

Brain metastases: Radiobiological, molecular and biochemical approach

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ABSTRACT

Radiotherapy has made remarkable technological progress in recent years. The accuracy of radiotherapy has improved significantly, and accordingly, the treatment of tumors with high-dose radiation has become possible. Stereotactic radiosurgery has become a rapidly accepted method for the treatment of solid small-sized tumors. Compared to conventional fractionation radiotherapy, stereotactic radiosurgery with a very high dose per fraction and hypofractionated radiotherapy provides satisfactory therapeutic efficiency with low toxicity as tumor cells can be ablated directly with this method. Stereotactic radiosurgery is known to induce radiobiological changes by playing an important role in tumor control, vascular endothelial damage and immune activation. Yet, the literature lacks a comprehensive review on the effects of stereotactic radiosurgery on molecular, genomic and biochemical structures. In this review, we discuss the role of radiobiology in stereotactic radiosurgery of brain metastases, radiobiological factors, genomic profile of brain metastases and biochemical factors.

Keywords: Biochemistry, brain metastases, genomic profile, molecular, radiosurgery.

Stereotactic radiosurgery (SRS) treatment of brain metastases is the most common indication in many treatment centers, but it is still in its experimental stage in glioma models. The response of glioma cell lines to varying doses of radiation also provided a platform for the response to SRS.^[1-4]

The role of SRS in terms of its benefit in controlling brain metastases has been well characterized in several Phase III randomized trials. The radiation therapy oncology group (RTOG) 9508 is a Phase III randomized study in which 333 patients with 1-3 brain metastases received either whole-brain radiotherapy (WBRT) at a dose of 37.5 Gy or a combination of WBRT and SRS. Intracranial metastasis control was superior in the combined modality arm.^[5] Another randomized study compared SRS with

the combination of WBRT and SRS in 132 patients with 1-4 brain metastases. In this study, combining WBRT with SRS alone had no overall survival benefit or differences in neurological deaths.^[6] Chang et al.^[7] reported a similar study comparing SRS with and without WBRT. They found that neurocognitive outcomes in patients receiving combined WBRT and SRS were worse in the fourth month.

Patients with brain metastasis should not be approached as a homogeneous group. The tumor histology varies greatly with different responses to treatment. Molecular sub-characterizations of breast cancer, melanoma, and lung cancer have changed treatments and outcomes.^[7] Focusing on the number of brain tumors has been an important inclusion criterion for almost all previous clinical trials, yet this factor may

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become less important when considering total tumor volume.

MOLECULAR PROPERTIES IN PROGNOSTIC CLASSIFICATION

Given its uniform association with poor clinical outcomes, brain metastases are a very common manifestation of cancer, which in the past was treated as a single disease. More sophisticated prognostic models and disease-specific treatment paradigms are being developed as knowledge about the biology and molecular basis of brain metastases are becoming clear. The therapeutic procedure in brain metastases proceeds from whole-brain radiotherapy and surgery to include SRS, targeted therapies, and immunotherapies, which are often used sequentially or in combination. Advances in neuroimaging have provided opportunities to accurately screen for the intracranial disease at initial cancer diagnosis, to precisely target intracranial lesions during treatment, and to help distinguish the effects of treatment from disease progression by incorporating functional imaging. Given the large number of treatment options available for patients with brain metastases, a multidisciplinary approach is strongly recommended to customize each patient's treatment to improve the therapeutic rate.^[8]

In the modern era, new prognostic criteria include data not only on primary tumor histology but also on clinically relevant molecular changes. Studies have shown that it provides important prognostic information in the classification of brain metastatic non-small cell lung cancer (NSCLC) patients in the presence of epidermal growth factor receptor (EGFR) mutation or anaplastic lymphoma kinase (ALK) rearrangement.^[9,10] Analogically, the presence of BRAF gene mutation has dramatic implications for prognosis in patients with brain metastatic melanoma.^[11-13] In patients with brain metastatic breast cancer, the presence of estrogen receptor (ER), progesterone receptor (PR) and HER2 are very important markers for the course of the disease.^[8]

