The radiobiological targets of SBRT: tumor cells or endothelial cells?

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Abstract: The development of stereotactic body radiation therapy (SBRT) and stereotactic radiosurgery (SRS) techniques has revolutionized the practice of radiation oncology. The radiobiological targets that alter the therapeutic response to SBRT remain a subject of debate. The prevailing perspective has been that the radiation-induced damage to endothelial cells and changes in microvasculature facilitate tumor response to SBRT. A provocative study by Moding *et al.* (PMID: 25761890), challenged this notion by elucidating the role of tumor cells versus endothelial cells in mediating sarcoma eradication following high-dose SBRT. Using dual recombinase technology, they generated primary sarcomas in genetically engineered mouse models (GEMMs). They also modulated the apoptotic pathway and radiosensitization profile using targeted mutations in either tumor cells or endothelial cells. Unlike transplanted tumor models, the findings here suggest that deletion of the pro-apoptotic gene *Bax* or of the DNA-damage response gene *ATM* in endothelial cells did not result in tumor eradication to high dose SBRT, despite extensive endothelial cell death. On the other hand, genetic targeting of *ATM* gene in tumor cells achieved local sarcoma control and tumor eradication. These findings imply that tumor cells rather than endothelial cells act as prime targets affecting a tumor eradication response to SBRT. The translational implications of these findings are of great potential significance. When targeting endothelial cells, delivery of SBRT irradiation can only result in tumor growth delay. The benefit of targeting *ATM* in this setting will be radiation dose dependent. Curative intent, tumor eradication and local control, on the other hand, are only possible by targeting tumor cells with high dose SBRT (50 Gy in 1 fraction) and with radiosensitization by *ATM* deletion. In the absence of radiosensitization, only palliation is possible with high dose SBRT. Whether these provocative findings can be extrapolated to other translational tumor models or proved valid in clinical trials remains the subject of future studies. The mechanisms by which tumors compensate to SBRT's endothelial cell damage, such as new vascular recruitment, and/or recruitment of other immune and stromal components, are also critical questions for the field of radiobiology to address. Such mechanistic understanding of the key cellular players mediating SBRT response in a model system that recapitulates human disease will be essential in designing targeted radiosensitizers ultimately aimed at improving the therapeutic ratio.

Keywords: Stereotactic body radiation therapy (SBRT); stereotactic ablative radiotherapy; stereotactic radiosurgery (SRS); dose; hypofractionated; radiosensitization; endothelial cell death; *ATM*

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Remarkable advances in the field of medicine and imaging diagnostics have led to the emergence of such techniques as stereotactic body radiation therapy (SBRT) and stereotactic radiosurgery (SRS). The advent of SBRT and SRS has brought a paradigm shift in the field of radiation therapy practice. Typically, with SRS and SBRT, cancer patients can now be effectively treated with a small number of high radiation doses. Dramatic improvements in tumor control have been achieved in several clinical studies following high-dose radiation therapy (1). However, the success of SBRT

has raised questions with regard to the radiobiological targets affecting tumor response following high-dose radiation therapy. The efficacy of SBRT irradiation in the curative setting also remains a question of significant clinical interest.

An elegant study by Moding *et al*., recently published in *Science Translational Medicine*, challenged the prevailing hypothesis that endothelial cells act as main contributors to radiation response in a sarcomatous mouse model (2). Their data provide provocative evidence supportive of a model whereby tumor cells, rather than endothelial cells, mediate SBRT cell killing of sarcomatous tumors (2). They also underscore the importance of using radiosensitizers in combination with high dose SBRT radiation for curative tumor eradication.

It has been well documented that a tumor microenvironment comprised of extracellular matrix, carcinoma-associated fibroblasts, immune cells, and endothelial cells plays a critical role in tumor initiation, progression, and metastatic spread (3). Changes in the tumor microenvironment also could have a marked impact on the therapeutic response in tumor cells (4,5). However, our understanding of the response of the tumor microenvironment, including the fate of microvasculature to high-dose SBRT, is still rudimentary. For a long time, the prevailing perspective in the field of radiation biology was that the ultimate outcome of SRS/SBRT is largely governed by radiation-induced damage inflicted on the endothelial cells and tumor vasculature (4,5). However, studies in the field of radiation biology have advanced our current knowledge, and alternate theories have emerged.

The study by Moding *et al*. used an ingenious dual recombinase technology to generate primary sarcomas in genetically engineered mouse models (GEMMs) with selective mutations in either tumor cells or endothelial cells (2). The remarkable use of GEMMs allowed for tumor formation in the native environment in immunocompetent mice. Unlike transplanted tumor models, GEMMs preserve the tumor stroma and microenvironment of human cancers more faithfully and can be advantageous in predicting the therapeutic response.

