Cancer Metastasis Rev (2007) 26:241–248
DOI 10.1007/s10555-007-9056-0

Hypoxia and radiotherapy: opportunities for improved outcomes in cancer treatment

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Published online: 14 April 2007

Abstract A large body of clinical evidence exists to suggest that tumor hypoxia negatively impacts radiotherapy. As a result, there has been longstanding active research into novel methods of improving tumor oxygenation, targeting hypoxic tumor cells, and otherwise modulating the effect hypoxia has on how tumors respond to radiation. Over time, as more has been learned about the many ways hypoxia affects tumors, our understanding of the mechanisms connecting hypoxia to radiosensitivity has become increasingly broad and complicated. This has opened up new potential avenues for interrupting hypoxia's negative effects on tumor radiosensitivity. Here, we will review what is currently known about the spectrum of influence hypoxia has over the way tumors respond to radiation. Particular focus will be placed on recent discoveries suggesting that hypoxia-inducible factor-1 (HIF-1), a transcription factor that upregulates its target genes under hypoxic conditions, plays a major role in determining tumor radiosensitivity. HIF-1 and/or its target genes may represent therapeutic targets which could be manipulated to influence hypoxia's impact on tumor radiosensitivity.

Keywords Hypoxia · Cancer · Radiation · HIF-1

1 Hypoxia and clinical tumor radiosensitivity

Dozens of clinical trials have been published demonstrating a significant direct relationship between tumor hypoxia and poor clinical outcomes after radiotherapy. The best-studied data come from trials utilizing the Eppendorf probe, a polarographic microelectrode which can accurately measure microregional tissue oxygen pressures. Passed through skin like a fine needle, these electrodes can be used to make several readings in a single sitting of superficial tumors in the clinic. In this way, investigators have identified tumors as well-or poorly-oxygenated, and then correlated oxygenation with local control, disease-free survival, and overall survival after radiotherapy. Tables 1 and 2 summarize the major studies published to date demonstrating the poor prognostic value of hypoxia for irradiated head & neck carcinomas, cervical carcinomas, and soft tissue sarcomas.

Clearly, these studies show that tumor hypoxia is correlated strongly with radioresistance. That hypoxia *causes* radioresistance is impossible to prove in the clinic, but this leap of faith is accepted by most. Causation seems likely partly due to the fact that hypoxia has been shown by many of the aforementioned studies to be an independent predictor of poor clinical outcomes. Moreover, a huge body of preclinical evidence shows that experimentally manipulating tumor oxygenation impacts radiosensitivity. The remainder of this article will focus on the importance of these preclinical data, their historical impact on the field, and their potential promise for improved radiotherapeutic outcomes in the future.

2 The oxygen enhancement effect

Historically, our understanding of the mechanism linking hypoxia to tumor radioresistance has been dominated by a physiochemical principle known as the oxygen enhancement effect. Damage to DNA is created by direct ionization

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Table 1 Summary of clinical Eppendorf data demonstrating a correlation between tumor hypoxia and radioresistance for head & neck tumors

Tumor	Author	*N*	Tx	Groups	Disease-free survival	Overall survival	Local control
HN 1°	Dunst et al. [39]	125	R	Above/below median HSV_5 (33%)	–	342 vs. 404 days $p=0.08$	–
	Nordsmark and Overgaard [40]	31	R	Above/below median $HF_{2.5}$ (15%), 2 years	–	–	45 vs. 90% $p=0.04$
	Stadler et al. [41]	59	R, C	HSV_5	–	Hazard Ratio (95%CI)=2.5 (1.2–5.1), $p=0.01$	–
	Nordsmark et al. [42]	35	R	Above/below median $HF_{2.5}$ (22%), 2 years	–	–	33 vs. 77% $p=0.01$
HN 1° or LNM	Nordsmark et al. [43]	397	R, C	Above/below median $HF_{2.5}$ (19%), 3 years	–	28 vs. 38 % $p<0.006$	–
	Brizel et al. [44]	63	R, C, S	Above/below median pO_2 (10 mmHg), 2 years	73 vs. 26% $p=0.005$	83 vs. 35% $p=0.02$	73 vs. 30% $p=0.01$
HN LNM	Rudat et al. [45]	194	R, C	Above/below median $HF_{2.5}$ (22%), 3 years	5 vs. 20% $p=0.004$	–	–
	Rudat et al. [46]	41	R,C	Above/below median $HF_{2.5}$ (21%), 2 years	–	10 vs. 20% $p=0.05$	–
	Adam et al. [47]	25	U, R, S	Above/below mean of median pO_2 (20 mmHg), 5 years	–	50 vs. 40% NS	60 vs. 30% NS
	Gatenby et al. [48]	31	R	CR vs. NR 90 days	–	–	CR $pO_2=20$ NR $pO_2=5$ $p<0.001$