GENOMIC PROFILE OF BRAIN METASTASIS

The genetic events that cause tumor progression and the spread of the disease to the

intracranial compartment have recently begun to be understood. Next-generation sequencing technologies provide important clues to better understand the genomic changes that cause cells from the primary tumor to spread to the brain and form metastases.^[14] Whole-exome sequencing of tissue samples from the patient enabled the identification of clinically relevant genetic changes. Using this information as “targetable” in treatment may increase intracranial response rates and provide disease control.^[15] Research in this area has significantly expanded our understanding of potentially actionable genetic changes in brain metastases and how they differ not only from primary tumors but also from those associated with extracranial metastases. For example, in a whole-exome sequencing-based analysis of 86 brain metastases that matched primary tumor and non-malignant tissue samples,^[16] 53% of brain metastases had at least one actionable change that was not detected in the primary tumor. In these mutations, there were changes in susceptibility to *CDK4/6* inhibitors in 51%, *PI3K-AKT-mTOR* inhibitors in 43%, and HER2 and/or EGFR inhibitors in 33%.^[16] Results from another study involving 61 patients with resected brain metastases from NSCLC have shown that the changes in *cyclin-dependent kinase (CDK)* and *phosphoinositide 3-kinases (PI3Ks)* signaling pathways were significantly different compared with primary tumor biopsies.^[17] Although these results have not impacted clinical practice to date, they have considered potentially a practice change and ultimately are decisive for the next generation of clinical trials. Multiple *CDK4/6* inhibitors have been approved for patients with hormone receptor-positive advanced-stage breast cancer, and a Phase II study (NCT02896335), which has been specifically designed to evaluate the role of *CDK4/6* inhibitors in patients with brain metastases, has been investigating the efficacy of palbociclib. In addition, targeting activating mutations in the *PI3K-AKT-mTOR* signaling pathway by administering a pan-AKT inhibitor has shown activity in a mouse xenograft model of brain metastatic breast cancer.^[18] Next-generation sequencing is increasingly used in clinical practice to guide the administration of systemic therapies; by this means, analysis of tissue samples from brain metastases could further

improve treatment choice. Given the impressive response rates with inhibitors of *NTRK1-3* and *ROS1*-encoded kinases in patients with and without brain metastases, tumor genomics can now provide dramatic and lasting responses in these patient subsets.^[19] An innovative designed genomic study (*NCT03994796*), sponsored by the National Cancer Institute, to investigate the efficacy of a variety of targeted therapies in patients with brain metastases as a result of solid tumors, includes categorizing patients according to the molecular changes detected in their brain metastases (in genes encoding components of *CDK* or *PI3K* signaling pathways, or in *NTRK1-3* or *ROS1*). Following the identification of specific changes, targeted therapy matching the primary endpoint of the objective response rate is administered.^[18]

THE 4R'S OF RADIOBIOLOGY IN SRS

Tumor cells with lethal damage would lead to deoxyribonucleic acid (DNA) breakage and cell death under conventional radiotherapy. However, tumor cells with non-lethal damage/potentially lethal damage would self-repair and continue to proliferate after a certain time due to insufficient radiation dose, resulting in tumor recurrence and metastasis.^[20,21] Since radiosensitivity is associated with the number of unrepaired residual DNA double-strand breaks, repair compromises the efficiency of radiation and reduces the radiosensitivity of tumors.^[22]

During SRS, high doses of radiation are administered per fraction and total doses are delivered in 2-5 fractions in a relatively short time, resulting in more necroptosis than apoptosis.^[23,24] Therefore, repair of tumor cells is almost impossible or occurs with a very low incidence. Thus, most tumor cells would undergo lethal damage leading to cell death.^[24,25]

SRS and redistribution

After irradiation, tumor cells in the G0 phase of the cell cycle would accelerate to the G2/M stage for rebalancing.^[26,27] Tumor cells in the G2/M phase are highly sensitive to radiation. During conventional radiotherapy, the sensitivity of radiation potentially increases as the proportion of tumor cells in the G2/M phase increases.^[28] Therefore, cell cycle redistribution improves the killing ability of multiple fraction conventional

radiation therapy.^[28] During SRS, the cell cycle is completely blocked at all phases after single high-dose ablation radiation. Therefore, redistribution of tumor cells is impossible, as both sensitive and insensitive tumor cells are killed directly.^[26]

