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Radiobiology of Stereotactic Body Radiation: Therapy/Stereotactic Ablative Radiotherapy, Potential Risk Factor for SBRT [Stereotactic body radiation therapy (SBRT)] Failure and Normal Tissue Response

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Abstract: The use of stereotactic ablative radiotherapy (SABR) or stereotactic body radiation therapy (SBRT) is expanding beyond its initial focus on treating tumours in the liver and lungs due to the remarkable success in controlling these malignancies. While technological advancements have been crucial to SBRT's success, biological constraints are currently limiting the use of highly effective SBRT regimens. Although SBRT will keep becoming more popular, there are still some questions about how normal tissues and tumours react to high dose per fraction radiation. This will help us understand how to improve the technology and prevent damage from being caused by too strong SBRT treatments. The models used to estimate biological consequences at high dose per fraction are undergoing a paradigm shift in radiobiology, even if the underlying mechanisms are still not fully understood. Additionally, SBRT may provide scheduling benefits that could be taken advantage of. Due to their serious adverse effects, radioprotective or hypoxic cell cytotoxic medicines that were either discontinued or were not utilised actively should be reevaluated. There is a mechanistic explanation that suggests SBRT is not suitable for tumours with low oxygen levels, therefore this may be particularly true in hypoxia. That idea, though, would be challenged by us.

Keywords: Radiobiology, Therapy/Stereotactic Ablative Radiotherapy, Risk Factor, Normal Tissue Response

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Introduction

The goal of stereotactic ablative radiotherapy (SABR) and stereotactic body radiation therapy (SBRT) is to target tumours with extremely high doses of ablative radiation while minimising the amount of healthy tissue in the treatment field. SBRT is quickly becoming a go-to method for treating tumours in all sorts of different parts of the body because of its reputation as a very effective targeted therapy. The primary factor that allowed for the deployment of SBRT—a targeted therapy that has demonstrated considerable promise—was the physical geometric avoidance of dose made possible by technological advancements [1-3]. The major airways, gut lumens, and ducts are just some of the normal tissues that have had serious clinical outcomes as a result of SBRT, and the influence of technological improvement alone is not going to be enough to solve the problem. It is still unclear if the negative effects of ablative radiation exposure can be mitigated or if there are biological consequences that can be used to our advantage in order to increase tumour killing or decrease normal tissue response. A concept in biological exploitation is the so-called threshold effect, which occurs when a tumour shows minimal response at low doses but a dramatic increase at high doses. This might be an effect on a large scale, or it could be an effect on the immune system that is not typically observed at typical therapeutic levels. One promising avenue for SBRT exploitation is the modulation of tumour and normal tissue responses through these threshold effects. The idea that tumour hypoxia will become a major obstacle to SBRT's application to some tumour sites when short course ablative fractionation strategies are applied is a hurdle to SBRT. Decades of real-world expertise in normal tissue sparing form the basis of conventional fractionated radiotherapy (CFRT). Although the first radiologists employed very high doses of radiation to sterilise ram testes, it wasn't until 1906 that Claudius Regaud demonstrated that skin could be safely exposed to radiation after many doses, as opposed to a single dose, during the procedure. To back up the usage of fractionated schemes, basic radiobiology proposed that the shoulders of in vitro survival curves described how normal tissue was spared during fractionated radiation doses, which led to cellular data supporting this theory. The therapeutic benefit was supplied by re-oxygenation and tumour cell cycle re-assortment [4], however this could theoretically be applied to tumour cells as well. The goal of fractionation regimens is not improved tumour management in and of itself, but rather a compromise between normal tissue sparing and tumour cure. Considering that, in the past, the volume of healthy tissue within a radiation field was far more than the volume of the tumour. The rationale for 2 Gy fractionated therapy regimens administered over several weeks can be reconsidered if the normal tissue in the radiation field is limited by technological advancements in dose delivery or is selectively protected by chemical or biologic methods [5, 6]. Also, this is supported by radiobiology, which shows that radiation doses beyond the shoulder of the survival curve are more effective per unit dose than those typically within the shoulder region.

Radioactivity at Elevated Dose

This raises the issue of how survival curves look at high doses. The clonogenic survival rate is an important metric to track since it is believed that the number of cell deaths caused by radiation-induced chromosomal abnormalities, which ultimately lead to reproductive death, is directly related to the efficacy of tumour control efforts. Apoptotic cell death, vascular damage, immunogenic effect, and abscopal effects are additional, albeit secondary, actions that may contribute to tumour control.

Mammalian Life Expectancy

When exposed to high dose rate, single fraction irradiation, the appearance of mammalian cell survival curves is not up for debate. It becomes a straight line when plotted on a semilog plot, where the x-axis represents the dose and the y-axis represents the logarithm of clonogenic survival. This was validated time and time again in the context of normal cells in vivo, tumour cells in vitro, and tumour cells in a lab. The survival curve is well-formed by a curved fit to the data at the more clinically relevant dose range normally utilised in CFRT.

Theoretical Frameworks

Because it allows us to anticipate how reality will behave in situations where data points are scarce, a mathematical model is helpful for simulating the reality of experimental data. The mathematical models that have been employed to

describe the survival curves of mammals are numerous. First, there is the empiricist perspective, and second [7-10], there is the mechanistic method. A key component of the empirical method is the capacity to use elementary mathematical functions to characterise experimental data. The ability to describe the underlying mechanism is of paramount importance in a mechanistic approach. Although the original explanatory model, which involved numerous critical targets leading to cell death, has been discarded, the historical mechanistic target theory parameters, D0 and N, are still empirically utilised to define high dosage survival curves. The ideal model would be easy to use, fit the experimental data well, and explain the mechanism at work; these are the two most important criteria [11-15]. It is intriguing to note that our understanding of how radiation destroys cells remains limited, which poses challenges when attempting to construct mechanistic models.

