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Hypoxia as a Cause of Treatment Failure in Non–Small Cell Carcinoma of the Lung

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Hypoxia is an important factor in tumor biology and is both a predictive and a prognostic factor in non–small cell lung cancer. The negative effect of low oxygenation on radiation therapy effect has been known for decades, but more recent research has emphasized that hypoxia also has a profound effect on a tumor's aggression and metastatic propensity. In this review, current knowledge on both these aspects of treatment failure in NSCLC due to hypoxia has been discussed, along with a presentation of modern methods for hypoxia measurement and current therapeutical interventions to circumvent the negative effect of hypoxia on treatment results.

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Hypoxia has been recognized as an important factor in tumor biology and therapy response since the first half of the 1900s, and hypoxia-mediated radiation resistance was first described by Gray et al[1] in 1953. Well-oxygenated tumors respond better to various therapies than hypoxic tumors do. Therefore, hypoxia is a predictive factor. However, more recently, emerging knowledge has underscored that hypoxia is indeed also a prognostic factor, independently of therapy, and that hypoxia in a tumor's microenvironment induces a more aggressive tumor phenotype.

A number of studies have suggested that hypoxia is highly prevalent in non–small cell lung cancer (NSCLC), based both on indirect measurements using hypoxia-dependent positron emission tomography (PET) tracers and on direct measurement of intratumoral oxygenation.[2,3] The exact prevalence is still not defined, because of both heterogeneous temporospatial oxygen distribution and different measurement methods and cut-off levels.

In this review, the factors involved in hypoxia-mediated therapeutic failures of NSCLC have been discussed in the context of therapy resistance and as a tumor biology phenomenon per se.

Measurement of Hypoxia

Direct tissue oxygenation measurement using an electrode such as the Eppendorf "histograph" should be regarded as the gold standard for hypoxia evaluation.[4] Such studies have shown that nonneoplastic tissues, with a few exceptions, are well oxygenated, with a pO_2 of more than 12.5 mm Hg. The oxygen concentration in arterial and venous blood is in the ranges of 75-100 and 30-40 mm Hg, respectively. Most tumors (including lung cancer) have a low pO_2 of 0-7.5 mm Hg.[5] Notably, tumor oxygenation is very heterogenous, both in time and space.

Invasive methods are of no practical use for evaluation of hypoxia in clinical lung cancer settings, even though this method has been employed intraoperatively on lung tumors.[2] Indirect measurements in lung tumors, using hypoxia tracers such as 18F-fluoroazomycin-arabinoside[3] (which bind only when pO_2 is less than 10 mm Hg) and 18F-HX4, 18F-fluoromisonidazole PET imaging, or magnetic resonance–based techniques such as blood oxygen level–dependent or dynamic contrast-enhanced methods, are more applicable in routine practice. Interestingly, Trinkaus and coworkers, using 18F-fluoroazomycin-arabinoside-PET, showed intratumoral hypoxia to be present in as many as 65% of NSCLC tumors evaluated pretreatment. After concomitant chemoradiation, normal oxygenation levels were found in most patients.[3] However, because of the heterogeneous distribution and

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instability over time of hypoxia, such indirect methods are limited by a lack of resolution and provide no information on temporal fluctuations. Repeated scans during the radiotherapy course could be a way to overcome this. Horsman et al[6] have provided a recent comprehensive review of hypoxia imaging techniques and their potential role in radiation planning.[6] They underscore the potential predictive value in noninvasive quantification of hypoxia but also admit that more clinical trials have to be performed to firmly prove the clinical value of reversing hypoxia or using hypoxia levels for dose-delivery guidance in radiation therapy.

Markers of hypoxia in tissues include pimonidazole, which accumulates in hypoxic regions and can be administered intravenously before carrying out a biopsy or surgical resection. High concordance has been shown between preoperative computed tomography parameters and hypoxia measured by pimonidazole staining in corresponding resected lung cancer tissue.[7] Other markers include immunostaining of the endogenous hypoxia-inducible factor (HIF) family of proteins and their transcriptional targets.[8] Prediction of tumor hypoxia may also now be based on gene signatures, as exemplified in studies on laryngeal cancer.[9]