SRS and reoxygenation

Given that oxygenated tumor cells are sensitive to radiation during conventional radiotherapy, tumor cells in a hypoxic state would be re-oxygenated and killed by radiation. Therefore, reoxygenation increases the killing effect in conventional fractionated radiotherapy.^[25] Reoxygenation can be reduced due to the relatively short duration of SRS. In addition, tumor hypoxia may persist after vascular damage caused by SRS.^[29,30] In such cases, increasing the radiation dose may provide a solution to overcome hypoxic radioresistance.^[31] Both oxygenated and hypoxic cells are ablated with high-dose radiation under SRS, resulting in highly efficient tumor death.

SRS and repopulation

Conventional radiotherapy leads to instability of cell populations and sensitive tumor cells rapidly enter a state of apoptosis. At the onset of homeostasis, fixed-stage tumor cells would proliferate to compensate for the loss of cell populations. Depending on fractionated radiation doses, total doses, and pathological types, repopulation of tumor cells usually occurs 2-3 weeks after conventional fractionated radiotherapy, with increased radiation resistance and reduced killing effects.^[25] The SRS treatment scheme is usually within 2-5 fractions and is completed within oneweek without the tumor cells having time to initiate the repopulation process.^[32,33]

For this reason, the 4R's of radiobiology contributes little to the killing effects of SRS as most of the tumor cells are ablated. Different patterns of intrinsic radiosensitivity between cells and tissues may play an important role in the tumor response demonstrated by Bergonie and Tribondeau^[34] in 1906. While the intrinsic radiosensitivity of tumor cells represents a component attributed to the therapeutic outcome of conventional multiple fraction radiotherapy, more research is needed for SRS.^[35] Based on the 4R's of radiobiology, the fifth R was first proposed by Steel et al.^[36] and emphasized

the intrinsic radiosensitivity of tumor cells that correlates with the radiation sensitivity of tumor cells. Brown et al.^[25,37] preferred the 5R's of radiobiology; however, this raised the question of whether there are any radiobiological factors yet to be identified.

DOSE-EFFECT RELATIONSHIP MODELS IN SRS

The linear-quadratic model (LQ model) can be applied in the treatment of cancers with conventional radiotherapy to calculate equivalent doses.^[38] The alpha/beta (α/β) ratio reflects the extent of biological effects on tissues and cells affected by fractionated radiation doses.^[39] Alpha/beta (approximately 10 Gy) in early responding tissue/tumor is greater than that in late responding tissue/tumor (approximately 3 Gy).^[40] The prerequisite for LQ model application is complete oxygenation of tumor cells with a fractional dose of less than 1-6 Gy during radiation.^[25] When the fractional dose is higher than 8-10 Gy, the LQ model is not suitable for estimating the effects of radiation.^[38,41] However, some clinical studies have found that the LQ model underestimates tumor control by the SRS.^[41,42] In 2004, to fully describe the biological effects of high dose per fraction, Guerrero and Li^[43] proposed improving the LQ model and offered the modified LQ model (MLQ model). In 2008 Park et al.,^[44] in 2010 Wang et al.^[45] introduced the general LQ model (gLQ model) that includes the entire dose range. However, the relationship between the biological effects of high-dose radiation per fraction and actual clinical efficacy could not be comprehensively explained by these models because indirect effects such as radiation-induced damage to blood vessels were not included.^[45]

POTENTIAL RADIOBIOLOGICAL FACTORS OF SRS

Stereotactic radiosurgery delivers high doses of radiation to directly destroy tumors.^[46] Recent clinical studies have confirmed that SRS not only directly eliminates tumor cells, but also induces indirect effects, including vascular endothelial damage and immune activation. Indirect tumor cell death caused by SRS plays a crucial role in tumor killing.^[47]

Vascular endothelial damage

As a homeostatic factor, endothelial apoptosis regulates angiogenesis-dependent tumor growth, which occurs only at radiation doses above 8-11 Gy.^[48] Other studies have found obvious vascular damage under high-dose radiation, particularly above 10 Gy, leading to hypoxia, acidification of the tumor microenvironment, and indirect death of tumor cells.^[49,50] High-dose radiation delivered by SRS increased vascular permeability and apoptosis along the ceramide pathway.^[51] Vascular endothelial damage intensified platelet aggregation and thrombosis, which further occluded the blood vessel. Also, blood vessel damage and ischemia caused by high-dose radiation lead to tumor necrosis. As a result, the anti-tumor effect of radiotherapy increased.^[48]

