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How to deal with the relationship between hypoxia and radiotherapy in the hypofractionated radiotherapy era?

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► ***Review article***

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ABSTRACT

Hypoxia, a common phenomenon in solid tumors can promote dysfunctional vascular growth and epithelial-to-mesenchymal transition, leading to cell mobility and metastasis. The decreased sensitivity of hypoxic tumor cells to ionizing radiation is one of the main factors affecting the effect of conventional radiotherapy. It is well known that conventional radiotherapy mainly reduces the effect of hypoxic radiation resistance by reoxygenation between fractions. With the improvement of radiation treatment planning and delivery, more and more cancer patients have been treated with hypofractionated radiotherapy (HFRT), which have achieved a much higher effect than conventional radiotherapy. Given that HFRT is delivered within one or a few fractions, does tumor hypoxia affect its efficacy? Is there any way to further improve the effect of HFRT? In this review, we focus on the interaction between HFRT and hypoxia, and how to optimize the regimen of HFRT to decrease the effect of hypoxia and improve the efficacy is discussed in detail.

Keywords: *Hypoxia, hypofractionated radiotherapy, radiobiology, fractionation schedules.*

INTRODUCTION

Tumor hypoxia is one of the main factors affecting the effect of radiotherapy. The conventional radiotherapy mode of 1-2 Gy is to reduce the effect of hypoxia on radiotherapy by reoxygenation of tumor cells between radiotherapy. HFRT reduces the number of fractions and overall treatment duration by using larger doses >2 Gy per fraction [(1)]. How does hypoxia and radiotherapy affect each other in HFRT? Better understanding of the interaction between hypoxia and HFRT is beneficial to optimize the radiotherapy plan and improve the outcome. So, here we make a review about the relationship and mutual effect between hypoxia and HFRT.

Hypoxia

Tumor hypoxia has been observed in many human cancers [(2)]. About 90% of solid tumors have lower partial pressure of oxygen than normal tissues [(3)]. Hypoxia is characterized by lower oxygen tension than normal (2.03-3.04kpa). "Intermediate" hypoxia (0.13-2kpa) plays an important role in enhancing tumor invasiveness and metastasis but does not interfere with radiation-related cell death. "Radiobiological" hypoxia (inhibiting radiation-induced cell death) occurs at oxygen

level below 0.13kpa. As oxygen is further reduced (less than 0.02 kpa), cancer cells exhibit survival-oriented mutations and maximal resistance to radiotherapy [4]. Tumor hypoxia can be defined as lower oxygen pressure in tumors than in surrounding normal tissues, but the most commonly used definition is pO2≤10mmHg [5].

Many methods can be used to detect the condition of hypoxia, but remain to be further improved. Direct measurements of tumor oxygenation have been performed predominantly with the Eppendorf histography. Exogenous markers have been used for the detection of hypoxia by immunohistochemical examination of hypoxic tumor areas (pimonidazole) or by positron-emission tomography (PET) imaging (misonidazole). Hypoxia-related proteins such as hypoxia-inducible factor-1a (HIF-1a) are considered as potential endogenous markers of hypoxia [6].

In individualized and complex environments, functional definitions may be more appropriate. Therefore, when oxygen supply does not meet the demand for oxygen, hypoxia-inducible Factors (HIF)-subunits become stable and tumor hypoxia begins [7]. More than 50% of solid tumors present with heterogeneous hypoxia, regardless of size and histological characteristics [8-10].

Tumor vasculature originates from host vessels and neovascularization induced by tumor angiogenesis factors [11]. The decrease of oxygenation in tumor cells is due to the disorder of the structure and function of tumor blood vessels, which inhibits the normal delivery of oxygen [12]. New vascular formation in tumor tissues is chaotic. In normal tissues, the branches of the blood vessels are strictly regulated, each cell needs to be within approximately 40 microns of adjacent capillaries to ensure that the cell has sufficient oxygen and nutrients. In solid tumors, this branch is more extensive because of the rapid proliferation of tumor cells. Rapid tumor proliferation means higher metabolic demand, which leads to excessive pro-angiogenic factors. Also, tumor vessels are constantly remodeled in solid tumors, resulting in loss of contact between endothelial cells and basement membrane and rupture of capillary beds. Then tumor blood vessels present large pores and leakage occurs [13]. Therefore, a highly vascularized tumor is not necessarily a highly oxygenated tumor [11].