Causes of Hypoxia

Oxygen molecules diffuse freely in normal tissues, with a diffusion range of up to 200 μm. In normal tissues, this range is sufficient to oxygenate all cells, owing to a dense network of capillaries. However, all solid tumors larger than 1 cm^3 contain hypoxic regions because of a number of factors: abnormal microvessel structure and function leading to increased diffusion distance from vessel to cell, increased oxygen demand because of increased cellular proliferation, reduced oxygen supply because of vascular constriction, and increased interstitial pressure, partly because of abnormal leaky vessels and resulting edema. Anemia and smoking, both frequently associated with patients with lung cancer, add to the reduced oxygen supply.[5] Based on this, the diffusion range of oxygen in tumor tissues may be much lower than in normal tissues, and even cells adjacent to blood vessels can be hypoxic. Interestingly, the presence of hypoxia does not correlate with tumor volume or metabolically active volume, implying that hypoxia is present in small and large tumors and is not an effect of size *per se*.[3]

Chronic vs Acute Hypoxia

Oxygen levels gradually decrease by distance from microcapillaries, and hypoxia is typically seen at 100-180 μm from the blood vessel. This effect leads to chronically hypoxic cells in this sector. However, acute hypoxia because of transient perfusion changes is also observed in tissues, and fluctuating blood flow is frequently observed in tumor tissues.[10] In acute hypoxia, the supply of other nutrients also tends to be reduced, leading to a potentially higher degree of therapy resistance than in chronic hypoxia, where supply deprivation is mainly confined to oxygen.

Experimental evidence exists of reduced expression of DNA repair genes in chronic hypoxia, but not in acute hypoxia, leading to a higher radiosensitivity in chronic hypoxic cells than in cells exposed to acute hypoxia.[11]

Hypoxia and Treatment Failure

Hypoxia may be responsible for treatment failure through 2 main mechanisms: (1) a treatment-related effect owing to reduced DNA damage and (2) an at least partially treatment-independent effect through upregulation of a number of factors, leading to a more aggressive tumor biology. The former has been known for decades, and an array of therapy-modulating perturbations have been tried. The latter, however, is more recently acknowledged, and therapies seeking to exploit these phenomena have just recently been introduced. Tumor hypoxia as a prognostic factor, or predictive factor in radiation therapy, has not been as extensively evaluated in NSCLC as in others such as head and neck cancers. Still, several studies confirm the detrimental effect also in NSCLC.[12-14]

Direct Influence of Hypoxia on Radiation Effect

Heavily charged ion beams induce cell death via direct DNA damage, but other radiation modalities, including protons and photons, kill mainly indirectly via production of free radicals (reactive oxygen species) that bind to DNA and induce strand breaks. These free radicals are produced either directly in the DNA or more commonly through reactions with water. Oxygen stabilizes the chemical bond breaks in DNA and makes the damage permanent or "fixed." Therefore, in the absence of oxygen, DNA is less vulnerable to permanent damage, leading to relative radioresistance.[15] It has been shown that radiation dose has to be increased by 2- to 3-fold to induce the same cell kill in a hypoxic milieus relative to aerobic settings.[5] However, it is important to underscore that the exact mechanisms behind this "oxygen effect" are still not completely understood, 60 years after the first observation of its existence.

Interestingly, mathematical modeling of various fractionation schemes for optimization of tumor control probability (TCP), taking into account hypoxia and reoxygenation as well as cell proliferation and nutrient supply, outputs an optimal daily dose of approximately 2 Gy. In this model, hypoxia predicts a dose increase requirement of approximately 30% to achieve similar TCP as seen under normoxic conditions.[16]

"Dose painting", using information from hypoxia imaging to guide extra dosing to hypoxic tumor regions, has been suggested as a method to overcome hypoxia-induced radioresistance in lung cancer.[17] However, promising current evidence for such heterogenous dose delivery is scarce, regarding both the dose levels potentially needed and the imaging limitations, as mentioned earlier.[6]

For extremely hypofractionated regimens, such as stereotactic ablative radiotherapy (SABR) of lung tumors, classical radiobiology laws may not be applicable.[18] Conventional wisdom points to the effect of reoxygenation of hypoxic regions as a major principle for the superiority of fractionated regimens over hypofractionated ones. However, arguments exist that the linear quadratic model is valid for SABR doses as for 2-Gy fractions, as long as the hypoxia factor is taken into account.[19] Hypoxia is also thought to contribute to the surprisingly (based on simple linear quadratic model calculations) high biologically effective dose needed to achieve sufficient TCP in SABR, as in lung cancer, where biologically effective dose less than 100 Gy leads to relatively high relapse rates.[20] Moreover, this implies that hypoxic radiosensitizers may also potentiate the effect of very high doses. Notably, ablative doses greater than 9 Gy facilitate cancer cell death via mechanisms not seen with conventionally fractionated regimens, such as acute vascular damage, thus modifying the effect of hypoxia.[21,22]