C Chemotherapy, *CR* Complete Responders, $HF_{2.5}$ % of pO_2 readings <2.5 mmHg, *HN* Head & Neck, HSV_5 Hypoxic Sub-volume below HF_5, *LNM* Lymph Node Metastasis, *NR* Non-responders, *NS* Not significant, *R* Radiation, *S* Surgery, *U* Untreated, – Not reported

Table 2 Summary of clinical Eppendorf data demonstrating a correlation between tumor hypoxia and radioresistance for head & neck tumors

Tumor	Author	*N*	Tx	Groups	Disease-free survival	Overall survival	Local control
Cervix	Fyles et al. [49]	106	R	Above/below HF_5 of 50% 3 years	37 vs. 67% $p<0.004$	–	27 vs 37% $p=0.12$
	Rofstad et al. [50]	32	R	Above/below median HSV_5 (40%), 5 years	20 vs. 75% $p=0.001$	30 vs. 80% $p=0.005$	40 vs. 85% $p=0.04$
	Sundfor et al. [51]	40	R, C, S	Above/below median HSV_5 (31%), 5 years	15 vs. 80% $p<0.0001$	Lower for high HSV_5 $p<0.0004$	Lower for high HSV_5 $p<0.0006$
	Fyles et al. [52]	74	R	Above/below HF_5 of 50% 3 years	34 vs. 69% $p=0.02$	–	–
	Knocke et al. [53]	51	R	Above/below median pO_2 (10 mmHg), 3 years	69 vs. 34% $p<0.02$	–	84 vs. 47% $p=0.053$
	Hockel et al. [54]	89	R, S	Above/below median pO_2 (10 mmHg), 2 years	70 vs. 40% $p=0.009$	70 vs. 35% $p=0.004$	–
STS	Nordsmark et al. [55]	28	R, S	Above/below median pO_2 (20 mmHg), 5 years	77 vs. 44% $p=0.05$	77 vs. 28% $p=0.01$	–
	Brizel et al. [56]	22	R, H	Above/below median pO_2 (10 mmHg), 1.5 years	70 vs. 35% $p=0.01$	–	–

C Chemotherapy, *H* Hyperthermia, HF_5 % of pO_2 readings <5.0 mmHg, HSV_5 Hypoxic Sub-Volume below HF_5, *R* Radiation, *S* Surgery, *STS* Soft Tissue Sarcoma, – Not reported

from radiation or is induced by interaction with oxygen centered radicals (e.g hydroxyl radical, superoxide anion etc.) formed by the ionization of water surrounding the DNA. Typically, DNA strand breaks result and those that are not repaired can lead to fatal chromosomal aberrations. The damage is more readily repairable in the absence of molecular oxygen. This is because if oxygen is available, it can react with the broken ends of DNA, creating stable organic peroxides, which are not as easily repaired by a cell. Studies dating back more than 50 years have shown that oxygenated cells are 2.5- to 3-times more radiosensitive than hypoxic cells [1, 2].

After the oxygen enhancement effect was discovered, studies were soon underway to find a way to exploit it for therapeutic gain. Since the prevailing thought was that oxygen essentially acts as a direct radiosensitizer, most strategies involved simply trying to increase oxygen availability in the tumor by either increasing the oxygen content in efferent tumor vessels or decreasing tumor oxygen consumption.