A prominent feature of cancer cells is their insensitivity to micro-environmental signals, resulting in continual proliferation and reduced cell death due to the accumulation of driver mutations and epigenetic changes [14]. This nature of the carcinogenesis process establishes a strong negative selective pressure that leads to cell adaptation and creates a heterogeneous tumor microenvironment in which the clone population of cancer cells produces a gradient of nutrients, pH, and metabolites that eventually produce hypoxia [15, 16].

Studies showed that α/β ratio was an important marker to evaluate repair ability of cells. The higher α/β ratio was, the more weakened repair ability of cells became [17]. And the sensitivity to radiation was directly affected by repair ability of cells. When tumor hypoxia happened, α/β ratio was increased, indicating that the sensitivity to radiation was decreased [18].

Effects of hypoxia on tumor and conventional radiotherapy

Oxygen supply is necessary for cell growth but is often reduced in solid tumors, especially at the center of the tumor mass [19]. Tumors must adapt to hypoxia to support their own growth and survival. Moreover, tumor hypoxia may be associated with resistance to radiation and chemotherapy [20-22]. Tatrai *et al.* showed that in different human tumor cell lines, hypoxic environment induced cell-type dependent changes and activated small GTPase, resulting in different migration and metastasis promotion responses [19]. Tumor hypoxia promotes dysfunctional vascular growth and epithelial-to-mesenchymal transition, leading to cell mobility and metastasis shown in figure 1. Hypoxia alters cancer cell metabolism and exacerbates therapeutic resistance by inducing cell quiescence [23]. So, the main reason for the failure of radiotherapy on severe hypoxic

tumors is the decreased sensitivity of hypoxic tumor cells to ionizing radiation [24].

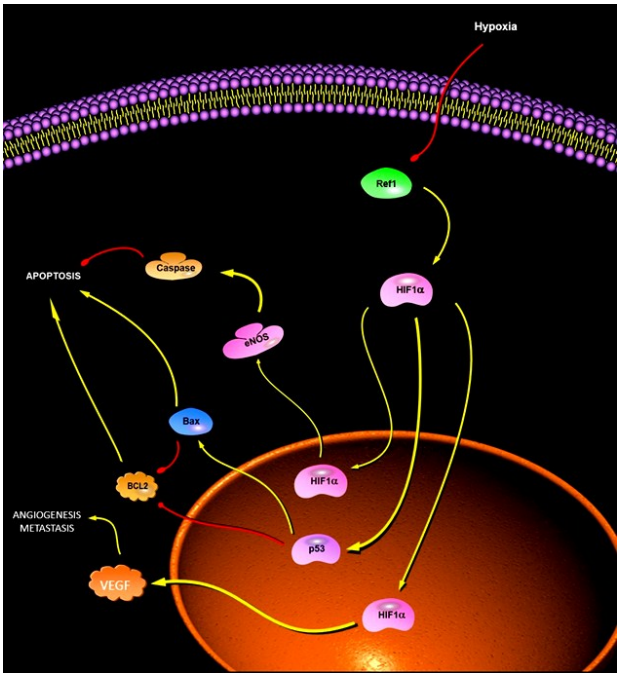

Figure 1. Hypoxia can affect cell apoptosis and metastasis of tumor via triggering HIF-1a, eNOS and VEGF signaling pathways.

The methods of reducing the effects of hypoxia on conventional radiotherapy

Under the conventional radiotherapy mode, by dividing the total dose, the reoxygenation of radiotherapy interval reduces the problem of hypoxic radiation resistance [25].

In the past century, radiologists have identified several factors that control the radiation response of tumors and normal tissues to fractionated radiotherapy. The most critical factors are 5"R", including repair of sublethal cellular damage, redistribution of cells within the cell cycle, reoxygenation of surviving cells, repopulation of cells after irradiation, and the radiosensitivity intrinsic to the cells [26].