Immune activation

Radiotherapy directly or indirectly activates such inflammatory cytokines as IL-1 and TNF, collects immune cells, causing an extensive CD8 (+) T-cell tumor infiltration and loss of myeloid-derived suppressor cells.^[52] Tumor cells are ablated, and tumor antigens are largely secreted under high-dose radiation. This in turn leads to immunogenic cell death and waterfall-like release of tumor necrosis antigens and adenosine triphosphatase (ATPase). Activation and release of these substances enhance human immune responses and uptake of immune cells into the microenvironment.^[53] Based on the clarified immune mechanisms, a combination of radiotherapy and immune therapy has been developed for the antitumor therapeutic approach.^[54]

BIOCHEMICAL APPROACH TO BRAIN METASTASES

It has been proven that brain irradiation can cause glioma formation years after exposure, even in areas where low doses are absorbed, but in general, the risk of secondary malignancy increases with higher treatment volumes and doses. A recent study, in particular, revealed that glial tumors tend to develop more frequently after exposure to high LET particle radiation (protons and carbon ions) compared to X-rays.^[55] However, previous research has suggested that

the use of protons may reduce the risk of secondary malignancies under the condition of active scanning rather than passive scattering transmission mode.^[56] Conversely, a history of allergy appears to reduce the risk of human glioma, possibly due to increased levels of immunosurveillance.^[57] Other than radiation, many genetic conditions increase the risk of glioma formation.^[58]

An increased risk of brain cancer has been reported after microwave radiation exposure, particularly affecting military personnel exposed to microwave/radiofrequency radiation.^[59] Even studies that found no association between microwave radiation and brain tumors suggested more research into longer exposure times and larger sample sizes.^[60] Microwave exposure can cause both oxidative and nitrosative stress in the brain. Therefore, these data may point to the role of oxidative and nitrosative stress in gliomagenesis.^[61]

Air pollution can certainly lead to proinflammatory activation and oxidative stress in metastatic cells, but more reliable models are needed to investigate these effects.^[62] Similarly, smoking, including the passive form, has been reported to be associated with the development of glioma in young people.^[63] Environmental metals such as arsenic, nickel, chromium, lead, and cadmium acting through oxidative stress can cause DNA damage and cause multiple epigenetic changes.^[64] Lead and cadmium, which are known for their particularly massive oxidative DNA damage, have been associated with gliomas in industrial workers, but the evidence is yet inconsistent.^[65] Prolonged exposure to cadmium can cause a decrease in intracellular glutathione concentration and an increase in the size of oxidative DNA lesions in cells.^[66] Methylation pathways affect the survival of glioma patients, just as widespread hypermethylation of CpG islands is associated with a better prognosis.^[67] It is also a known fact that methylation of CpG islands in the promoter region of O(6)-methylguanine-DNA methyltransferase (MGMT) (which suppresses MGMT transcription) increases chemosensitivity to alkylating agents such as temozolomide. Therefore, DNA methylation profiling has been proposed as a useful tool for classifying tumors.^[68]

MOLECULAR MECHANISMS OF FREE RADICAL GENERATION IN TUMOR CELLS

Free radicals can form in the mitochondria or cytoplasm of metastatic cells. Mitochondria are the main active site of such reactive oxygen species (ROS) productions as superoxide, hydroxyl radical and hydrogen peroxide.^[69] In addition, superoxide can react with nitric oxide producing peroxynitrite (ONOO-) and hydrogen peroxide (H₂O₂) reacts with reduced transition metals to give a hydroxyl radical. Consistently, a high iron requirement has been detected in glioma cells.^[70] Malignant cells also produce structurally high concentrations of H₂O₂. The ROS-mediated mitochondrial DNA (mtDNA) damage can cause respiratory chain dysfunction, leading to higher levels of free radicals in cells.^[71] The free radicals formed as a result can act at close range, damage the membranes of normal cells, and activate tumor growth and formation.^[72] Cytokines also contribute to oxidative stress, and macrophages that secrete them have been shown to actively contribute to glioma invasion.^[73] On the other hand, mitochondria can effectively deal with ROS induced by inflammatory stimuli in glioma cells, confirming mitochondrial antioxidant defenses as a prospective therapeutic target.^[74,75]