Some of the earliest work towards this end was done using hyperbaric oxygen to radiosensitize cervical or head and neck cancers [3, 4]. Though there was some initial success with this technique, recent studies have indicated that combining radiation with hyperbaric oxygen results in significant normal tissue toxicities that need to be taken into account. A meta-analysis revealed that hyperbaric oxygen improved five year survival for irradiated head and neck tumors but also increased the incidence of severe radiation toxicity, with numbers needed to treat and harm of 5 and 8, respectively [5]. Early studies were also done using transfusion of red blood cells to increase the oxygen carrying capacity of blood and, thereby, increase the tumor tissue pO_2. Again, there was some initial success with this method [6]. Recently, however, red cell transfusion has been largely supplanted by the administration of erythropoiesis-stimulating factors. Unfortunately, the wisdom of giving erythropoietin alongside radiation is currently in question after a large randomized study showed no benefit and, moreover, suggested potential harm for head and neck patients treated this way [7]. It is unclear at this point, then, what the future will hold for these earliest strategies.

Over time, methods for improving tumor oxygenation have become somewhat more sophisticated. One approach currently undergoing clinical investigation combines inhaled hyperoxic gases with vasodilating drugs to maximize oxygen delivery to tumors. The inhaled gas commonly used here is called carbogen, a hyperoxic gas containing a small fraction of carbon dioxide—typically 2–5%. The high oxygen concentration is used to increase the O_2 content of circulating blood. The mechanism by which CO_2 improves oxygen delivery is controversial but

may involve decreased tumor oxygen consumption, respiratory stimulation, increased oxygen–hemoglobin dissociation, and/or vasodilation [8–10]. Carbogen is typically combined with nicotinamide, a vitamin B_3 derivative that is thought to act by improving tumor perfusion to increase tumor oxygenation [11]. Carbogen and nicotinamide are now being used in combination with accelerated radiotherapy—a regimen known as ARCON—in multiple clinical trials. Kaanders et al. have reviewed some of the clinical data on ARCON to date which, while showing some increases in normal tissue toxicities, appears promising for both head and neck as well as bladder carcinomas [12].

Other methods that can increase oxygen delivery include agents that right shift the hemoglobin saturation curve combined with high oxygen content gas breathing [13], artificial blood substitutes [14, 15], and calcium channel blockers that decrease red cell rigidity and improve microcirculatory flow [16].

As an alternative to increasing oxygen delivery to tumors, other investigators have focused on finding novel means of killing hypoxic tumor cells in order to reduce their contribution to radioresistance. The first work in this area led to the development of the electron-affinic nitroimidazoles. These chemicals are similar to oxygen in their capacity to react with and stabilize free radical species, and early evidence suggested they might preferentially radiosensitize tumor over normal tissue [17]. This selectivity is due to the fact that these compounds become active through a bioreductive process that can only be carried out in hypoxic tissue. Though individual studies on combining the nitroimidazoles with radiation were mixed, a meta-analysis including 50 randomized trials and more than 7,000 patients showed improvements in local control and overall survival (OR 1.17 and 1.13, respectively) [18]. Other bioreductive agents that act as direct hypoxic cytotoxins have also been developed, in part, to improve tumor radiosensitivity. Unlike the nitroimidazoles, these compounds do not interact with the free radical species generated by radiation but, rather, exert their effect by causing DNA damage selectively in hypoxic cells. Tirapazamine is one of the front-runners in this field, and has shown benefit over fluorouracil for head and neck cancer patients when combined with cisplatin and radiation [19].

Hyperthermia has also been shown to improve tumor oxygenation in pre-clinical models and in clinical trials [20]. In two human trials, the degree of improvement in oxygenation has been correlated with anti-tumor effect [21, 22]. The mechanism by which hyperthermia exerts this effect is not known, but may be related to either improvements in perfusion and/or reduction in oxygen consumption rates.