Quadratic Linear Model

In the field of radiation survival cure fitting, the Linear Quadratic model (LQ model, alpha-beta model) is among the most widely used methods. Up to 8-10 Gy and in the CFRT range, it performs admirably. Though it is probably oversimplified, the original model—which used a linear and quadratic term to describe the survival curve—fits the data very well in the shoulder region and lends itself well to rational explanations of how chromosomal aberrations are induced, whether by single or double events, and whether or not this leads to cell death. Mathematical ease and its connection to the biologically effective dose (BED) formalism, which establishes a relationship between effective doses, are its appealing features. Potentially, the LQ model could work well in therapies that use more than one modality [14-17], in cases where there are effects that aren't visible in CFRT regimens (such as when the endothelial cells and microvacular function regulate tumour cell radiosensitivity) or when new targets for immune modulation mediate antibody-dependent cytotoxicity. A survival curve may be better fit by a LQ model with a supporting mechanism if it reveals such threshold effects and an increasing effectiveness per unit dose when this threshold dose is crossed.

Recommendations for SBRT Dose Models

The fundamental LQ model demands a constantly bending curve, which is not feasible for the SBRT dose range. Consequently, it is unable to adequately represent the curve. There have been several efforts to develop a mathematical model that can explain survival in the high dose range commonly employed in SBRT. There are two distinct methods, once again. Attempting to preserve mathematical simplicity at the expense of explaining the underlying mechanism is one strategy. We can still characterise and compare radiosensitivity and forecast results when parameters are varied, so it may still be valuable even if we can't understand the mechanism. Alternatively, we may simplify the mathematics by making certain adjustments to the LQ model so that it better fits the SBRT scenario, although this would increase its explanatory power at the expense of simplicity. In most cases, this is accomplished by adjusting the beta term, which causes the LQ curve to become more linear. Although this modification is usually somewhat mathematically complex, it is valuable since it can provide more insight into the mechanism. A model that embraced the first method, which is descriptive, was the Universal Survival Curve model. A straight line in the high dose range and the LQ model in the shoulder are piecewise combined at a point called the transition dose [18-21], creating an abrupt yet differentiable junction. Its benefits include that the LQ model is maintained in the CFRT range, that the SBRT range uses well-defined target theory parameters D0 and N, and that both regions are quite simple. The capacity to create a straightforward, equivalent dosage formalism that evaluates two distinct SBRT dose-fractionation algorithms is the model's strong suit. On the other hand, some have pointed out that the sudden transition makes it mathematically ugly, especially as the data fit at the transition dose range might not be as good as the data fit in either of the clinically important regions. The generalised linear quadratic model (gLQ) was one model that used the second, explanatory, method. We begin our derivation on the mechanistic premise that, within the SBRT dose range, the pool of sublethal lesions is exhausted and transformed into permanent damage. This flattens the curve by adding a reduction term to the dose rate factor. The reason behind the straightening of the survival curve may be better understood with the help of this model or its more extensive variants. The more thorough version also lets you foretell how changing other simulation parameters will play out. The danger of drawing too broad conclusions from experimental results and assuming overly complex or deceptive mechanisms is inherent to every mechanistic method [22, 23]. Words like "sublethal lesions" have not yet been fully defined, for instance. Goodness of fit to existing experimental data allows one to compare models using either an empirical or a mechanistic approach. A successful

model will fit the data well across the relevant dose range and produce accurate results when extrapolated and interpolated from the data that is already available. But the intended usage is more crucial [24, 25]. Different models would be preferred by programmers simulating the effects of a heterogeneous, ablative radiation dosage and by physicians doing back-of-the-envelope calculations to generate isoeffect dose-fractionation schemes.

Resolving Biological Barriers to SBRT

There are biological barriers that, if removed, would allow for more widespread use and improved efficacy of SBRT, even though we may expect to see more technological advancements in dose delivery. Negative reactions in healthy tissues and low oxygen levels in tumours are two of the biggest obstacles in biology. Every one of them will be covered thoroughly.

