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Therapeutic Targets in Malignant Glioblastoma Microenvironment

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Abstract

There is considerable evidence that the tissue microenvironment can suppress cancer, and that microenvironment disruption is required for cancer growth and progression. Distortion of the microenvironment by tumor cells can promote growth, recruit nonmalignant cells that provide physiological resources, and facilitate invasion. Compared to the variable routes taken by cells to become cancers, the response of normal tissue to cancer is relatively consistent such that controlling cancer may be more readily achieved indirectly via the microenvironment. Here, we discuss three ideas about how the microenvironment, consisting of a vasculature, inflammatory cells, immune cells, growth factors and extracellular matrix, might provide therapeutic targets in glioblastoma (GBM) in the context of radiotherapy (RT). First, that viable therapeutic targets exist in the GBM microenvironment; second, that RT alters the microenvironment of tissues and tumors; and third, that potential benefit may be achieved by targeting the microenvironments induced by RT.

Tissue microenvironments comprise cooperating cells that provide a complex milieu that supports and directs the specific functions of the organ parenchyma. In cancer there must be subversion of the normal microenvironment until the cancer itself becomes an aberrant organ. Cancer cells recruit, enlist and beguile normal cells to elude or alter signals from the microenvironment. By inducing aberrant behaviors of normal cells, cancer cells restyle the tissue to support their growth at the expense of the host. If the genesis of tumors requires the complicity of normal stromal cells, what changes in a tissue permit the growth of cancer? Is the process irreversible? What are the critical molecules and mechanisms? The recognition that tumor cells depend on tissue microenvironments provides the rationale for new therapies that target the microenvironment to interrupt or reverse this process. Furthermore, looking outside the genome provides a route that circumvents the cell-to-cell and patient-to-patient variability of genomic targets [1,2].

The brain is a highly structured tissue. The parenchyma consists of neurons, which are supported by the glial cells: astrocytes, oligodendrocytes and microglia. The tissue is supplied by a unique vasculature that creates a privileged site due to the blood-brain barrier. Glioblastoma (GBM) invades the parenchyma and destroys functional architecture, eventually superseding compensatory mechanisms to give rise to clinical consequences. There are several notable features of GBM. First, although extremely invasive, GBM rarely

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ever shows distant metastases. Second, GBM is characteristically highly and aberrantly vascularized. Third, these tumors are extremely resistant to radiotherapy and chemotherapy and eventually recur, almost always at the site of origin.

This review will focus on the GBM microenvironment in the context of radiotherapy (RT). Radiation oncology is predicated on two essential goals: to kill cancer cells while sparing normal tissue. This is achieved in part by taking advantage of the physical attributes of ionizing radiation (IR) using sophisticated planning and delivery techniques, which make it possible to deliver the radiation dose to the tumor while limiting the dose to surrounding healthy tissues that are sensitive to radiation [3]. Further benefit can be realized by understanding the biological response of tumors and the surrounding normal tissue so that they can be manipulated to increase tumor radiation sensitivity or inhibit deleterious normal tissue effects. We have proposed that the radiation-induced microenvironment is a key regulator of cellular responses, and that it could provide novel routes for manipulating the response to IR [4]. We will discuss viable microenvironment targets that can be disrupted therapeutically in GBM, then introduce the idea that RT alters the biology of tissues and tumors and conclude with speculations on potential benefits that may be achieved by targeting the microenvironments induced by RT in GBM.

Therapeutic inhibition TGF β in GBM

The extracellular growth factor transforming growth factor β (TGF β) is a constituent of the tumor microenvironment that mediates a neoplastic plexus, driving cancer cells toward more aggressive behaviors and supporting their survival, while simultaneously limiting suppression by the host and initiating normal tissue complications from therapy (reviewed in [5]). Many malignant cells secrete TGF β that acts on the host to suppress anti-tumor immune responses, to enhance extracellular matrix production, and to augment angiogenesis. TGF β is often elevated in the plasma of breast, lung, hepatocellular carcinoma and prostate cancer patients (reviewed in [6]). Levels of systemic TGF β have been used as a surrogate of tumor load and/or response to therapy because tumors, or the tissue response to tumors, is the presumed source [7,8]. Chronic exposure to TGF β can elicit phenotypic transformations under certain conditions, or in certain cells, leading to the mesenchymal-like transformation of mammary epithelial cells [9] or myofibroblast characteristics in stromal cells [10]. These and other aspects of TGF β biology make it a high-value target of the microenvironment.