Theoretical models have indicated that reduction in oxygen consumption rate is a much more efficient means of reducing hypoxia than increasing perfusion or blood oxygen content. Thus, methods that combine metabolic inhibition with high oxygen content gas breathing my ultimately prove optimal if one wants to pursue this strategy [23, 24]. Some studies at the preclinical level have been conducted using this strategy [25].

3 Revisiting the oxygen enhancement effect

Over the period of time the above studies were being carried out, our understanding of the relationship between cancer and hypoxia deepened. As described elsewhere in this issue, it has become clear that hypoxia is not only an important cause of treatment resistance but also a powerful stimulus of many critical tumor phenotypes. These discoveries have prompted many to question whether the link between hypoxia and radioresistance is completely explainable by the oxygen enhancement effect as described above or, rather, whether hypoxia also influences radiosensitivity through biological effects.

This question was addressed experimentally in a study by Shrieve and Harris more than two decades ago [26]. They investigated how the duration of hypoxia influenced cellular radiosensitivity. If the effect of hypoxia on radiosensitivity were only as important as its physiochemical effects, the duration of oxygen deprivation prior to irradiation would be irrelevant—a tumor could be made acutely or chronically hypoxic, and the effect on radiosensitivity would be identical. If radiosensitivity were also influenced by the biological impact of tumor hypoxia, however, the duration of hypoxia would likely be important. Longer periods of oxygen deprivation would allow the biological effects of hypoxia to manifest themselves and exert a more pronounced influence on radioresponsiveness.

The Shrieve and Harris experiment demonstrated that cellular radiosensitivity increases with the duration of hypoxia. The fold-increase in radiosensitivity over acutely hypoxic cells levels off at 1.43 by 24 h of hypoxia. They also showed that the effect persists after the cells are reoxygenated. These results have since been validated by our group and by others [27]. We set out to study how the biological effects of chronic hypoxia influence cellular radiosensitivity in the absence of the radiochemical effects of acute hypoxia. Therefore, the cells in our studies were irradiated under normal oxygen conditions; the variable we studied was the oxygen concentration cells were exposed to prior to irradiation. Using this model, three separate cell lines were found to be radiosensitized by hypoxia [28]. This serves as an important point of comparison for the Shrieve

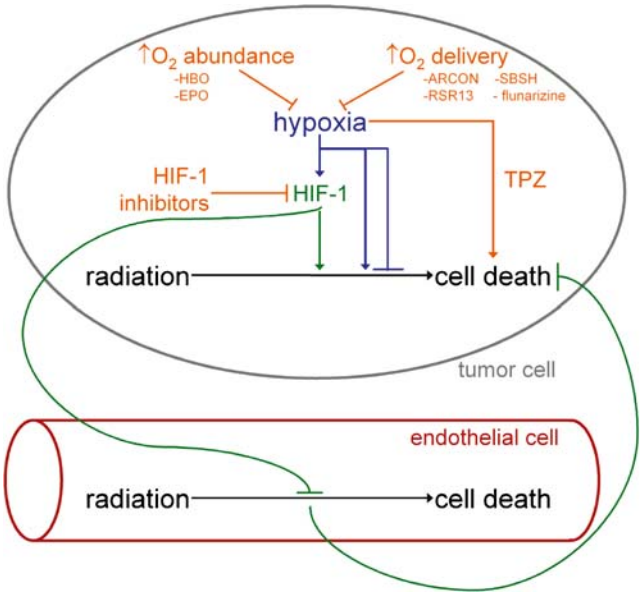

Fig. 1 Schematic representation of the relationship between hypoxia and radiosensitivity, and therapies that can alter the associated pathways. Hypoxia has direct (*blue*) and indirect (*green*) effects on radiosensitivity, which can be both protective and sensitizing. Various therapeutics (*orange*) can intervene by increasing tumor pO2, blocking HIF-1 activity, or directly killing hypoxic cells. *HBO* hyperbaric oxygen, *EPO* erythropoietin, *ARCON* accelerated radiotherapy, carbogen, and nicotinamide, *SBSH* bovine hemoglobin, *TPZ* tirapazamine

and Harris experiment described above. Not only are chronically hypoxic cells more radiosensitive than acutely hypoxic cells, they are also more radiosensitive than normoxic cells when both are irradiated under oxygenated conditions.

