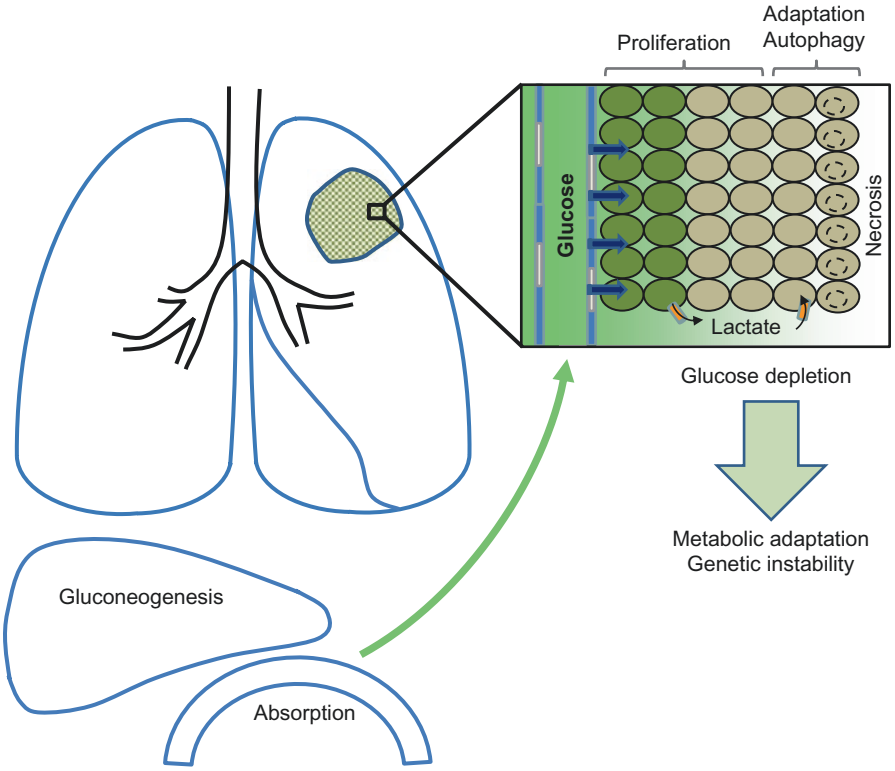

Fig. 18.2 Causes and consequences of glucose deprivation in lung cancer. The blood glucose is kept relatively constant by absorption from the intestine and by gluconeogenesis, which takes place mainly in the liver. Glucose is avidly consumed by cancer cells. High glucose consumption and reduced blood flow cause steep glucose gradients in solid cancers. In the low glucose microenvironment, alternative carbon sources and energy fuels, like lactate, are used by cancer cells, or autophagy is initiated. *Open circles* symbolize autophagic vacuoles

similar to other solid cancers, the glucose concentration is consistently lower than in corresponding normal lung tissue, as shown, e.g., by in vitro magnetic nuclear spectroscopy of excised tissues [24–26]. Similar results have been obtained in other solid human cancers [27–29]. The concentration of glucose was estimated to be 3–10 times lower in tumors compared to corresponding normal tissues [30].

Using imaging bioluminescence, which allows the histographical mapping of glucose and lactate concentrations in tissue sections at a high spatial resolution, glucose levels were shown to approach zero in the viable tumor area of some experimental tumors [31, 32]. At present, it is poorly understood how changes in blood glucose affect tumor glucose levels. However, when given intravenously, glucose is trapped intracellularly as glucose 6-phosphate in many cancers. This is the underlying mechanism of tumor imaging by ^{18}F-deoxyglucose positron-emission tomography (FDG-PET), a routinely used diagnostic imaging technique in the clinical staging of cancer patients [33].

Glucose deprivation in cancers is regarded as a consequence of high glucose consumption. In 1968, ascites fluid in the peritoneal cavity of mice inoculated with Ehrlich ascites tumor cells was shown to virtually lack glucose, in contrast to peritoneal fluid from non-inoculated mice [34]. This had been attributed to the high glycolytic activity of tumor cells [34]. When two different human cervical cancer cell lines were grown as subcutaneous tumors in SCID mice, xenografts formed from the cell line with higher glycolytic activity and OC316 displayed significantly reduced glucose levels compared to xenografts from the less glycolytic IGROV-1 cell line [35]. Similar to other aggressive cancers, invasive non-small cell lung cancers and small-cell lung cancers typically show a high maximum standardized uptake value (SUVmax) in FDG-PET, indicating high glucose uptake, while the noninvasive bronchioloalveolar carcinoma and seldom metastasizing typical carcinoid tumors generally show low uptake of FDG [9, 36].