The conventional radiotherapy model is based on the classical radiobiology of 5"R" to optimize the treatment plan. The resistance of radiotherapy was overcome mainly by redistribution of cells within the cycle and reoxygenation of hypoxic cells between fractions. The sensitivity of cells to radiation therapy varies with their position in the cell cycle [27]. During conventional radiotherapy, tumor cells increase their probability of being in a sensitive phase during one or more fractions by progression of the cell cycle between fractions [28]. Hypoxia can be transient because of fluctuations in tumor blood flow or be chronic because of increased demand for oxygen within the tumor and the irregularity of tumor blood vessels [29]. In traditional radiotherapy, transient hypoxia during radiotherapy can be alleviated by radiation fractionation, which allows surviving cells to be reoxygenated between fractions [28].

Hypofractionated radiotherapy

With the improvement of radiation treatment planning and delivery, it has become possible to deliver radiation more accurately to tumors while limiting the dose to normal tissue around them. These advances have improved the treatment and have been able to provide a small number (≤5) highly accurate high-dose radiation to the target at certain anatomical sites [30]. These techniques, which have been termed stereotactic body radiation therapy (SBRT) or stereotactic ablative radiation therapy (SART) for extracranial treatment and stereotactic radiosurgery (SRS) for intracranial treatment, are increasingly being used in different clinical settings to improve local control of cancer [31]. Conventional radiotherapy is aimed to use several principles of radiobiology to complete a small daily dose in a few weeks, which is significantly different form HFRT [28]. In conclusion, the development of HFRT is based on the progress of radiation physics such as image guidance and precise radiotherapy, which makes it possible to locate, plan and treat the tumor target area accurately, thus realizing the high-dose irradiation of tumor and minimizing the radiation dose received by normal tissue around tumor.

Different Understandings of Radiobiology of Hypofractionated Radiotherapy

Despite the wide-spread adoption of HFRT in the clinic, divergent views existed about the mechanisms by which HFRT enhances local control [32-51] shown in table 1.

The classical radiation biology theory (5"R") and linear-quadratic (LQ) model are the basis of conventional radiotherapy models. Some studies showed that the LQ model may not be suitable for the accurate evaluation of the killing effect of tumor cells by HFRT, and some researchers reported that it remains to be revised to meet the needs of clinical biologically effective doses conversion [52-54]. For example, Sheu *et al.* found that when a single dose was greater than 10Gy, the LQ model significantly underestimated the killing of cells by HFRT [55]. These studies suggest that, in addition to the classical LQ model, there may be other mechanisms such as changes in tumor cells and microenvironment involved when the dose is greater than a certain fraction.

Table 1. Studies supporting or not supporting that linear-quadratic model is suitable for the therapeutic evaluation of SBRT.

Studies not supporting that linear-quadratic model is suitable for the therapeutic evaluation of SBRT	Studies supporting that linear-quadratic model is suitable for the therapeutic evaluation of SBRT
Song CW *et al.,* 2019. [29]	Torok JA *et al.,* 2019. [39]
Bodo S *et al.,* 2019. [30]	Moding EJ *et al.,* 2015. [40]
Song CW *et al.,* 2015. [31]	Shuryak I *et al.,* 2015. [41]
Sperduto PW *et al.,* 2015. [32]	Brown JM *et al.,* 2014. [42]
Park HJ *et al.,* 2012. [33]	Mehta N *et al.,* 2012. [43]
Kirkpatrick JP *et al.,* 2008. [34]	Brenner DJ, 2008. [44]
Garcia-Barrps M *et al.,* 2003 [35]	Krause M *et al.,* 2007. [45]
Szeifert G *et al.,* 2002. [36]	Hoinkis C *et al.,* 2005. [46]
Kocher M *et al.,* 2000. [37]	Budach W *et al.,* 1993. [47]
Clement JJ *et al.,* 1978. [38]	van der Kogel AJ, 1985. [48]