These data are important for several reasons. First, they imply that hypoxia exerts biological effects on tumors that influence radiosensitivity, following the reasoning outlined above. Second, they show that not all effects hypoxia has on tumors are radioprotective. These conclusions strongly suggest that our understanding of the relationship between oxygen and radiosensitivity needs revisiting.

4 Hypoxia, HIF-1, and radiosensitivity

Though the cellular response to hypoxia has been an area of intense research for many years, not until very recently has work focused on the biological mechanisms linking hypoxia to radiosensitivity. Our group has focused on this area in an attempt to uncover molecular signals that might regulate this relationship.

As discussed elsewhere in this issue, there are many signaling pathways regulated by hypoxia that influence various important cellular processes. One of the best-characterized of these pathways is the one controlled by hypoxia-inducible factor-1 (HIF-1). HIF-1 is a heterodimeric transcription factor that is stabilized by low oxygen tensions and functions to regulate the expression of greater than 100 gene products whose functions help to protect cells from hypoxic stress. As reviewed elsewhere [29], HIF-1 affects many processes—including glycolysis, mitosis, apoptosis, and angiogenesis—which have been shown to influence radioresponsiveness and might, therefore, serve as a link between HIF-1 activity and tumor radiosensitivity.

Making their potential link even more plausible, HIF-1 activity is known to be influenced by radiation, itself. Between 24 and 48 h after irradiation, HIF-1 activity in a tumor will increase approximately two-fold [30]. Interestingly, this phenomenon occurs only *in vivo*, as it relies on microenvironmental mechanisms that take place only in intact tumor tissue. Tumors tend to undergo transient increases in oxygen tension after they are irradiated. This effect, called reoxygenation, is a result of the cytotoxic effect of radiation. By killing off space-occupying and oxygen-consuming tumor cells, radiation increases perfusion into and decreases oxygen consumption within the tumor, resulting in increased tissue pO_2.

It is as a result of this reoxygenation effect that HIF-1 activity increases in irradiated tumors [30]. Reoxygenation causes an increase in oxidative stress, likely through mechanisms related to hypoxia-reperfusion injury, which directly stabilizes the HIF-1 complex. Reoxygenation also leads to the reversal of certain mechanisms of translational suppression brought on by hypoxia. One of these mechanisms, the so-called stress granule, inhibits the expression of various proteins regulated by HIF-1. By depolymerizing stress granules, then, reoxygenation also increases HIF-1 activity in an indirect fashion.

The next logical question is whether HIF-1 activity has any significant effect on outcome for the irradiated tumor. The simple answer to that question is that inhibiting HIF-1 activity seems to have a radiosensitizing effect on tumors [28, 30, 31], suggesting that HIF-1 may be partially responsible for the biological link between hypoxia and radioresistance. But just as the relationship between radiosensitivity and the biological effects of hypoxia are complex, so is the interplay between radiosensitivity and HIF-1. Echoing the Shrieve and Harris experiment discussed above, not all HIF-1-mediated effects are radioprotective for tumors—some appear to be radiosensitizing.

In fact, HIF-1 may be responsible for the radiosensitizing influence of hypoxia's biological effects. HIF-1 acts to radiosensitize tumors through several different pathways [28]. It enhances the degree of p53 phosphorylation in irradiated cells, leading to increased caspase 3/7 cleavage and a greater likelihood of undergoing apoptosis. As a result, a smaller proportion of cells become apoptotic after being irradiated in tumors wherein HIF-1 has been inhibited. HIF-1 also drives glycolysis in oxygen-deprived

cells and, as radiosensitivity tends to correlate with glycolytic rate, this too has a radiosensitizing effect on tumors. Similarly, HIF-1 maintains the mitotic drive in tumor cells starved of nutrients. Since starved cells tend to arrest in a radioresistant phase of the cell cycle, this effect of HIF-1 activity is also radiosensitizing. These effects may all tend to make tumors subjected to HIF-1 inhibition less likely to respond to radiotherapy.