The Response of Normal Tissues

Four patients with tumours located centrally (within two cm of the bronchial tree) may have died as a result of the high rate of late toxicity (Grade III) that emerged by the end of year two in the landmark study using SBRT to treat lung tumours. This was due to the inability to exclude certain normal tissues within the lung. Participants in this trial underwent radiation treatment for up to 66 Gy spread out over two weeks. Local tumour control remained above 80% despite these side effects. For tumours positioned in the centre, it is possible to increase the number of fractions and decrease the dose per fraction (5 fractions of 8-10 Gy), although this results in a lower local control rate. Since ablation of tubular tissues affects all downstream structures, the argument goes [26-29], this kind of unfavourable reaction could be the result of the responsive tissue's design. There are three potential causes of these serious side effects, which restrict the use of full power SBRT in treating non-small cell lung cancer (NSCLC) in clinical settings: (1) Irradiation of an excessively large partial volume of a sensitive structure with a dosage equivalent to or lower than the mean dose supplied to the target volume. Variations in cellular radiosensitivity between individuals;(1) irradiation of a small volume of the target structure with a high dose owing to inhomogeneity in the dose distribution, which is more noticeable in SBRT than in CFRT settings;(2) optimisation of the dose distribution in the target volume being more important than the integral dose delivered outside the target volume; and(3) large integral doses in large volumes. In the short term, the normal tissue toxicity indicated earlier can be mitigated by limiting therapy to distal tumours. Radiotherapy, especially high-dose-per-fraction techniques like SBRT and CFRT, can be effective, although typical tissue toxicity can hinder its use. Radiation pneumonitis is a model of a typical normal tissue response. V20, the lung volume receiving 20 Gy or more, is a predictor of pneumonitis, and the risk of pneumonitis increases with treatment volume during CFRT. Interestingly, the incidence of pneumonitis was very low in two trials that used SBRT. It is possible that the low V20 values for SBRT in comparison to CFRT explain why the pneumonitis observed was sporadic across all dosage levels. Lung fibrosis is a similar case. In the lung parenchyma surrounding the treatment site, lung fibrosis at high doses per fraction can develop as a late reaction; nonetheless [30, 31], it is as likely to occur with large doses of CFRT. To keep tumour management intact while avoiding normal tissue harm, two options are available: (a) Clarification of the typical responses of tissues to SBRT. A more reasonable characteriszation of tolerable quantities and dosages can be achieved through this enhanced understanding. (b) Achieving tumour control while taking medication to lessen radiation's negative effects on healthy tissues, without compromising on this goal.

Radiation safety

Among the first radioprotectors, amifostine (Ethyol) is the only one to have received FDA approval. According to clinicaltrials.gov, 74 studies were conducted using amifostine in conjunction with radiation therapy, chemotherapy, or combination therapy, with the majority of these trials operating outside of the restrictions set by the FDA for this drug. Acute myelogenous leukaemia, breast cancer, prostate cancer, and malignancies of the head and neck are among the cancers that are targeted. The traditional pharmacologic radioprotector is amifostine. It has to be present at or around the time of irradiation because of its reputation as a radical scavenger. Additionally, it is thought to possess properties that prevent mutations. Anaphylactic reactions have been reported in extremely uncommon instances involving amifostine and CFRT, for instance, due to the former's toxicity. Radiation can cause cumulative exposure effects such as severe hypotension, vomiting, allergic responses, weakness, lethargy, erythema, or fever; as a result, cessation rates as high as 40% have been recorded after each daily radiation portion when administered as a bolus or subcutaneously

[32-36]. The possibility of tumour sparing has also raised some concerns. As a result, researchers have conducted two meta-analyses: one to find out how much of an impact amifostine had on response rates in non-small cell lung cancer (NSCLC) and another to see how much of an impact amifostine had on late normal tissue effects (xerostomia). Amifostine was found to considerably decrease radiation side effects in one meta-analysis, but to have no influence on tumour response rates in the other. A radio/chemotherapy study for head and neck squamous cell cancer found little effect on normal tissue toxicity, however individual studies assessing normal tissue response outside of xerostomia are often positive. Adding amifostine to SBRT regimens may have further benefits. The fact that SBRT is not a long-term, daily regimen suggests that radioprotectors such amifostine may be better tolerated. In a research conducted by Koukourakis (2002), post-operative breast cancer patients were treated with a hypofractionated and expedited radiotherapy schedule, which was aided by the administration of Amifostine. Each of the twelve daily fractions was preceded by a 1,000 mg infusion of amifostine in this instance. Amifostine is usually administered to patients at doses ranging from 300 to 500 mg/m2. Only seven percent of patients were able to stop using amifostine, according to the authors. Results from this study indicate that short-term administration of high dosages is tolerable. To mitigate the potential toxicity of SBRT to healthy tissues, preclinical investigations evaluating the radioprotective effects of amifostine administered via big bolus or infusion following high doses per fraction are necessary. On the other hand, a C3H mouse model found a delayed radioprotective effect by increasing MnSOD, also known as SOD2. This kind of delayed response would be great for CFRT-treated normal tissue, but Grdina et al. also demonstrated increased MnSOD levels in the transplantable murine sarcoma SA-NH 24 hours after amifostine injection. Grdina et al. recommended limiting amifostine administration to every 72 hours to prevent tumour radioprotection, which could be an advantage for SBRT, and this result starts the debate about potential tumour radioprotection.

Lessening the Impact on Healthy Tissues

With the DHS's focus on radioprotection and mitigation for first responders and the general public, this method is particularly appealing for the development of new or improved agents. The first surge of free radicals generated by radiation exposure is not what a mitigating chemical aims to target, unlike amifostine. Alternatively, the proliferation and differentiation of epithelial cells can be stimulated to reduce the risk. To illustrate this point, consider keratinocyte growth factor; another example would be the regulation of pro-inflammatory cytokines and chemokines that trigger the recruitment of macrophages in response to late-stage normal tissue injury. These factors can persist for a long time after radiation treatment has ended. In addition, while minimising free radical damage during radiation has always been a priority, recent research suggests that hypoxia and oxidative stress might be to blame for radiation damage that manifests later on, so it makes sense to continue targeting free radicals even after treatment has ended. Compounds like AEOL10150, which imitate SOD but may have other, less understood chemistries, and the expression of superoxide dismutase (SOD) both provide protection against radiation damage. The use of radioactive doses to ablate tumours while limiting normal tissue in the field is one logistical advantage of SBRT that could be useful for radioprotectors and radio-mitigators. Another advantage is that therapy only takes two weeks instead of six, which means that normal tissue can recover in between fractions. Lastly, RT fractions are not given daily [37-41], so normal tissue can recover faster. Because they wouldn't have to be administered every day, radioprotective chemicals with their own set of toxicities might be employed more successfully in a shorter treatment duration, allowing for a higher dose to be administered. For improved tolerance, consider implementing a recovery interval between fractions. However, as SBRT shortens the duration of treatment overall, mitigating drugs could be administered early.