In the case of GBM, there is a significant literature documenting the production of TGF β production in brain cancer, and evidence of its activity in experimental models in vivo. While the interest in TGF β inhibition as a targeted therapy for other tumor sites is relatively recent [11], there has been a decade of studies in GBM from a few laboratories that provide the rationale for TGF β inhibition. TGF β has a primary role in autocrine brain tumor growth regulation; notably, although TGF β 1 has some role, the predominant isoform in GBM appears to be TGF β 2. Using 12 glioma cell lines, Bogdahn and colleagues showed that exogenous TGF β 1 either stimulated or inhibited proliferation, whereas TGF β 2 stimulated the proliferation of all [12]. As in other tissues, TGF β appears to suppress proliferation in normal brain, but tumors are stimulated and produce significant amounts. However, inactivating mutations in the TGF β signaling pathway of GBM are infrequent.

As in other tumors, brain tumors develop resistance to the anti-proliferative effects of TGF β rather than decrease TGF β production, suggesting a survival advantage. Malignant gliomas escape from immune surveillance, which resides mainly in the T-cell compartment. This T-cell suppression has been attributed to the release by the glioma cells of immunosuppressive factors like TGF β and prostaglandins (reviewed in [13]). TGF β further compromises the

immune privileged brain since it can inhibit proliferation of lymphocytes, reduce immune cell activation, block anti-tumor activity, and inhibit antigen representation (reviewed in [14]). Adenoviral gene transfer of soluble TGF β receptor-II blocked ligand binding and reduced SMAD2 phosphorylation and TGF β -dependent reporter activity in human glioma cell lines [15]. Furthermore, soluble receptor expression enhanced glioma cell lysis by natural killer cells in vitro and markedly delayed growth of intracerebral LN-308 glioma xenografts in nude mice. Brain tumors have highly aberrant vascular beds. TGF β also has complex effects on vascular cells and angiogenesis [16]. Tumor-derived TGF β can also promote angiogenesis by inducing VEGF in both endothelial cells and glioma cells [17]. Mice in which TGF β is over expressed in the brain have an aberrant extracellular matrix [18]. A notable target of TGF β is tenascin, an extracellular matrix proteoglycan that is induced in brain tumors and has itself been used as therapeutic target [19].

TGF β 1 has been shown to promote the survival of embryonic, neonatal, and adult neurons, assist in neurite outgrowth, protect against experimental allergic encephalomyelitis, and inhibit microglial and astrocyte proliferation (reviewed in [20]). Homozygous deletion of TGF β 1 in mice results in embryonic death due to vascular defects or perinatal death due to gross inflammation [21]. Likewise, recent studies of the TGF β 1 null in a Rag2 null T and B-cell deficient background resulted in an extensive inflammatory response in otherwise uninjured brain parenchyma [22]. Gliosis, evidenced by increased astrocyte GFAP and CD44 immunoreactivity and abnormally proliferative activated microglia that express of phagocytic markers, was accompanied by local disruption in axonal transport and focal demyelination. Furthermore the response to brain injury was further compromised. There is some concern about the consequences of limiting the activity of a growth factor whose action is essential to normal development and that plays key roles in wound healing and inflammation. However, the key to rational targeting, as in most things, is moderation. The high levels of both protein and activity in the context of cancer elicit very different effects than those found in normal tissues where TGF β activation is highly controlled. Targeting TGF β in cancer therapy should seek to reduce, rather than eliminate, cytokine activity, which in doing so becomes a feasible objective with limited toxicity [5,23].

There has been significant progress using oligonucleotide TGF β 2 antisense compound AP12009 by Schlingensiepen and colleagues [24,25]. Initial studies in 12 glioma cell lines, employing phosphorothioate antisense oligodeoxynucleotides that specifically targeted the coding sequences of TGF β 1 or TGF β 2 mRNA, showed that TGF β 2 inhibition reduced the cell proliferation in all glioma cell lines, compared to controls, while TGF β 1 only affected five cell lines [12]. AP 12009 has been shown to specifically block TGF β 2 using in vitro assays of patient-derived malignant glioma cells as well as peripheral blood mononuclear cells. Recently this group reported AP 12009 treatment of patients with recurrent or refractory malignant WHO grade III or IV glioma in three Phase I/II-studies [25]. These investigators reported prolonged survival compared to literature data and response data and two patients experienced long-lasting complete tumor remissions. The authors suggest that the complete remissions as well as the observed time course are consistent with the proposed reversal of tumor-induced immunosuppression and the restoration of an effective anti-tumor immune response.