In cancer cells, glucose is metabolized primarily by glycolysis [37–40]. This "aerobic glycolysis," described by the Nobel laureate Otto Heinrich Warburg as early as 1924, is observed in cancer cells but also in other highly proliferative cells and ensures the generation of building blocks for cell growth. It enhances flux of glucose to glycolytic metabolites and further along to the oxidative and non-oxidative branches of the pentose phosphate pathway (PPP), thus providing NADPH (reduced nicotinamide adenine dinucleotide phosphate) and ribose [37–40]. Furthermore, glucose is diverted to the synthesis of glycerol, serine, and hexosamines [41, 42].

Low availability of glucose dramatically reduces the flux via glycolysis. The fact that glucose consumption decreases with reduced glucose availability in tumor tissue was already observed by Warburg [43]. However, since glucose was regarded as a major fuel for cancer cells, the mechanisms of adaptation of cancer cells to glucose deprivation and a possible contribution of a glucose-deprived microenvironment to carcinogenesis have long been neglected.

18.2.3.2 Impact of Glucose Deprivation on Carcinogenesis

It has been suggested that glucose deprivation, on the one hand, and hypoxia, on the other hand, select for particular genetic abnormalities in cancer cells [44]. In a study by Yun et al. [44], colon cancer cell lines that survived glucose limitation (0.5 mM) have been shown to possess activating mutations in the gene encoding KRAS (4.4% of the clones) or BRAF (0.8% of the clones). In contrast, no KRAS or BRAF mutations were identified in clones generated in the presence of high (25 mM) concentrations of glucose. When cells with mutant KRAS or BRAF alleles were mixed with an excess of cells containing wild-type KRAS or BRAF alleles, respectively, and were incubated in either low (0.5 mM) or high (25 mM) concentrations of glucose, cells with mutant KRAS or BRAF alleles overtook the population in low-glucose conditions, but not in high-glucose conditions [44].

In a study published by Schlappack et al. [45], murine cancer cells formed a 5 to more than 20 times higher number of lung metastases after injection into mouse veins after exposure to glucose starvation (0 mM) for 48 h. Exposure of cells to low pH also had a metastasis promoting effect in that study. This suggests that glucose deprivation may enhance metastasis formation in some cancers. However, further studies are warranted to clarify the role of glucose depletion in cancer progression.

Only a limited number of studies assessed the correlation between glucose deprivation and tumor aggressiveness. Higher grade breast cancers exhibited lower glucose levels compared to lower grade breast cancer [46]. In contrast, the glucose levels in human cervical cancer xenografts from two different cell lines either forming spontaneous metastases or not forming spontaneous metastases were not significantly different [47]. In this study, however, metastasis formation was associated with increased lactate levels in the primary tumor, which correlated with the hypoxic fraction [47]. To the best of our knowledge, no studies on the relation between glucose levels in lung cancer tissue and survival have been published.

18.2.3.3 Survival of Cancer Cells Under Low Glucose

How cancer cells, which are reprogrammed to utilize high amounts of glucose, adapt to a decline in extracellular glucose levels is poorly understood. Recent studies show that cancer cell metabolism is more complex and intricate than previously thought and that metabolic flexibility allows cells to survive conditions of nutrient shortage [48]. Using a small interfering RNA (siRNA) screen, it has been found that cancer cell lines are dependent on respiratory chain proteins for survival and growth under chronically reduced glucose conditions (0.75 mM glucose) [30]. On the other hand, storage of glucose in the form of glycogen, which is activated by hypoxia, was shown to protect cancer cells from acute glucose deprivation [49].

Alternative fuels are utilized by cancer cells for biomass and energy production under glucose starvation, e.g., acetate, fatty acids, and amino acids. This is accomplished by altered expression of central metabolic enzymes but also by enhanced expression of membrane transporters (for review see [48]). Metabolic adaptation in tumors also involves metabolic symbiosis between cancer cells in different tumor compartments or between cancer cells and stroma cells, which excrete metabolites used by cancer cells (for review see [50, 51]). However, the use of alternative carbon sources for the generation of glycolysis-derived metabolites, like ribose (for DNA synthesis) and glycerol, would require the action of a gluconeogenesis enzyme, phosphoenolpyruvate carboxykinase (PEPCK) [52, 53]. We have recently shown that the mitochondrial isoform, of PEPCK and PCK2, is expressed and active in lung cancers, mediating the conversion of lactate into the glycolytic/gluconeogenic intermediate phosphoenolpyruvate under glucose deprivation [54]. Subsequently, PCK2 has also been found to play a role in the survival of other cancer cell lines and to be activated by endoplasmic reticulum stress and glutamine deprivation [55]. Silencing of PCK2 using shRNA led to significantly decreased growth of lung cancer cell xenografts in vivo [56].