Tumor cells produce high levels of ROS. In particular, glioblastoma stem-like cells (GSCs) produce high levels of oxidative stress, in part due to their high metabolic demands and hypoxic conditions.^[76,77] In glioma cells, such as complexes I and III of the respiratory chain that particularly harbors mtDNA mutations, can produce large amounts of ROS because the activity of complexes I and III appears to be elevated in a few glioma cell lines, while coenzyme Q10 (CoQ10) levels are found to be decreased relative to the control group.^[78]

ROS are also by-products of cyclooxygenase 2 (COX-2) activity, which plays a role in tumorigenesis and progression of glioblastoma. Its inhibition reduces growth and increases autophagy in U87MG and T98G cells.^[79] Most of these effects have been attributed to a reduced COX-2-dependent ROS generation.^[80]

The ROS supports epidermal growth factor (EGF), platelet-derived growth factor (PDGF),

PI3K/AKT and mitogen-activated protein kinase (MAPK) signaling. It can also inhibit apoptosis of the Rel family of transcription factors, nuclear factor-kappaB (NF- κ B) activated by itself, by inducing antiapoptotic genes.^[81]

Free radicals activate many factors that are beneficial for gliomas. ROS activates NF- κ B, while expression of NF- κ B has been shown to promote cell proliferation. NF- κ B is extremely beneficial in this regard as it is activated by a mild oxidative stress. It can protect cells against excessive oxidative stress by regulating superoxide dismutase 2 (SOD2). Activation of NF- κ B by many inducing stimuli can be inhibited by antioxidants. As another important transcriptional factor, Nuclear factor-erythroid 2 (NF-E2) p45-related factor 2 (Nrf2) has been shown to be activated by oxidative stress. Glutathione S transferase (GST) transcriptionally mediates the activation of GSH for enzyme-conjugating anticancer drugs and elimination.^[82]

Furthermore, ROS has an important function in metastasis formation. Oxidative stress regulates the expression of intercellular adhesion protein-1 (ICAM-1), induces matrix metalloproteinase (MMP13), MMP-3 and MMP-10 as well as MMP-2 and MMP-9.^[83] Likewise, NO stimulates the expression of several MMPs in gliomas.^[84] Induced by ROS-activated ERKs, Metalloproteinase-9 is involved in the invasion and migration of U87 glioma cells.^[85] Also, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase inhibitors and ROS scavengers reduce the invasion/migration of malignant glioma cells.^[86]

In addition, the immune system relies on oxidative stress to attack neoplastic cells. Activated NK cells, T lymphocytes and macrophages can kill neoplastic cells via toxic reactive oxygen species or TNF α , leading to hemorrhagic necrosis in tumors.^[87-89] Therefore, approaches to increase oxidative stress to facilitate tumor-killing should be examined for immune system involvement.

On the other hand, macrophages in the tumor microenvironment produce ROS and reactive nitrogen species (RNS), which lead to cancer progression. Whether ROS stimulates macrophages to kill glioma cells or exploit their growth depends on the macrophage functional phenotype. The dominance of M1 (inflammatory, antitumor responses) or M2 (cytoprotective,

immunosuppressive) macrophages in glioma may dictate pro-death or pro-survival ROS-mediated responses of macrophages to glioma cells.^[73,90-92]