Hypoxia-Inducible Factor

HIF-1 is an intracellular protein whose transcriptional activity is increased in response to various cellular stresses, including hypoxia.[23,24] HIF-1 consists of a labile unit (HIF-1α) and a stable unit (HIF-1β), which heterodimerize to be transcriptionally active. In normoxia, HIF-1α undergoes proteolysis induced by hydroxylation of the oxygen-dependent degradation (ODD) domain by the prolyl-4-hydroxylase domain family of proteins which are activated in the presence of molecular oxygen. Following ODD hydroxylation, ubiquitination via the von Hippel-Lindau complex occurs, resulting in a very low level of HIF heterodimers in aerobic conditions.[25] In hypoxia, degradation of the α-unit is reduced, leading to an increased level of the functional heterodimer, which via binding to hypoxia response elements induces expression of a multitude of genes. Notably, in extreme hypoxia or anoxia, HIF-1α level is again low, probably because of lack of glucose, which is required for the stabilization of the α-subunit.[26] HIF-1 is also regulated by other factors apart from or in concert with molecular oxygen, including oncogenes, free radicals, and growth factors, which may act via interaction with heat shock protein 90 or phosphorylation.[27,28] For instance, in certain hypoxic experimental settings, free radicals are required to stabilize HIF-1α, probably via cytochrome c–mediated electron shuttling.[29] Furthermore, nitric oxide (NO) may also affect HIF-1α degradation via modification of the ODD and redistribution of oxygen from mitochondria to the cytosol.[30] In irradiated tumors, the protein kinase B/mammalian target of rapamycin pathway is shown to regulate the HIF-1 level.[31] In addition, the NOTCH pathway is activated in hypoxic NSCLC and induces radioresistance. High NOTCH expression correlates with poor prognosis, and preclinical inhibition of NOTCH indicates a therapeutic potential.[32] Additionally, cycling hypoxia (recurrent acute hypoxia) results in the upregulation of HIF-1 to a level above what is seen in chronic hypoxia.[33] Finally, long noncoding RNAs are also involved in HIF-1 regulation in lung cancer.[34] In fact, underscoring the complexity of HIF regulation, several studies show no correlation between HIF-1 level, or level of HIF-1-regulated proteins, and grade of hypoxia in tissues, measured by agents such as pimonidazol.[35]

Hypoxia response elements are found in the promoter or enhancement regions of various families of genes involved in anaerobic metabolism,[36] angiogenesis,[37-39] antiapoptosis,[40] and invasion and metastasis.[41] Thus, on hypoxia-mediated HIF-1 stabilization, a number of pathways are activated that are involved in radioresistance, but which also are responsible for an aggressive phenotype. One of the key downstream factors upregulated by HIF-1 is miR-210, which is involved in a multitude of hypoxia pathways[42] and also found to be of prognostic relevance in lung cancer.[43]

Lysyl oxidase (LOX) is upregulated in hypoxia via HIF-1 and has also been shown to be an independent prognostic marker in lung cancer.[44,45] LOX exerts its effect locally by modifying the tumor microenvironment by cross-linking of matrix proteins and by stimulating migration and invasive behavior.[46] Furthermore, hypoxia-induced secreted LOX can act far away from its secretory origin, preparing the metastatic niche by recruiting bone marrow cells and stimulating endothelial cells to support establishment of distant metastases.[47,48] Blockade of LOX has experimentally been shown to reduce the metastatic propensity of tumors, implying that LOX can serve as a target for metastasis-preventive therapy.[47]

HIF-1–mediated signaling regulates virtually every step of the metastatic cascade, from migration toward blood vessels to intravasation through HIF-induced leaky endothelial cells. Further, HIF-1 inhibits anoikis of circulating tumor cells, and hypoxic primary tumors secrete factors that permeabilize the endothelium at distant premetastatic sites. Finally, as mentioned previously, secreted LOX may have prepared the metastatic "soil" in the distant organ. For a detailed overview, refer to the study by De Bock et al.[49] Adding to the complexity, every element of the stromal compartment is also influenced by hypoxia, including fibroblasts, immune, lymph, and blood cells, each playing an important role in tumor progression. These aspects have been recently reviewed by Casazza et al.[50]

It is of special interest in lung cancer epidermal growth factor receptor (EGFR) is involved in several aspects of hypoxia. Recently, hypoxia was shown to stimulate invasion via invadopodia formation by histone deacetylase–mediated EGFR activation.[51] Furthermore, hypoxia may induce EGFR activity via reduced endocytosis, which also may influence other membrane-bound growth-stimulating, and thereby cancer-promoting, proteins.[52] Resistance to gefitinib, extensively used in EGFR-mutated lung cancer, is also induced by hypoxia via upregulation of insulinlike growth factor.[53] Finally, EGFR has been shown to suppress specific tumor-suppressing microRNAs in response to hypoxic stress through posttranslational regulation of Dicer regulator AGO2.[54]

HIF and Radiation Therapy

A number of HIF-1–upregulated genes contribute to radioresistance, perhaps most important is the shift from glucose

metabolism to a glycolytic phenotype, which was recently reviewed by Meijer et al.[55] This effect increases the cell's antioxidant capacity via accumulation of redox buffers such as NADH/NAD^+ and glutathione, and thereby reduces the level of free oxygen radicals produced by radiation, thus protecting the DNA from damage.