Therapeutic inhibition of aberrant vasculogenesis and invasion in GBM

Since malignant gliomas are highly vascular and express VEGF, targeting the vascular endothelium offers an interesting option in these patients. There is preclinical evidence suggesting that the blockade of VEGF can result in growth arrest and regression of the malignant glioma (Stefanik 2001). Advances in magnetic resonance imaging have allowed us to measure and quantify changes in tissue perfusion and the integrity of the blood-brain

barrier following VEGF blockade [26,27]. Bevacizumab (Avastin, Genentech), a humanized immunoglobulin G1 monoclonal antibody that inhibits VEGF, has shown its effectiveness in malignant glioma [28]. In two recently reported small Phase II trials in recurrent glioblastoma, the combination of Bevacizumab and irinotecan resulted in radiological response of 47–67% and a 6-month survival of 62–77% [29,30]. Based on these encouraging results, a small pilot study that looked at the feasibility of using Bevacizumab along with radiation therapy and Temozolomide in newly diagnosed gliomas was recently reported [31]. Radiographic responses were noted in 13 of 14 assessable patients. The pVEGFR2 staining was seen in 7 of 8 patients at the time of initial diagnosis. One-year progression-free survival and overall survival rates were 59.3% and 86.7%, respectively. Other clinical trials are underway to further evaluate the role of anti-angiogenic therapy in newly diagnosed gliomas.

Although the radiological and clinical responses are very impressive with anti-angiogenic therapy, the changes are transient and most patients with glioma recur within months. The predominant pattern of relapse is local, but with continued follow-up, the incidence of diffuse relapse increases. In a large series of 61 patients with recurrent high grade gliomas treated with Bevacizumab therapy to date, 35 of the 50 recurrences (70%) were local [31]. The remaining 15 patients (30%) recurred as extensive gliomatosis with a component of local recurrence. Biopsy sampling of the tumor following Bevacizumab therapy in five of these patients revealed a potential correlation between diffuse relapse and an invasive mesenchymal tumor phenotype as demonstrated by increased expression of D2–40 (podoplanin), CD34, and fascin, markers of mesenchymal expression, suggesting transition to a more invasive mesenchymal phenotype [32]. Similarly in newly diagnosed patients treated with Bevacizumab therapy, of the six patients who experienced relapse, three (50%) failed as diffuse disease indicating a similar transition [28]. A similar increase in a diffuse pattern of relapse has also been seen by other groups [33].

The impact of anti-angiogenic therapy on cell migration is not clear. It is possible that following an initial response, GBM tumor cells can migrate along the outside of blood vessels (perivascular invasion), using them as conduits into normal tissue, or infiltrate through the extracellular matrix [34]. In either case they depend on normal vasculature for sustenance. It is also recognized that gliomas have both stem cell and more differentiated tumor cell components [35,36]. It has been shown that blocking angiogenesis in an immunodeficient nude rat model result in angiogenic-independent GBM stem cell tumor growth with the up regulation of proinvasive genes [37]. A possible uncoupling of angiogenesis and invasion in these stem cells may result in a subsequent diffuse relapse too. Either way, a possible change in phenotype with therapy may have an impact on the choice and timing of the anti-angiogenic agent or the possible use of invasion blocking agents along with anti-angiogenic therapy in future trials.

A recent study conducted at Ohio State University found that lithium, which is a FDA approved drug for bipolar disease, potentially blocked glioma cell invasion in vitro using spheroid, wound-healing, and brain slice assays [38]. Lithium is known to activate $\beta\beta$ -catenin signaling [39]. The effects observed were dose dependent and reversible. In addition, there was little effect on cell viability at lithium concentrations that inhibited migration, showing that this was a specific effect. Lithium treatment was associated with a marked change in cell morphology, with cells retracting the long extensions at their leading edge, suggesting that the effects of lithium on glioma cell invasion could be mediated through glycogen synthase kinase-3 (GSK-3). GSK-3 is a protein kinase found in eukaryotes that regulate multiple processes, including metabolism, cell division and cell death. The role of GSK-3 in cancer cell migration is not clear yet. However, this study showed an inverse correlation between the degree of GSK-3 inhibition as measured by a luciferase reporter

assay of $\beta\beta$ -catenin transcriptional activation with the degree of invasion, suggesting a direct link between GSK-3 activity and the rate of glioma invasion. A multi-institutional phase II trial of bevacizumab with lithium, temozolomide and radiation therapy in newly diagnosed GBM will open soon (AN, personal communication). In an EORTC phase II trial, cilengitide, a V3 and V5 integrin inhibitor when used at 500mg IV 2 times/week with standard chemo-radiation in GBM was found to be well tolerated [40]. With this approach, they reported a median progression free survival of 7.9 months and a one-year survival of 63.5%.