Since previous reports suggested that endothelial cell death and microvascular damage played a role in tumor control following radiation therapy (4,6,7), the authors mutated the proapoptotic gene *Bax* in endothelial cells of GEMMs. They observed that *Bax* deletion in endothelial cells did not enhance radiation-induced endothelial cell apoptosis or, more importantly, tumor response to SBRT (2).

Perhaps more intriguing was the ultimate lack of effect on local *in vivo* tumor control in their model system when altering the radiosensitization profile of endothelial cells, despite evidence of induction of cell death. They showed that targeted deletion of the *ATM* gene, a master regulator of DNA damage response pathway (8), in endothelial cells resulted in increased cell death following high-dose SBRT (2). However, this failed to translate into *in vivo* difference in tumor eradication (failure to triple in size after 18 weeks of radiation treatment) or local control (absence of tumor volume tripling) outcomes between the animals where endothelial cells were *ATM* deleted versus the control group. The same finding was observed with a single high SBRT dose of 50 Gy in one fraction or with hypofractionated SBRT dose of 20 Gy in 4 fractions.

To appreciate this finding, one has to examine the earlier work of Moding and colleagues using different dosing and fractionation regimens (9). They showed that if endothelial cells are targeted at SBRT dosing of 20 Gy or a more conventional fractionation of 30 Gy in 10 fractions, improved tumor growth delay (55% longer time to tripling in size) is seen if radiosensitization is employed through *ATM* gene deletion (9).

The above findings suggest a distinction between tumor eradication and tumor growth delay upon targeting endothelial cells and highlight the importance of radiation dose and of using radiosensitizers. Importantly, they carry potentially translational significance, particularly given that targeting endothelial cells with anti-angiogenic agents such as VEGF inhibitors is not uncommon. Targeting endothelial cells is never curative, as it will not eradicate sarcomas even if *ATM* deletion is present. However, if palliation or growth delay is the intent of treatment, then targeting endothelial cells will be of benefit. Whether or not radiosensitization (*ATM* deletion) is necessary in palliative treatment when endothelial cells are targeted will simply depend on the radiation dose being used. At dosing of 20 Gy in a single fraction or at conventional fractionation of 30 Gy in 10 fractions, radiosensitization by targeting *ATM* would be necessary. However, at dosing of 50 Gy in a single fraction or 20 Gy in 4 fractions, targeting *ATM* in endothelial cells will not add any more benefit.

Strikingly different results were obtained in tumor cells. Specifically, Moding and colleagues showed that altering the radiosensitization profile by targeted *ATM* deletion in tumor cells resulted in a significant tumor eradication following high-dose SBRT (2). This was evident both in the *in vitro* and the *in vivo* model systems used in this study. Without radiosensitization and in the control group, only tumor growth delay in response to 50 Gy can be observed.

Two provocative findings are generated from these results. First, it is tumor cells rather than endothelial cells that act as important targets mediating sarcoma eradication by SBRT (2). Second, and equally important, tumor eradication in the high dose SBRT setting is only achieved in combination with radiosensitizers such as *ATM* inhibitors (2).

This is provocative as, for example, up to one third of patients with medically inoperable early stage lung cancer are treated with high dose SBRT per practice guidelines (10). Similarly, in the treatment of solitary or oligometastasis, high dose SBRT is used in the absence of radiosensitizers (11). The results by Moding and colleagues, as they aptly note out in their discussion, are limited to sarcomas, and the biological effects of high-dose SBRT may vary based on tumor type or target tissue (2). It nevertheless raises the question of whether better clinical outcomes would be achieved if high dose SBRT were coupled with radiosensitizers in other tumor models.

Whether altering the radiosensitization profile in sarcoma cells by methods other than *ATM* deletion or other non-DNA repair pathways would have resulted in tumor eradication remains to be shown. It would have been interesting for the authors, for example, to examine whether targeting *Bax* in sarcoma cells would have had a similar effect on local control as that observed with *ATM* deletion. When the pharmacological inhibitor of *ATM* was used, a significant increase in TUNEL staining was observed, suggesting increased cell death (2). Translationally, it would be important to determine whether combining the targeting of the apoptotic pathway with high dose SBRT would have resulted in tumor eradication.