Although each of these potentially disadvantageous effects of HIF-1 blockade have been experimentally verified, when tumor cells deficient in HIF-1 activity are grown in vivo and irradiated they respond better than do their normal controls. How can it be that HIF-1 inhibition is radioprotective for the tumor cell but radiosensitizing for the tumor? The answer seems to come from what lies in between. A tumor is clearly more than the sum of the tumor cells within; it is also made up of vital stromal compartments whose importance is becoming ever clearer over time. One crucial element of the tumor stroma is the vasculature, long recognized for its importance in feeding the growth of cancerous tissue [32].

It has recently been hypothesized that the tumor vasculature may also be important in determining how the tumor responds to radiotherapy. In an experimental model, tumors whose vessels were made artificially resistant to radiation-induced cell death were, themselves, significantly less responsive to the effects of radiation [33]. This finding suggests that the response of the tumor vasculature to ionizing radiation may be an important component in determining the radiosensitivity of the tumor as a whole.

HIF-1 plays a role in this relationship by promoting tumor cells to express cytokines that have radioprotective effects on neighboring endothelial cells. These cytokines, including VEGF and others like it, send anti-apoptotic signals to tumor vessels making them resistant to radiation. Blocking these cytokines can result in dramatic increases in the radiosensitivity of tumor vasculature and, as a result, increased overall tumor radioresponsiveness [34–38]. As might be expected, given that it is a major regulator of endothelium-signaling cytokines, inhibiting HIF-1 also causes tumor vasculature to become significantly sensitized to ionizing radiation [28, 30]. The effect can be quite dramatic—tumors whose vessels normally change little after irradiation will undergo nearly complete vascular regression when irradiated in combination with HIF-1 blockade. Since tumor cells are somewhat reliant on their vasculature for survival, this effect translates into a significant increase in overall tumor radiosensitivity. Importantly, though the effect of HIF-1 blockade on tumor vessel radiosensitivity appears large, it seems to have little or no impact on the stability of irradiated normal tissue vasculature [30]. Consequently, HIF-1 blockade may represent a plausible strategy to widen the therapeutic window for ionizing radiation by exploiting the importance of the endothelial cell as a target for radiotherapy.

The preclinical work done by our group suggests that all of the HIF-1-dependent modifiers of radiosensitivity discussed above are relevant for the irradiated tumor. The fact that tumors seem to respond better to radiation when HIF-1 has been inhibited implies that the radiosensitizing effects of HIF-1 blockade (i.e. endothelial sensitization) outweigh the radioprotective effects (i.e. decreased apoptosis, mitosis, and glycolysis).

However, HIF-1 blockade may not turn out to be a radiosensitizer in the clinic, where tumor biology is likely to be different in important ways. For this reason, it may become necessary to select patients undergoing radiotherapy for whom HIF-1 blockade is most likely to deliver a therapeutic advantage. For example, tumors without functional p53 may respond better to the combination of HIF-1 blockade and radiation as the loss of apoptotic potential may not be as significant. Also, well-oxygenated tumors may not benefit much from this treatment as the degree of HIF-1 activation in these tumors—pre-and post-radiation—is likely to be modest. As HIF-1-inhibiting agents are being readied for clinical use, it is hoped that these early data may help in guiding how they might be best combined with radiotherapy.

5 Summary

It seems there are many ways in which hypoxia influences radiosensitivity, and just as many ways to therapeutically intervene in these pathyways (Fig. 1). Continued research is underway in the area of molecular mechanisms explaining the effect that hypoxia has on tumor radiosensitivity. As more pathways are uncovered, new therapeutic targets may offer promising opportunities to improve radiation response rates by minimizing the radioprotective effects of hypoxia while leaving intact its potential to radiosensitize.

Acknowledgments This work was supported by the Duke SPORE for breast cancer, NIH grant CA40355, the Howard Hughes Medical Institute, and the Duke Medical Scientist Training Program grant.

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