These studies underscore that vasculature is the Achilles heel of tumors [1]. Furthermore, poorly regulated growth of tumor vasculature leads to contorted and ineffective aberrant vascular networks in GBM resulting in hypoxia.

Therapeutic inhibition of molecular pathways associated with hypoxia

Hypoxia is associated with resistance to both radiotherapy and chemotherapy [41] with important consequences in tumor biology. Over expression of HIF-1 is associated with a poor prognosis in several cancers (reviewed in [42]). Tumor cells sense and adapt to hypoxic environments by means of HIF-1, a heterodimeric nuclear transcription factor. HIF-1 consists of two subunits: HIF-1 α and HIF-1 β . The HIF-1 α subunit determines HIF-1 activity in response to changes in local O₂ levels. Under normoxic conditions, the α subunit of HIF-1 is rapidly degraded, thus rendering HIF-1 inactive. During hypoxia, however, HIF-1 α remains intact and binds to the constitutively expressed β subunit to form HIF-1 in the cell nucleus, where it induces many genes under the regulation of hypoxia response elements. Some of these genes encode for CXCR4 and its ligand, SDF-1 [43,44].

Previous studies by our group and others suggest that hypoxia stimulates glioma cell migration in vitro and invasion in orthotopic glioma models and human GBM [43,45,46]. HIF-1 α is commonly observed at the invading margins of human and murine brain tumors. For example, GFP labeling of GL261 glioma cells identified most HIF-1 α -expressing cells as those invading into the surrounding brain [46]. HIF-1 up regulates a variety of genes whose products play a well-established role in glioma invasions that include CXCR4, SDF-1, VEGF, and MMP [47].

SDF-1, also known as CXCL12, is a ligand of the chemokine receptor CXCR4, also known as fusin [48]. Knockout mouse experiments demonstrated that CXCR4 and SDF-1 are required for normal embryonic development of the nervous, gastrointestinal, hematopoietic, and cardiovascular systems [49]. Abundant experimental evidence suggests that the CXCR4/SDF-1 pathway is a crucial component of invasion in gliomas. Primary human GBM and glioma cell lines over express CXCR4 and SDF-1 [43,47,50]. SDF-1 has been shown to exert proliferative, anti-apoptotic, and chemotactic effects on glioma cell lines in vitro.

We and others have shown that hypoxia and HIF-1 α up regulate CXCR4 mRNA and protein in glioma cells and stressed the role of CXCR4 in glioma cell invasion [43,47]. For example, CXCR4-expressing human and rodent glioma cells demonstrate enhanced invasive capacity as compared to noninvasive tumor cells in vitro and in vivo [47]. We have previously shown that when exposed to hypoxia, glioma cells in which HIF-1 α expression was inhibited by RNA interference failed to show CXCR4 up regulation under hypoxic conditions while CXCR4 inhibition prevented migration [43]. Moreover, the capacity of CXCR4 to mobilize tumor cells has been linked to increased production of members of the matrix metalloproteinase (MMP) family [51]. MMPs are known to be overexpressed in gliomas, and their production has been shown to promote glioma cell invasiveness [52]. These studies indicate that the hypoxia and HIF-1-induction of CXCR4, the engagement of

the CXCR4 receptor by SDF-1 and CXCR4 signaling may mediate glioma invasion, at least in part, directly through up regulation of MMP production [43,47].

Focal adhesion kinase, a non-receptor-type tyrosine kinase, is expressed in most tumor and normal cells and is required for cell proliferation, migration, and survival (reviewed in [53]). Some studies have shown the association between FAK and vascularity [54], and proliferation and migration of gliomas. FAK can be activated upon ligand binding and clustering of integrin receptors as well as activation of other cell surface receptors such as EGFR or VEGF. FAK participates in multiple cell functions required for cell proliferation, survival, migration motility, and invasion. Experiments designed to disrupt FAK signaling, such as over expressing a kinase-dead FAK mutant resulted in anti-proliferative activity and reduced cell migration in vitro [55]. FAK also promotes secretion of MMPs while inhibition resulted in decreased levels of MMP secreted into the media and decreased invasion [55].