SBRT and Hypoxia in Tumours

A major obstacle to the efficient use of high dose per fraction radiation to treat lung tumours is tumour hypoxia. Conventional wisdom held that "diffusion-limited" hypoxia occurred when big, locally progressed solid tumours were isolated from the normal vasculature and subjected to a persistent drop in oxygen content. New research has significantly complicated this oversimplified theory by showing that acute hypoxic responses can be caused by perfusion-limited hypoxia as well as diffusion-limited hypoxia, by transient blockages or changes in blood flow. The fact that hypoxic tumours are far more resistant to chemotherapy and radiation is now commonly acknowledged. Recent research has shown that tumour hypoxia is complex and ever-changing, and that tumour cells exposed to even mild hypoxia may be far more resistant to radiotherapy and chemotherapy than cells in anoxic or extremely hypoxic environments. How tumours adapt to low oxygen levels has been the subject of a large amount of research. The

growing body of research suggests that hypoxic tumours have a distinct range of gene expression patterns and DNA damage responses (DDRs), which could impact tumour resistance to radiation or chemotherapy and potentially promote a more aggressive tumour model. The gold standard for treating hypoxic tumours in the past has been traditional hyper-fractionated radiation. There has been no experimental or clinical determination of the efficacy of hypofractionated radiation regimens like SBRT in the context of tumour hypoxia. In contrast to traditional hyper-fractionated radiation [42, 43], SBRT is anticipated to have a far lower efficacy in managing hypoxic tumours. Hypoxic tumours pose significant obstacles to both conventional and hypo-fractionated radiation treatments. On the other hand, their hypoxia-adaptive responses could present some interesting possibilities for improving SBRT for hypoxic tumours.

One Possible Risk Factor for the Failure of Stereotactic Body Radiation Therapy (SBRT) Is Tumour Hypoxia

The small number of fractions used in SBRT raises concerns that it might not be able to effectively treat hypoxic tumours. The conventional wisdom holds that the tumor's hypoxic, radiosensitive areas can be targeted and destroyed in successive fractions of radiation, while the tumor's hypoxic, radioresistant cores can be gradually exposed to oxygen gradients that increase their radiosensitivity. With hypo-fractionated radiation, the tumor's re-oxygenation and radiosensitization won't happen as quickly. Therefore, hypoxic tumours may be less effectively treated with SBRT hypo-fractionated regimens. Mathematical models indicate that compared to many fractions of radiation, hypo-fractionated radiotherapy may be three orders of magnitude less effective in reducing clonogenic survival. The impact of oxygen on radiosensitivity, especially at high doses, is another argument against SBRT for hypoxic tumours. When exposed to ionising radiation, cells in the body release free radicals, which then chemically react with DNA to form DNA radicals. Many DNA lesions, most notably DNA double strand breaks (DSBs), are produced when DNA radicals are fixed (made permanent) in an oxygen-containing environment. By comparing the radiation dose needed to achieve a surviving [44-46] fraction in an oxygen-free environment with the dose needed to achieve the same surviving fraction in an oxygen-containing environment, the oxygen enhancement ratio (OER) measures the impact of oxygen on tumour cell radiation sensitivity. The OER for tumour cells falls around between 2.5 and 3.0. The OER is much greater with high radiation doses ([6-8 Gy]), according to the studies. The majority of SBRT protocols use fractions with extremely high doses (22-30 Gy) every time.

Significant DNA damage and radiosensitivity are more likely to occur at these fractional dosages, which increase the oxygen need. Clinical investigations have also demonstrated that individuals with tumours placed in the centre are at increased risk of potentially fatal toxicity, even with regimens with such high doses per percent. Tumour control is negatively impacted by merely reducing the dose per fraction of SBRT. As a result, tumor-selective reduction of radioresistance is an obvious requirement to supplement SBRT. Radiosensitizing strategies like these need to take tumour hypoxia into consideration for them to work.

Eliminating Hypoxia from Tumours

Various methods for making hypoxic tumours more radiosensitive have been developed in the past few decades. First, there is the idea of reversing hypoxia with oxygen. Second, there are agents that imitate oxygen. Third, there are pro-drugs that activate when hypoxia is present. Fourth, there are agents that target HIF-1a. Fifth, there is a developing notion called contextual synthetic lethality. But, it should be mentioned that the majority of these trials used CFRT in conjunction with the specific hypoxic cell radio-sensitizers. Looking again at these approaches could reveal SBRT-specific advantages that could be tested in future clinical or preclinical trials.

Gases of Oxygen and Substitutes for It in the Treatment of Hypoxia

Several preliminary clinical studies utilised hyperbaric oxygen chambers to provide fractionated radiation to patients, driven by the understanding that hypoxic tumours are radioresistant. An increase in blood oxygen levels was thought to have the desired effect of radiosensitizing tumours by increasing their availability of oxygen. Part of the reason these experiments failed was that they failed to take into consideration the then-unknown idea of dynamic variations in perfusion-limited tumour hypoxia. Radiosensitization, which involves chemical molecules that mimic oxygen, became a focal point in the early 1960s. The premise that nitroimidazole-based medicines like metronidazole, misonidazole, and etanidazole could radiosensitize hypoxic tumours by reacting with and stabilising radiation-induced DNA free radicals was the basis for their development.