On the other hand, some studies confirmed that the LQ model is suitable for 10 Gy [56], or even a single dose of 15 to 20 Gy [47]. The LQ model can also predict the effect of HFRT with the reference of biologically effective doses (BED), and it is suitable for predicting the effect of different radiotherapy modes. There is no need to modify or replace the model [57]. For now, there are still many studies trying to modify the LQ model in order to find the most suitable predictive model for HFRT, but it does not go far beyond the traditional LQ model [58, 59]. Brown *et al.* combined the standard theory of radiobiology with the preclinical and clinical studies of HFRT and concluded that in the HFRT model, there is no need to change the LQ model, nor to introduce other biological mechanisms beyond the classic radiobiological theory 5"R". For most tumors, the standard radiobiology concepts of the 5R's are sufficient to describe the clinical effects of HFRT, and the excellent results obtained from clinical studies are those from the much larger BED that are delivered with HFRT [45]. Furthermore, the tumor control probability (TCP) model for predicting lung SBRT, which is closest to clinical observations, is also based on a LQ model of cell killing [18].

Interaction between hypoxia and hypofractionated radiotherapy

Compared with conventional radiotherapy, emerging radiotherapy techniques provide a more valuable physical advantage for patients with isolated tumors [60, 61]. HFRT produces excellent local control rates (>90%) in many prospective clinical trials of lung tumors [62-67]. However, the local control rate of cancer patients in daily clinical practice is not as high as in prospective studies. As the total radiation dose is completed in only a few fractions, the possibility of reoxygenation between fractions is reduced, and the therapeutic effect is affected. So, hypoxia may be an important cause of resistance to HFRT. But on the other hand, HFRT can cause endothelial cell and vascular damage [37], and aggravate hypoxia, which is not conducive to the repair of sublethal cellular damage and leads to the indirect death of tumor cells shown in figure 2.

Effect of hypoxia on hypofractionated radiotherapy

It has been suggested that the effect of tumor hypoxia on single high-dose radiotherapy may outweigh the effect on conventional radiotherapy because the important benefit of reoxygenation between fractions has been lost [45]. Preclinical and modeling studies have shown that tumor hypoxia can lead to significant

resistance to single high-dose radiotherapy [68, 69]. With fractionated radiotherapy, the effects of hypoxic radiation resistance were reduced by reoxygenation between fractions [70]. Compared with conventional radiotherapy, HFRT has technical feasibility and logical advantages, but the potential reoxygenation is reduced because the total dose is accomplished within a few fractions [71]. Therefore, the radiation resistance of hypoxic tumor cells is more serious in HFRT [12, 45, 72]. A recent LQ modeling study of tumor hypoxia suggested that HFRT limited the potential for reoxygenation between fractions and therefore could lead to a significant reduction in tumor cell kill ratio in comparison with conventional radiotherapy [12]. Lindblom *et al.* calculated cell survival in the simulated tumors with a modified LQ model taking into account different radiosensitivities of chronically and acutely hypoxic cells. The simulated treatments were evaluated by calculating the TCP. They found hypoxia could have impact on the outcome of HFRT [73].

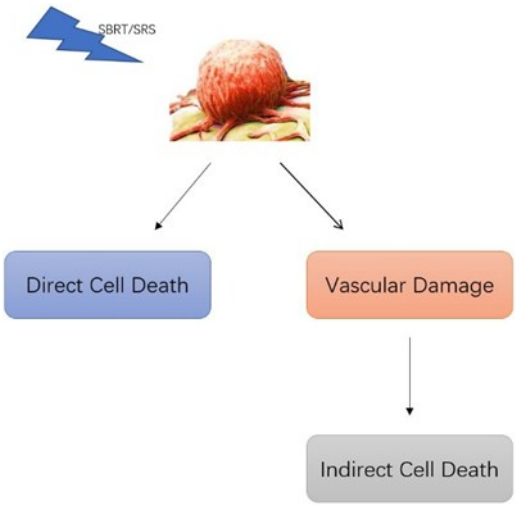

Figure 2. Tumor cells are directly and indirectly killed by SBRT or SRS.