Previously, we have shown that several molecules are associated with glioma invasion, including integrins, FAK, and HIF-1 [46,56]. We showed that anti- $\beta 1$ and anti- αv integrin antibodies inhibited migration of glioma cell lines in vitro using an aggregate migration assay that can assess the invasive characteristic of gliomas. Second, immunohistochemistry of GBM with specific anti-FAK antibodies detected FAK expression primarily in the tumor periphery in infiltrating glioma cells. Furthermore, hyperplastic vessels demonstrate strong immunoreactivity for FAK. These results suggested that FAK might play a dual role in the invasion of glioma cells into the surrounding brain and in the angiogenic activity associated with the development of GBM.

Human gliomas contain mutations and deletions of the PTEN tumor suppressor gene, a negative regulator of PI3K signaling [57]. PTEN plays an important role in cell migration of a variety of cell types including glioma cells (reviewed in [58]). The loss of PTEN correlates with activation of Akt as assessed by positive immunostaining of the tumors for phospho-Akt. Thus, deregulation of the PI3K pathway is an important signaling pathway driving human gliomagenesis. Most growth factors exert their biological effects primarily by activating the ERK and the PI3K signaling pathways [59]. ERK1/2 belongs to the family of mitogen-activated protein kinase, which also includes stress-activated protein kinases/JNK and p38 MAPK. GBM show a high degree of ERK activation [60], while ERK activation is low in low-grade gliomas [61]. Treatment of CXCR4 positive glioma cells with SDF-1 ligand results in phosphorylation and activation of both Akt and ERK1/2 and induces their proliferation in a dose-dependent manner [50]. Similarly, VEGF signaling activates the ERK and PI3K pathways [62]. In addition, both ERK1/2 and PI3K pathways have been implicated in the regulation of VEGF expression [63]. Interestingly, the biological interactions between CXCR4/SDF-1 and VEGF occur through the Akt signaling pathway [64]. Thus, targeting FAK, Akt and ERK signaling in GBM to limit migration of glioma may be more readily achieved by targeting the external ligands that stimulate them.

Radiation-induced microenvironments

Targeted RT attempts to control the tumor by causing death of most cancer cells, which in turn cures many patients. However, this goal is rarely attained in GBM. Newly diagnosed patients have a median survival of 9–36 months. Notably almost all tumors recur at the site of origin, which means that they recur within the irradiated microenvironment. Very few studies have attempted to define the biology of irradiated brain, or its impact on the execution of specific functions. One notable exception is the demonstration that irradiation alters neural precursor cell regulation [65]. This study demonstrates that the irradiated brain microenvironment is actually detrimental to the maturation of transplanted neural precursor cells.

Although the focus has been on DNA damage as the root cause of cell death, in the last decade it has become abundantly clear that radiation has long term consequences beyond cell kill and mutation. Global analysis of gene expression patterns reveals dynamic and complex radiation responses, both in tissues and normal cells and in cancer and cancer cells [66,67,68]. Hence, the ultimate consequence of radiation therapy is a composite of the results of genetic damage, cell loss and induced gene products.

This multifactorial tissue and tumor response to radiation may ultimately compromise the flow of information among cells. Taking an integrated view of the complex cellular processes resulting from tumor response to radiation could provide novel targets for therapeutic intervention. The first problem is to understand what changes occur and why. Studies in other organs provide an example of how radiation can persistently alter tissue and affect cell fate, but more is needed to understand the biology of irradiated GBM and brain.

Radiation-induced TGF β

The potential for functional alterations in the radiation-induced microenvironment is exemplified by TGF β . IR induces TGF β activation in vitro and in vivo in normal and cancer cells [69,70,71,72,73,74,75]. Following just TGF β in irradiated tissues has provided new insight into its action and novel mechanisms regulating tissue response to radiation. The potential for TGF β inhibition during RT to block normal tissue toxicity from fibrosis after treatment of solid tumors in other organs is now well-recognized (reviewed in [23,76,77]). Several rodent models of compromised TGF β signaling provide significant experimental support for this hypothesis [78,79,80,81]. The rationale is that blocking TGF β induced by RT inhibits a deleterious cytokine cascade that stimulates inflammation, extracellular matrix remodeling and fibrosis that compromise tissue function in some patients [82,83]. The question is whether this knowledge can be used to improve cancer treatment, specifically that for GBM.