These hypoxic cell sensitizers showed impressive effectiveness in lowering the OER of several tumours from 3.0-3.5 to 1.5 in preclinical cell line-based and animal models, without affecting normoxic cells' radiosensitization. In the context of SBRT, one important finding from these animal research stands out. Although it was unfathomable for clinical use at the time, most investigations found that combining metronidazole with a single big dose of radiation (about 25 Gy) significantly reduced tumour radiosensitivity. On the other hand, depending on the intervals between the radiation fractions, a combination of several fractions of low dosage radiation had only a small effect [47, 49]. When tested in conjunction with CFRT, the medications failed to improve radiation therapy in clinical studies, and toxicity at effective concentrations was a key drawback. A later meta-analysis did find that nitroimidazole with radiation improved local control slightly more than radiation alone, but the difference was statistically significant. The radiosensitizing activity of nimorazole, a second-generation nitroimidazole molecule, was independent of fraction size, and compared to other radiosensitizers, it has a less steep dose-response relationship. These two facts have rekindled interest in the drug, especially in Denmark. Nimorazole is water-soluble, which means it can be used at extremely low doses without causing any harm. When combined with radiation, nimorazole has demonstrated impressive efficacy in treating head and neck cancer in clinical trials. Compared to individuals who received a placebo in addition to radiation, those who had nimerazole and CFRT had far better five-year loco-regional control with much less toxicity, according to a multi-center double-blind Phase III study including 422 patients with head and neck cancer.

Although nimorazole and other medications may improve conventional hyperfractionated radiation, additional research is needed to determine whether radiosensitizers that were successful with single high doses could be even more effective when added to SBRT.

Examining Tumour Hypoxia with the Use of Pro-Drugs

The initial generation of nitroimidazole medications were quite dangerous, so researchers started looking for alternatives. These new compounds may be inactive pro-drugs in oxygen-containing cells, but when they were in an oxygen-free condition, they could selectively harm hypoxic tumours. Molecular oxygen is required for the formation of superoxides by these medications in oxic cells. They are transformed into free radicals when oxygen is not present; they serve as substrates for one electron reductases. Tiravasamine, AQ4N, and NLCQ-1 are three such pro-drugs that are presently undergoing clinical studies, with reports of toxicity for two of them. The loco-regional control of 54 patients with Stage III and IV squamous cell head and neck carcinoma who were selected for hypoxia and who received tirapazamine in addition to radiotherapy or cisplatin was significantly lower than that of patients who received either radiation or chemotherapy alone, according to phase II clinical trials. Nevertheless, tirapazamine did not show any advantage when added to chemotherapy or radiation in a randomised multinational Phase III trial with about 861 patients undergoing treatment for head and neck cancer who were not selected for hypoxia. Based on the available evidence [50-53], it appears that the synergistic effects of pro-drugs in conjunction with conventional radiotherapy are only observed in tumours with severe hypoxia. In tumours with normal oxygen levels, the medication has no effect and conventional radiotherapy has only a modest effect on tumour control. When coupled with high dose per fraction radiation, these pro-drugs may have an even greater impact; after all, SBRT's radiation component has shown better loco-regional control than traditional radiotherapy on its alone.

Using HIF-1 as a Target in Hypoxic Cancers

Cancer cells respond to low oxygen levels in various ways. The stabilisation and activation of the transcription factor hypoxia-inducible factor 1 (HIF-1) is an important part of the adaptive response to hypoxia. HIF-1 causes apoptosis and necrosis to occur, as well as p21/p27 mediated cell cycle arrest at the G1/S checkpoint and a drastic shift in global gene expression and metabolic pathways. One reason HIF-1a has become a promising therapeutic target is the significant role it plays in tumour hypoxia. Clinical data demonstrating a distinct advantage of combining HIF-1a inhibitors with chemotherapy or radiation has not yet converged, despite the fact that numerous candidate inhibitors of HIF-1a demonstrate substantial sensitisation of hypoxic tumours to these treatments in pre-clinical investigations using cell lines and animal models.

Aiming at the Hypoxic Tumor-Specific DNA Damage Response

Using the DDR pathways specific to hypoxic tumours could be a technique to overcome radiation resistance caused by hypoxia. Cells undergo a cascade of events known as DNA damage response (DDR), which includes DNA repair, cell cycle arrest, apoptosis, senescence, and necrosis in reaction to DNA double-strand breaks (DSBs) caused by genotoxic stress. The two primary routes for DSB repair, homologous recombination (HR) and non-homologous end-joining repair (NHEJ), work hand in hand. The DNA-end binding Ku complex, the protein kinase DNA-PKcs, putative DNA-end processing enzymes (e.g., Artemis, Wrn, Tpd1), and the DNA ligase, IV/XRCC4/XLF complex are all involved in the DNA-end processing and ligation that occurs during NHEJ, which is active throughout the cell cycle. HR is a more precise and time-consuming technique, but it can only work in the late stages of the cell cycle (S/G2) and requires an unbroken sister chromatid. HR starts with the Mre11/Rad50/Nbs1 (MRN) complex recognising DSBs, then RPA is bound to a single strand, Rad52 is loaded and RPA is displaced, then Rad54, Rad51, and Rad51 paralogs generate strand invasion and D-loops. Other components, including Brca1, Brca2, and CtIP, play ancillary functions. Tumour DDR complexity changes dramatically between low and extremely high radiation exposures. Moreover, the onset, maintenance, and release of radiation-induced cell cycle arrest are facilitated by the Ataxia telangiectasia mutant protein (ATM) and the ATM and Rad3-related (ATR) proteins. Hypoxic tumours exhibit a distinct spectrum of DDR compared to oxic cells, as has been seen by multiple groups.