Autophagy is a tightly regulated pro-survival pathway that captures, degrades, and recycles intracellular proteins and organelles in lysosomes [57, 59]. It involves the action of specific autophagy-executing proteins, e.g., microtubule-associated protein 1 light chain 3 (MAP1LC3, best known as LC3). Nutrient depletion is the most potent known physiological inducer of autophagy. Acute autophagy induction is critical for mammalian cells but also for yeast cells to survive nutrient depletion, which is attributed to the recycling of intracellular components into metabolic

pathways. However, the exact substrates that are degraded by autophagy and the metabolic pathways supported remain to be identified [57–59]. In cancer, autophagy has both tumor-suppressing and tumor-promoting functions. Healthy cells are thought to be protected from malignant transformation by autophagic responses, and carcinogenesis may involve a temporary loss in autophagy competence. Conversely, autophagy promotes tumor progression and therapy resistance in a variety of models [57, 59].

The concept that glucose deprivation induces autophagy which promotes survival was challenged by a report showing that glucose deprivation did not induce autophagy in four different cancer cell lines, and autophagy inhibition did not alter apoptosis and necrosis induction by low glucose [60]. In some cancer models, the p53 status switches the role of autophagy during tumor development. Mutant p53 ameliorated the inhibition of tumor growth by autophagy inhibition in some models, including a genetically engineered lung cancer model with lung-specific expression of mutant Kirsten rat sarcoma (KRAS), making tumors less autophagy dependent, but not in other models [57]. Thus, the protective role of autophagy under glucose starvation in cancers may depend on their genetic background or on other unknown factors like availability of alternative carbon and energy sources.

18.2.4 Lactate

Lactate, the glycolysis end product, is known to accumulate in cancers [61, 62]. It is produced primarily via glycolysis and exported into the extracellular space by monocarboxylate transporters, most importantly MCT4 [63]. Lactate has been shown to exert multiple effects on cancer cells, mostly by reducing the pH, including enhancement of invasion and metastasis, induction of apoptosis resistance, and others (for review see [63]). Elevated lactate levels were shown to correlate with poor prognosis and poor disease-free survival in several epithelial cancers, such as cervical, head and neck, NSCLC, and breast cancers [63].

Lactate is not only a metabolic waste product but may be consumed by cancer cells, especially under low glucose concentrations. This phenomenon was described in SiHa cervix squamous cell carcinoma cancer cells [64] and p53−/− HCT116 colon carcinoma cells [65]. We have shown that this is also an important mechanism in lung cancer cells [54]. Lactate is transported into the cell via a bidirectional transporter and monocarboxylate transporter 1 (MCT1) [66] and oxidized to pyruvate via lactate dehydrogenase [64], the same enzyme that catalyzes the reverse reaction during glycolysis. Thereafter, lactate may be converted to acetyl-CoA and serve as an energy fuel [64, 65] or feed into biosynthetic pathways. Due to the important role of MCTs in regulating local lactate accumulation and use, inhibitors of MCTs have been considered as anticancer therapeutic drugs. AZD3965, an MCT1 inhibitor, reduced SCLC tumor growth in a mouse model in vivo [67]. Clinical trials with AZD3965 in different cancers are on the way [67] (www.clinicaltrials.gov).

18.2.5 Therapeutic Strategies Targeting Hypoxic and Metabolic Adaptation in Lung Cancer

Therapeutic approaches to target the cancer stroma or cancer metabolism or to affect the metabolic microenvironment are intensively studied. A detailed discussion is beyond the scope of this chapter. Briefly, targeting metabolic tumor cell vulnerabilities present in rapidly growing tumors [48] or tumor cell vulnerabilities induced by anti-angiogenic therapies (e.g., activation of HIF) is under preclinical evaluation [20]. Furthermore, the use of hypoxia-activated prodrugs and HIF1α inhibitors in hypoxic cancers, like lung cancer, is a promising approach [20]. Cancer hypoxia, assessed by novel hypoxic tracers, is increasingly taken into account in the planning of radiotherapy [68]. On the other hand, tumor hypoxia may be potentially predictive for the efficacy of anti-angiogenic therapies, which is currently investigated in clinical studies [20]. Thus, analysis of the metabolic cancer microenvironment might not only help to uncover metabolic vulnerabilities in cancer cells but may also help in the clinical routine to predict response to therapy.

In summary, there is a remarkable heterogeneity among different tumors and within single tumors with respect to the access to O_2, glucose, and maybe other essential substances. Cancer cells use specific enzymes like PEPCK allowing them to make use of fuels like lactate under glucose-deprived conditions. However, this makes them also vulnerable to specific approaches using such enzymes as therapeutic target. Cancer cells may use hypoxia-induced metabolic changes to attain resistance to chemotherapy. The investigation of the basic mechanisms in vivo, but also in well-defined in vitro models, mimicking the in vivo situation, will be essential for future research.

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