Effect of hypofractionated radiotherapy on hypoxia

Studies have shown that secondary or indirect cell death induced by vascular injury plays an important role in the high-dose response of tumors to HFRT [35, 36, 74-76]. It has been reported that a single exposure to an experimental rodent tumor of 10 Gy or more can cause severe vascular damage, leading to indirect tumor cell death [41, 77-82]. Other reports also suggest that high-dose irradiation-induced endothelial cell death and vascular dysfunction can lead to secondary cell death in various types of tumors [39, 83, 84]. Song *et al.* found that a single dose of 15 to 30 Gy induced a dose-dependent secondary cell death in FSaII tumors of C3H mice, considering the possible deterioration of the intratumor microenvironment due to vascular damage. After irradiation with 15 or 20 Gy, the survival rate of FSaII tumors decreased for 2 to 3 days, and began to recover thereafter in some but not all tumors. While after irradiation with 30 Gy, cell survival rate decreased continuously for 5 days. In some tumors, the cell survival rate of 5 days after 20 to 30 Gy irradiation was 2 to 3 logs less than that immediately after irradiation. 20 Gy irradiation significantly reduced blood perfusion, up-regulated HIF-1a and increased expression of carbonic anhydrase-9, suggesting that irradiation increased tumor hypoxia [34].

Recent studies of radiation-induced changes in tumor blood vessels have shown that a single dose of 5 to 10 Gy causes relatively mild vascular damage, whereas a higher dose of radiation more than 10 Gy per fraction causes severe vascular damage [36]. Song *et al.* observed in their reoxygenation studies that high-dose exposure caused vascular damage to the tumor, leading to the death of hypoxic cells that escaped the direct effects of radiation. Therefore, it is concluded that the decrease of hypoxic cell fraction in tumor after high dose irradiation is not only due to the reoxygenation of hypoxic cells, but also partly due to the indirect death of hypoxic cells [41, 81]. In analyzing the radiobiological mechanism of SBRT and SRS, song et al. also showed that in addition to killing tumor cells directly, using high-dose irradiation also caused indirect tumor cell death through vascular damage [32]. Keladaoj *et al.* used dynamic positron emission tomography images to prospectively observe hypoxic volume in the tumor after a single high dose of radiotherapy by injecting 18F-fluoromisonidazole into patients with early NSCLC cancer. It was found that high single doses of radiation may induce an elevated and, in some cases, persistent state of tumor

hypoxia in NSCLC tumors [85].

How to reduce the effect of hypoxia on hypofractionated radiotherapy

Although many clinical studies have demonstrated the superior efficacy of HFRT, many aspects still need to be optimized. One of the most important issues is to set an optimal fractionation schedule (including prescription dose, number of treatment fractions and interval) for HFRT to mitigate the effect of hypoxia on radiotherapy.

Studies have shown that a single dose of 24 Gy caused transient vascular dysfunction associated with adhesion of platelets and leukocytes to vascular endothelium, and increase of vascular permeability [86]. It doesn't seem to be a good way to get a daily dose like conventional radiotherapy. In HFRT, tumor hypoxia should not be ignored. A new fractionation paradigm of 12 fractions of approximately 12 Gy followed by more moderate dose fractions of 5-6 Gy could increase the therapeutic ratio. This option has the advantage of not only providing the largest dose of radiotherapy when the tumor is resistant to treatment due to hypoxia, but also allowing a degree of reoxygenation within a time frame, but limiting the time for tumor regrowth [87].

Harriss-Phillips *et al.* simulated SART on hypoxia and well-oxygenated tumors using probabilistic parameter distributions and LQ versus linearquadratic-cubic (LQC) methods, and evaluated the optimal fractionation schemes using BED comparisons. The results showed that the complex temporal dynamics of tumor oxygenation combined with the probabilistic cell dynamics in radiotherapy model required a complex stochastic model to predict the killing of tumor cells. For HFRT, a high dose in the first week, followed by a milder dose, may be beneficial because a high proportion of hypoxic cells can be eradicated early, while maintaining a relatively low BED required, with normal tissue toxicity in tolerable levels [87].