One piece of evidence is that hypoxic cells do not undergo DNA double-strand breaks. Secondly, although there is brief hypoxia (<12 h), the survival of the organism depends on HR, not NHEJ. In contrast, DNA-PKcs and other NHEJ components are significantly up-regulated under extended hypoxia (>12 h), while other HR pathway components, including MRE11 and RAD50, are down-regulated. As a result of hypoxia, ribonucleotide reductase activity decreases, leading to nucleotide insufficiency. This, in turn, causes DNA synthesis to quickly come to a stop and cell cycle arrest through replication fork-bound RPA and activation of the ATR/Chk1/Cdc25 pathway. Interestingly, ATM is phosphorylated in a manner that is independent of DSB and MRN complexes, and this activity activates Chk2 kinase. However, the ATM-Chk2 pathway fails to induce cell cycle arrest. Under hypoxia, 53BP1 foci do not form because no DNA DSBs are present, but cH2AX foci can be found at locations of replication forks that have stalled, which is in sharp contrast to IR, which causes the creation of both 53BP1 and cH2AX foci at DNA DSBs. A DDR shift involving a substantial down-regulation of HR enzymes is triggered by persistent hypoxia, according to the accumulating evidence from these investigations.

A Method for Radiosensitizing Hypoxic Tumours Using Contextual Synthetic Lethality

Mutations in BRCA-1 and BRCA-2 severely impair the HR DNA repair mechanism in cancer. In the particular setting of HR deficiency, two seminal investigations established substantial synthetic lethality linked to inhibitors of Poly ADP-Ribose Polymerase (PARP). In tumours that lack BRCA1 or BRCA2, the teams of Bryant et al.(2005) and Farmer et al.(2005) demonstrated that inhibiting PARP had a strong and selective anticancer effect. The conditional lethality of PARP inhibition in HR-deficient cells is driven by NHEJ up-regulation, as recently shown by Patel et al. (2011). Thirdly, Johnson et al. (2011) found that cells that are BRCA-proficient become more sensitive to PARP inhibition when CDK1 activity is impaired. Hypoxic tumours' distinctive DDR is strikingly similar to the HR-deficient state common in BRCA-deficient malignancies. There is strong evidence in favour of using a synthetic lethality strategy comparable to PARP inhibition to target hypoxic tumours. First, a condition analogous to that of BRCA-deficient cells is achieved when HR enzymes are drastically down-regulated by prolonged hypoxia. The second possibility is that hypoxic tumour cells' selective activation of NHEJ enzymes can enhance a synthetic lethality effect analogous to PARP inhibition. Third, synthetic lethality could target a wider spectrum of tumours that have an HR deficiency in hypoxia, rather than just a subset of mutations like BRCA, due to the simultaneous down-regulation of numerous components of HR in this environment. Lastly, hypoxic tumours with down-regulated ChK1 could mimic the synthetic lethality-friendly conditions seen in BRCA-positive cells. Radiotherapy and such a contextual synthetic lethality technique may have a very synergistic effect.

Because SBRT targets the tumor's oxic parts, and synthetic lethality can radio-sensitize its hypoxic sections, a synthetic lethality technique paired with high dose per fraction SBRT is expected to be highly effective in tumour control. The main drawback of SBRT for treating lung malignancies is its toxicity, especially in cases when the tumour is located in the centre of the lung. Reduced tumour control may occur as a result of dose reduction efforts to reduce toxicity, particularly when tumour hypoxia is present. Because the decreased fraction number could potentially

hamper re-oxygenation, tumour hypoxia presents a particular problem in the treatment of lung malignancies with SBRT. A safer and more successful approach to treating lung malignancies with SBRT with a decreased dose per fraction may be possible thanks to the advantages of combining hypoxic cell radiosensitizers. A strong case exists for taking advantage of hypoxic tumours' DNA damage response. There was very little harm, at least when PARP inhibition was considered. To confirm whether this method, when coupled with SBRT, is safe and successful in controlling hypoxic tumours, further clinical and preclinical trials are obviously required. At the same time, it might be worthwhile to look at alternative approaches, like nimerazole, an oxygen mimic, or HIF1a inhibitor. It would appear that nimerazole is the sole hypoxic cell radiosensitizer that, when coupled with radiation, is both effective and safe, at least according to the Head and Neck cancer experience in Denmark. It seems that hyperfractionated radiation did not work with drugs like metronidazole unless the patient was given extremely high and harmful doses of the drugs. It may be necessary to reevaluate their efficacy when used in conjunction with SBRT, though. Combining these medications with a single high-dose of radiation had impressive results, at least in preclinical trials, even at low, non-toxic concentrations. We may soon be able to better evaluate tumour hypoxia and its role in radiation failure because to developments in noninvasive hypoxia tumour imaging. It is important to conduct clinical trials on individuals specifically chosen for tumour hypoxia in order to assess the effectiveness of hypoxia-radiosensitizing techniques. Tirapazamone is just one example of how randomised trials in populations with both hypoxic and well-oxygenated tumours can produce misleading data about prognosis. Patients with lung cancer who are at risk for harm from SBRT could benefit greatly from a technique that eliminates hypoxia-associated tumour radio-resistance.