Furthermore, accumulation of lactate acid because of glycolysis induces stromal proteinases, thereby facilitating tumor cell migration, inhibits immune cell activity and stimulates tumor-promoting macrophages, and induces angiogenesis, all contributing to an aggressive phenotype of lung tumors.[55,56]

Chronic hypoxic tumor cells can be categorized depending on the HIF-1 level and the degree of pimonidazole staining. Recently, dynamic in vivo studies have shown that HIF-1-negative or pimonidazole-positive cells are more radioresistant than other cells and that the HIF-1 level in these surviving cells increases in response to radiation-induced reoxygenation. Furthermore, cells acquiring HIF-1 secrete vascular endothelial growth factor, resulting in vascular protection, and migrate toward blood vessels, resulting in a metastatic phenotype.[26] Interestingly, the grade of hypoxia in preirradiated lung tumors may also be visualized using radiolabeled 2-nitroimidazoles, such as 18F-HX4-PET/computed tomography, and has recently shown to be of potential value in discriminating tumor areas for increased dosing.[57]

Hypoxic tumors reoxygenate after radiation therapy, owing to reduced demand because of cell death and increased perfusion in tissues.[58] Based on this, one would expect HIF-1α levels to decline after radiation, but the opposite is observed. This phenomenon is primarily caused by (1) increased level of free radicals and (2) liberation of "stress granula" content, both leading to stabilization of the HIF-1α subunit.[59] The initial HIF-1 increase occurs within hours of radiation. A few days thereafter, increased NO produced by infiltrating macrophages induces a second peak of HIF-1 stabilization, via NO-mediated prevention of HIF degradation through nitrosylation of a cystein residue in the ODD.[60] Both the initial and the later increase of HIF levels may contribute to a more aggressive phenotype and ultimately to treatment failure as cells become more prone to invasion and metastasis.

Counteracting Hypoxia

Several hypoxia sensitizers are currently in clinical trials, but so far, none are in routine use in lung cancer.[61] Notably, a number of studies on radiation therapy combined with various hypoxia-directed therapies, have been conducted for NSCLC, including those assessing carbogen,[62] tirapazamine, (a cytotoxin selectively targeting hypoxic cells),[63,64] and angiogenesis-directed therapies, such as anti-vascular endothelial growth factor, endostatin, or thalidomide.[65,66] The results have been mainly disappointing, producing significant toxicity but insignificant survival gains. However, it should be noted that most, if not all, of these studies included patients regardless of the presence of tumor hypoxia. The results may have been different had tumor hypoxia been an inclusion criteria.

The most promising strategies today might be HIF-1 inhibitors and drugs targeting glucose metabolism, which should be further examined in the context of radiation therapy in patients with hypoxic tumors.[55] These studies should not only be confined to fractionated therapy but may likely also have a positive effect on SABR.

Heavy charged particle radiotherapy acts independently of oxygen, as mentioned earlier, but emerging evidence points to the involvement of the HIF-1 signaling pathway also in this modality. In contrast to what is seen with photon radiation, induction of HIF-1 is not seen with carbon ion therapy; on the contrary, a significant downregulation was seen in a NSCLC model. Furthermore, the protein kinase B/mammalian target of rapamycin pathway, presumably inducing angiogenesis, was also not affected by carbon ions, in contrast to the inducing effect of photons.[67] Thus, heavy charged particle irradiation is a promising strategy also in lung cancer.

Conclusion

In conclusion, several lines of evidence point to tumor hypoxia as a major cause of therapy failure and tumor aggression in NSCLC involving a multitude of factors. As knowledge emerges, it is evident that the relatively simple "oxygen effect" attributed to radioresistance in hypoxic tumors is not the sole cause of treatment failures. Given the still dismal prognosis of NSCLC, further research into possible strategies to circumvent the negative effect of hypoxia is highly warranted. Despite an increasing recognition of the amazingly complex biology of this phenomenon, the opportunities of therapeutic interventions are also ample.

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