Multiple theories exist for compensatory responses negating the effect of endothelial cell damage on tumor control, some of which were discussed in the manuscript. These are imperative, as they shed light on potential combination therapies that could improve local control to SBRT by overcoming compensatory responses to endothelial cell damage. The contribution of other stromal cell population might be one responsible mechanism for tumor relapse following high-dose radiation therapy. Strong evidence suggests that immune cell components plays a role in mediating anti-tumorigenic effects in response to SBRT (12). This theory is supported by the generation of a tumor microenvironment that can elicit an immunological response (1,7). Thus, to uncover the immune aspects of SBRT-mediated killing of tumor cells, it would be important to target the immune cells and determine the effects in response to high-dose SBRT.

Similarly, revascularization of tumors following radiation therapy from outside the radiation field is another factor that could explain sarcoma recurrence despite high dose SBRT. In an earlier study, Moding and colleagues (9) validated that endothelial cell death that accompanies radiosensitization mediated by *ATM* deletion, translated into a functional change in the vasculature in the irradiation field 24 h following treatment with a single 20 Gy dose. One can assume that, at the curative 50 Gy (or 20 Gy in 4) dosing reported in this manuscript (2), similar revascularization is present within the radiation field when radiosensitization is utilized. In mice where tumors recurred despite endothelial cell death and radiosensitization, the source of neovessels in relapsing tumors could be surviving endothelial cells still capable of establishing a tumor vasculature during post-radiation recurrence (13). This, however, is unlikely, given the high curative dosing used here. Whether recruitment of "distal" stroma from outside the field in the form of inflammatory bone marrow-derived cells (13) plays a role in this context remains a subject for future study.

Cancer stem cell clearance could also be a pivotal factor contributing to the tumor response following SBRT (7) that could negate any effect of endothelial cell damage. Cancer stem cells have been shown to occupy the perivascular niche in tumors (12). These cells display an increased activation of AKT/mTOR pathway regulating cell proliferation and cell survival (12). Previous studies have reported that these cancer stem cells confer radioresistant characteristics and might be responsible for tumor recurrence following fractionated radiotherapy (7,12). Following a low dose of radiation (2 Gy), these perivascular cells show cell cycle arrest within 6 hours of irradiation but re-enter cell cycle and start proliferating in 72 h, ultimately affecting the treatment response (7). Thus, one of the implications of high-dose SBRT could be the ablation of this self-renewing population of radioresistant cancer stem cells, leading to tumor growth eradication (7).

The clinical applicability of the findings shown in this manuscript will be limited largely by concern over radiosensitization of normal tissues by direct targeting of *ATM*. When pharmacological inhibition of *ATM* was used following whole heart irradiation, they showed that radiosensitization there is far less impressive than it is in sarcoma cells (2). It is important to note, though, that the dose there was only 20 Gy in a single fraction and not the "curative" 50 Gy in a single fraction. A single dose of 20 Gy, however, only resulted in growth delay, not tumor eradication, despite the presence of pharmacological inhibitor of *ATM*. Similarly, they showed in their earlier work that

ATM deletion does not radiosensitize heart cells at 20 Gy in a single fraction (9). For radiosensitization to occur with *ATM*, the cells have to be proliferating and progressing through the cell cycle (9). In other words, loss of *ATM* does not affect all tissues equally. At doses of 50 Gy in a single fraction required for tumor eradication, the therapeutic index would therefore be largely determined by the volume of tumor and proximity to critical structures, particularly proliferative, non-quiescent tissue. The requirement of such a high dose of 50 Gy in single fraction or 80 Gy in four fractions in combination with *ATM* targeting would likely be prohibitive in the clinical setting. Testing the benefit of other radiosensitizers that may not be as prevalent in normal tissue in a similarly elegant manner would be of clinical importance.

In short, the findings documented by Moding *et al*. (2) have challenged a fundamental assumption of SBRT radiobiology. The mechanistic understanding provided by using such systems as GEMMs, which better recapitulates human disease, allows for designing future studies aimed at improving tumor control outcomes. Studying key compensatory mechanisms that could explain the inherent lack of tumor control when endothelial cells are targeted with high dose SBRT will be critical for developing better therapeutic strategies. Immunotherapy, blockade of tumor-promoting effects of TGF-β, and targeting tumor revascularization from outside the radiation field could provide potential therapeutic benefit when combined with radiosensitizers and high dose SBRT. Finally, this study challenges the current practice of using high dose SBRT alone in a curative intent setting without radiosensitizers. Future studies in other tumor models aimed at expanding the generalizability of these findings into translational models, particularly those where high dose SBRT remains the standard of care, are warranted.

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Footnote

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