REFERENCES

1. Abraham RT (2001) Cell cycle checkpoint signaling through the ATM and ATR kinases. Genes Dev 15:2177–2196
2. Abraham RT (2004) PI 3-kinase related kinases: ‘big’ players in stress-induced signaling pathways. DNA Repair (Amst) 3:883–887
3. Begg AC, Sheldon PW, Foster JL (1974) Demonstration of radiosensitization of hypoxic cells in solid tumours by metronidazole. Br J Radiol 47:399–404
4. Fu XL, Huang H, Bentel G, Clough R, Jirtle RL, Kong FM, Marks LB, Anscher MS (2001) Predicting the risk of symptomatic radiation-induced lung injury using both the physical and biologic parameters V(30) and transforming growth factor beta. Int J Radiat Oncol Biol Phys 50:899–908
5. Fujino M, Shirato H, Onishi H, Kawamura H, Takayama K, Koto M, Onimaru R, Nagata Y, Hiraoka M (2006) Characteristics of patients who developed radiation pneumonitis requiring steroid therapy after stereotactic irradiation for lung tumors. Cancer J 12:41–46
6. Garcia-Barros M, Paris F, Cordon-Cardo C, Lyden D, Rafii S, Haimovitz-Friedman A, Fuks Z, Kolesnick R (2003) Tumor response to radiotherapy regulated by endothelial cell apoptosis. Science 300:1155–1159
7. Grdina DJ, Murley JS, Kataoka Y, Baker KL, Kunnavakkam R, Coleman MC, Spitz DR (2009) Amifostine induces antioxidant enzymatic activities in normal tissues and a transplantable tumor that can affect radiation response. Int J Radiat Oncol Biol Phys 73:886–896
8. Greijer AE, van der Wall E (2004) The role of hypoxia inducible factor 1 (HIF-1) in hypoxia induced apoptosis. J Clin Pathol 57:1009–1014
9. Guerrero M, Li XA (2004) Extending the linear-quadratic model for large fraction doses pertinent to stereotactic radiotherapy. Phys Med Biol 49:4825–4835
10. Hackenbeck T, Knaup KX, Schietke R, Schodel J, Willam C, Wu X, Warnecke C, Eckardt KU, Wiesener MS (2009) HIF1 or HIF-2 induction is sufficient to achieve cell cycle arrest in NIH3T3 mouse fibroblasts independent from hypoxia. Cell Cycle 8:1386–1395
11. Hammond EM, Giaccia AJ (2005) The role of p53 in hypoxiainduced apoptosis. Biochem Biophys Res Commun 331: 718–725

12. He J, Luster TA, Thorpe PE (2007) Radiation-enhanced vascular targeting of human lung cancers in mice with a monoclonal antibody that binds anionic phospholipids. Clin Cancer Res 13:5211–5218

13. Henk JM, Bishop K, Shepherd SF (2003) Treatment of head and neck cancer with CHART and nimorazole: phase II study. Radiother Oncol 66:65–70

14. Hill RP, Marie-Egyptienne DT, Hedley DW (2009) Cancer stem cells, hypoxia and metastasis. Semin Radiat Oncol 19: 106–111

15. Johnson N, Li YC, Walton ZE, Cheng KA, Li D (2011) Compromised CDK1 activity sensitizes BRCA-proficient cancers to PARP inhibition. Nat Med 17:875–882

16. Kang S (2003) Overexpression of extracellular superoxide dismutase protects mice against radiation-induced lung injury. Int J Radiat Biol Oncol Phys 57:1056–1066

17. Kataoka Y, Basic I, Perrin J, Grdina DJ (1992) Antimutagenic effects of radioprotector WR-2721 against fission-spectrum neurons and 60Co gamma-rays in mice. Int J Radiat Biol 61:387–392

18. Kataoka Y, Perrin J, Hunter N, Milas L, Grdina DJ (1996) Antimutagenic effects of amifostine: clinical implications. Semin Oncol 23:53–57

19. Kavanagh BD, Newman F (2008) Toward a unified survival curve: in regard to Park et al. (IntJ Radiat Oncol Biol Phys 2008;70:847–852)

20. Krueger et al. (Int J Radiat Oncol Biol Phys 2007;69:1262–1271) Int J Radiat Oncol Biol Phys 71:958–959

21. Kawase T, Kunieda E, Deloar HM, Seki S, Sugawara A, Tsunoo T, Ogawa EN, Ishizaka A, Kameyama K, Takeda A, Kubo A (2007) Experimental stereotactic irradiation of normal rabbit lung: computed tomographic analysis of radiation injury and the histopathological features. Radiat Med 25:453–461

22. Komaki R, Lee JS, Milas L, Lee HK, Fossella FV, Mooring D, Tucker SL, Cox JD (2004) Effects of amifostine on acute toxicity from concurrent chemotherapy and radiotherapy for inoperable non-small-cell lung cancer: report of a randomized comparative trial. Int J Radiat Oncol Biol Phys 58:1369–1377

23. Koukourakis MI (2002) Hypofractionated and accelerated radiotherapy with amifostine cytoprotection (HypoARC): a new concept in radiotherapy and encouraging results in breast cancer. Semin Oncol 29:42–46

24. Koukourakis MI, Patlakas G, Froudarakis ME, Kyrgias G, Skarlatos J, Abatzoglou I, Bougioukas G, Bouros D (2007) Hypofractionated accelerated radiochemotherapy with cytoprotection (Chemo-HypoARC) for inoperable non-small cell lung carcinoma. Anticancer Res 27:3625–3631

25. Li C, Jackson RM (2002) Reactive species mechanisms of cellular hypoxia-reoxygenation injury. Am J Physiol Cell Physiol 282:C227–C241