Animal studies found that the tumor perfusion of hoechst33342 dye was significantly reduced and vascular morphology changed in lung cancer-bearing mice at 6 hours after high-dose radiotherapy. However, 2 days after radiotherapy, hoechst33342 perfusion and cd31 density partially recovered. The results suggested that a single high dose irradiation produced rapid but reversible vascular collapse in the tumor [88]. A prospective study of six patients with NSCLC tumors receiving SBRT-eligible using non-invasive methods showed that NSCLC patients with detectable baseline levels of tumor hypoxia might have higher levels of tumor hypoxia (by a factor of up to 2.7) 2 days after receiving the first fraction of SBRT. It was believed that given this phenomenon of increased hypoxia volume at 2 days after SBRT treatment, tumor oxygenation should be fully taken into account in the formulation of the optimal hypofractionated schedules. To overcome hypoxic radiation resistance, the SBRT delivery schedule for patients with more hypoxic tumors could be altered from 3 times per week to once per week for 3 weeks [85]. Increasing the time between fractions may allow for more reoxygenation to occur and may improve clinical outcomes. In addition, five fractions of 10 Gy delivered every other day (excluding weekends) improved local control compared with consecutive daily fractions [89, 90]. Shibamoto *et al.* summarized the radiobiological properties of HFRT, and based on these considerations, they suggested that lung tumors larger than 2 cm in diameter were irradiated 60 Gy in eight fractions delivered three times a week [72]. Meanwhile, some reports suggested that the current reference lung SBRT schedule (18 Gy × 3) represents overdosage, at least for smaller tumors. Taking into account changes in tumor oxygenation, it is recommended to increase the treatment rate by doing more than three times (such as 10 Gy ×5 or 6 Gy × 8) rather than the current reference schedule (18 Gy × 3) [18].

Pre-treatment assessment of tumor oxygenation using hypoxic imaging is feasible. A study has explored the feasibility of using a method for calculating the dose required for hypoxia subvolume on 18F-HX4 positron emission tomography (PET) in NSCLC. It was found that the method to account for heterogeneous and dynamic hypoxia in target

volume segmentation and dose prescription based on 18F-HX4-PET imaging appeared feasible in NSCLC patients, and the distribution of oxygen partial pressure within hypoxic target volumes could impact the required prescribed dose more than the size of the volume [91]. In turn, hypoxic imaging can be used to develop personalized treatments. For example, lowering the prescribed dose reduces the risk of normal tissue complications in patients with low levels of hypoxia. This strategy was successful in patients with head and neck cancer who selectively received reduced dose to neck nodes based on hypoxic imaging and achieved 100% local control [92]. Selecting tumors with low resistance to radiotherapy for dose reduction can improve the eligibility of patients with more central lesions for SBRT [85].

In addition to optimizing the dose segmentation, the sensitizer for HFRT is also a hot topic. Hypoxia-selective drugs, such as tirapazamine, can counteract the radiation-protective effects of tumor hypoxia after delivery of the first fraction [93]. Alternatively, the use of a hypoxic cell radiosensitizer immediately before SBRT dose delivery can sensitize patients with hypoxic tumors [12, 94]. It was found that dolanidazole, a hypoxic cell radiosensitizer combined with a single 25Gy fraction, improveed 3-year survival for pancreatic cancer [95].

Other methods of increasing sensitivity in HFRT, such as manipulating the cell cycle phase [95] and blocking the mechanisms of tumor repopulation[98,99], are not covered in this paper.

CONCLUSION

This paper discusses the current understanding of radiobiology of HFRT, the interaction between hypoxia and HFRT, and the methods to improve the curative effect of HFRT. The radiobiology of HFRT is controversial, but for most tumors the standard radiobiology concepts of the 5R's are sufficient to explain the clinical data besides possible anti-tumor immunity in certain tumors. As to the interaction between hypoxia and HFRT, hypoxia also affects the efficacy of HFRT, and in turn, HFRT, through its effect on tumor blood vessels, can aggravate tumor hypoxia, leading to secondary death of tumor cells after radiotherapy. According to the understanding of the above problems, some schemes for optimizing HFRT have been proposed in recent years. The topic about hypoxia and HFRT gains more and more attention recently. Further research remains necessary tobetter understand the phenomenon of hypoxia, clarify the hypoxia-inducible responses and signaling pathways, and find more constructive strategies to improve the effect of HFRT.

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