Nitric oxide synthases (NOS), commonly expressed in metastases, and the primary type of RNS that interacts with O₂ yielding ONOO, and the nitric oxide (NO) radical use L-arginine to generate NO.^[93] Nitric oxide is more effective than vitamin E in quenching superoxide. Nitric oxide reacts with O₂ to form other oxides of nitrogen, such as nitrogen dioxide (NO₂), free radicals, which can react with NO to give N₂O₃ (dinitrogen trioxide). Also, a decrease in tetrahydrobiopterin results in the cleavage of NOS and the production of O₂ and free radicals (NOO) instead of NO, but its role in glioma biology remains largely unexplored. However, it is known that exogenous BH₄ can promote the proliferation of C6 glioma cells, suggesting the indispensability of properly functioning NOS in this process.^[94] Nitric oxide is more effective than vitamin E in quenching superoxide. Nitric oxide reacts with O₂ to form other oxides of nitrogen, such as NO₂, free radicals, which can react with NO to give N₂O₃ (dinitrogen trioxide). Also, a decrease in tetrahydrobiopterin (BH₄) results in the cleavage of NOS and the production of O₂ and NOO instead of NO, but its role in glioma biology remains largely unexplored. However, it is known that exogenous BH₄ can promote the proliferation of C6 glioma cells, suggesting the indispensability of properly functioning NOS in this process.^[94] Nitric oxide-depleting myeloperoxidase (MPO) can maintain long-term NOS activity by preventing NO feedback inhibition.^[95] On the other hand, myeloperoxidase has an important role in the host's defense against glioblastoma multiforme (GBM), as its inhibition worsens survival after radiation therapy in an animal model of glioblastoma.^[96] Therefore, NO is involved in numerous processes that promote glioma growth. Angiogenesis is involved in the maintenance, differentiation and therapeutic resistance of glioma-initiating cells.^[84] The RNS can also disrupt the Keap1-Nrf2 complex, leading to prolonged Nrf2 activation and, hence, tumor cell survival.^[97,98] Although oxidants play an indispensable role in glioma cell signaling and survival, they also make cells vulnerable to oxidative damage.

Cancer cells chronically develop higher levels of ROS than normal cells, but this may be offset by higher levels of total antioxidant capacity. Glioma neoplastic cells have high levels of some antioxidants (SOD2, CAT, GST, GSH) compared to normal cells. Both SOD and glutathione peroxidase play important roles in the susceptibility and protection of GBM cells to oxidative stress. The deficiency of these enzymes leads to ROS/RNS accumulation and cell death.^[99-101] Although glioblastoma cell lines (T98G, U87MG) were found to have more intracellular GSH than some other malignant cells, GSH levels were also found to be reduced in glioma compared to peritumoral tissue.^[102,103] Similarly, catalase has been found to be structurally expressed in gliomas, whereas its inhibition sensitizes rat 36B10 glioma cells to oxidative stress and radiation.^[104] Catalase detoxifies high levels of H₂O₂ in glioma cells, causing autophagic cell death.^[105]

Conclusion

The rational design of therapeutic strategies is highly dependent on a comprehensive understanding of the genomic context in which tumors arise and proliferate. Increasing awareness of genomic heterogeneity challenges previous assumptions that differences in response at metastatic sites reflect only pharmacokinetics. There is increasing recognition of genomic heterogeneity and molecular mismatch between primary tumors and brain metastases. Technological advances in SRS have improved outcomes, reduced treatment time, and mitigating adverse effects. With multidisciplinary treatment, the understanding of the effect of tumor biology on patient outcomes and toxicities improves, and an individualized treatment approach gains importance. Advances in precision medicine would enable us to better characterize the risk of brain metastases for each patient, predict a patient's prognosis and develop strategies to mitigate toxicities, as well as highlight the future need for molecular profiling by enabling the identification of potential future targets for intracranial guided therapies.

When the accumulation of free radicals exceeds their elimination, oxidative stress occurs. Both antioxidants and pro-oxidant approaches have been tried for the treatment of glioma,

specifically to reduce oxidative stress in the tumor environment and to induce excessive oxidative stress in tumor cell bodies. For this purpose, either strong antioxidants or strong oxidants can be administered. Mild antioxidants or oxidants can paradoxically protect glioma cells. Antioxidants may be particularly suitable for the prophylactic approach, thereby preventing cancerous signals.

Depending on the level of ROS/RNS and the type of ROS/RNS production, how they promote glioma cell survival or act as antitumor agents should be better understood. In particular, nitrosative stress has not been adequately studied in this context. Studies have shown that modulating endogenous antioxidant levels can pave the way for overcoming chemoresistance, while therapeutic levels of ROS can act in synergy with the Warburg effect inhibition.

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