26. Lin A, Lawrence TS (2006) An anaphylactoid reaction from amifostine. Radiother Oncol 79:352

27. Maj JG, Paris F, Haimovitz-Friedman A, Venkatraman E, Kolesnick R, Fuks Z (2003) Microvascular function regulates intestinal crypt response to radiation. Cancer Res 63:4338–4341

28. Marks LB (1995) Extrapolating hypofractionated radiation schemes from radiosurgery data: regarding Hall et al., IJROBP 21:819–824; 1991 and Hall and Brenner, IJROBP 25:381–385;

29. McGarry RC, Papiez L, Williams M, Whitford T, Timmerman RD (2005) Stereotactic body radiation therapy of early-stage non-small-cell lung carcinoma: phase I study. Int J Radiat Oncol Biol Phys 63:1010–1015

30. McKenna F, Ahmad S (2009) Toward a unified survival curve: in regard to Kavanagh and Newman (Int J Radiat Oncol Biol Phys 2008;71:958–959

31. Mell LK, Malik R, Komaki R, Movsas B, Swann RS, Langer C, Antonadou D, Koukourakis M, Mundt AJ (2007) Effect of amifostine on response rates in locally advanced non-small-cell lung cancer patients treated on randomized controlled trials: a meta-analysis. Int J Radiat Oncol Biol Phys 68:111–118

32. Moulder JE, Cohen EP (2007) Future strategies for mitigation and treatment of chronic radiation-induced normal tissue injury. Semin Radiat Oncol 17:141–148

33. Mueller-Klieser W, Schlenger KH, Walenta S, Gross M, Karbach U, Hoeckel M, Vaupel P (1991) Pathophysiological approaches to identifying tumor hypoxia in patients. Radiother Oncol 20(Suppl 1):21–28

34. Muniyappa H, Song S, Mathews CK, Das KC (2009) Reactive oxygen species-independent oxidation of thioredoxin in hypoxia: inactivation of ribonucleotide reductase and redoxmediated checkpoint control. J Biol Chem 284:17069–17081

35. Overgaard J (1994) Clinical evaluation of nitroimidazoles as modifiers of hypoxia in solid tumors. Oncol Res 6:509–518

36. Overgaard J, Overgaard M, Nielsen OS, Pedersen AK, Timothy AR (1982) A comparative investigation of nimorazole and misonidazole as hypoxic radiosensitizers in a C3H mammary carcinoma in vivo. Br J Cancer 46:904–911.

37. Puck TT, Marcus PI (1956) Action of X-rays on mammalian cells. J Exp Med 103:653–666

38. Rades D, Fehlauer F, Bajrovic A, Mahlmann B, Richter E, Alberti W (2004) Serious adverse effects of amifostine during radiotherapy in head and neck cancer patients. Radiother Oncol 70:261–264

39. Rischin D, Peters L (2005) Tirapazamine, cisplatin, and radiation versus fluorouracil, cisplatin, and radiation in patients with locally advanced head and neck cancer: a randomized phase II trial of the trans-tasman radiation oncology group. J Clin Oncol 23:79–87

40. Rischin D, Peters LJ, O'Sullivan B, (2010) Tirapazamine, cisplatin, and radiation versus cisplatin and radiation for advanced squamous cell carcinoma of the head and neck: a phase III trial of the trans-tasman radiation oncology group. J Clin Oncol 28:2989–2995

41. Robbins ME, Diz DI (2006) Pathogenic role of the reninangiotensin system in modulating radiation-induced late effects. Int J Radiat Oncol Biol Phys 64:6–12

42. Rubin P, Johnston CJ, Williams JP, McDonald S, Finkelstein JN (1995) A perpetual cascade of cytokines postirradiation leads to pulmonary fibrosis. Int J Radiat Oncol Biol Phys 33:99–109

43. Saha D, Watkins L, Yin Y, Thorpe P, (2010) An orthotopic lung tumor model for image-guided microirradiation in rats. Radiat Res 174:62–71

44. Sasse AD, Clark LG, Sasse EC, Clark OA (2006) Amifostine reduces side effects and improves complete response rate during radiotherapy: results of a meta-analysis. Int J Radiat Oncol Biol Phys 64:784–791

45. Semenza GL (2007) Evaluation of HIF-1 inhibitors as anticancer agents. Drug Discov Today 12:853–859

46. Semenza GL, Shimoda LA, Prabhakar NR (2006) Regulation of gene expression by HIF-1. Novartis Found Symp 272:2–8

47. Thompson LH, Schild D (2002) Recombinational DNA repair and human disease. Mutat Res 509:49–78

48. Till JE, Mc CEA (1961) A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat Res 14:213–222

49. Timmerman RD, Story M (2006) Stereotactic body radiation therapy: a treatment in need of basic biological research. Cancer J 12:19–20

50. Timothy AR, Overgaard J, Overgaard M (1984) A phase I clinical study of Nimorazole as a hypoxic radiosensitizer. Int J Radiat Oncol Biol Phys 10:1765–1768

51. Vujaskovic Z, Anscher MS, Feng QF, Rabbani ZN, Amin K, Samulski TS, Dewhirst MW, Haroon ZA (2001) Radiationinduced hypoxia may perpetuate late normal tissue injury. Int J Radiat Oncol Biol Phys 50:851–855

52. Wang JZ, Huang Z, Lo SS, Yuh WT, Mayr NA (2010) A generalized linear-quadratic model for radiosurgery, stereotactic body radiation therapy, and high-dose rate brachytherapy. Sci Transl Med 2:39ra48

53. Weterings E, Chen DJ (2008) The endless tale of nonhomologous end-joining. Cell Res 18:114–124.