Rapid activation of TGF β by IR was first demonstrated by an increase in immunoreactivity of epitopes that are masked by the TGF β complex [69,84]. Dose dependent TGF β activity is evident at three days post-irradiation following 10 to 500 cGy in mouse mammary gland [70]. The three mammalian TGF β genes (TGF β 1, TGF β 2, TGF β 3) share a high degree of sequence homology in terms of the ligand and some functional overlap. Each is synthesized as a large polypeptide that is processed by intracellular proteolysis to yield a C-terminal peptide that dimerizes to form the mature ~24 kDa cytokine. The remaining N-terminal peptide forms a ~75 kDa glycosylated homodimer called the latency associated peptide (LAP) [85]. Non-covalent association of LAP with its respective TGF β cytokine forms the latent TGF β complex, referred to as the small latent complex. LAP acts as a chaperone to ensure proper folding of TGF β and contains the signal peptide for secretion. The so-called large latent complex where LAP is covalently bound via a disulfide linkage to a protein called latent TGF β binding protein facilitates latent TGF β sequestration within the extracellular matrix. Thus, biological activity of TGF β is restricted by the fact that it is secreted and sequestered in this latent state. Release of TGF β from its LAP, referred to as activation, is required before the cytokine can bind to cell surface receptors, which underscores the mode of activation as a critical determinant of biological activity [86].

It is likely that radiation-induced activation of TGF β is mediated by reactive oxygen species (ROS). We demonstrated that latent TGF β can be efficiently activated in solution as a consequence of exposure to ROS generated by metal-ion catalyzed ascorbate oxidation, without any deleterious effect on the activity of TGF β itself [87]. We have recently shown that the oxidation of specific amino acids within LAP- β 1 causes a conformational change in the latent complex, allowing release of active TGF β 1 [88]. This mode of activation has been

confirmed in recent studies using asbestos generated ROS [89]. Given the knowledge of the ROS mechanism of activation, and that radiation generates ROS, it is reasonable to assume that TGF β 1 is activated by RT in GBM.

Does TGF β contribute to tumor control or work against it? Several studies suggest that radiation-induced TGF β could have a deleterious effect. Teicher and colleagues showed that tumors secreting high levels of TGF β are more resistant to chemotherapy, such as cis-platinum. Cis-platinum treatment of breast cancer cells increased both TGF β mRNA levels and the secretion of active TGF β leading to growth arrest and repair of damage, thus rendering these cells resistant to cis-platinum killing [90]. Furthermore, treatment of cells with anti-TGF β antibodies restored cellular sensitivity to cis-platinum [91]. Treatment of animals bearing cis-platinum-resistant tumors with TGF β neutralizing antibody or with a TGF β inhibitor restores drug sensitivity of the tumor [92,93,94]. Proteomic profiling of TGF β treated cells suggests that TGF β can inhibit DNA repair genes [95]. Treatment of mink lung epithelial cells with 5ng/ml TGF β causes down regulation of Rad51, an essential component of the DNA double-strand break repair machinery, in a Smad dependent manner through ubiquitylation and proteosomal degradation of Rad51 protein.

Recent studies have identified a mechanism directly linking TGF β to the DNA damage response and radiosensitivity [96,97]. Human cells in which TGF β signaling was inhibited showed reduced p53, chk2 and rad17 phosphorylation compared to controls [96]. All of these proteins are substrates of ATM, a serine/threonine protein kinase required for the rapid response to IR induced DNA double strand breaks [98]. Consistent with the hypophosphorylation, ATM auto-phosphorylation was decreased by more than 50%. Treatment with the TGF β type I receptor kinase inhibitor also reduced the formation of nuclear foci of the histone variant H2AX by irradiation, which is rapidly phosphorylated by ATM. Moreover, three of 4 breast cancer cell lines are radiosensitized by TGF β inhibition as measured by clonogenic assay (Barcellos-Hoff et al. unpublished).

Thus, one can ask would TGF β 1 activation in irradiated brain have a positive or deleterious effect on function and tumor regrowth? TGF β 1 activity is extremely restricted in normal brain, but is induced by wounding and inflammation [22]. Expression of active TGF β in astrocytes blocked the generation of new neurons by affecting neural progenitors [99]. Furthermore, TGF β 2 induces MMP-2 expression and suppresses tissue inhibitor of metalloproteinases (TIMP)-2 expression, which promotes the invasion of glioma cells in a Matrigel invasion assay [100]. Thus, emerging evidence suggests that TGF β can inhibit the effectiveness of RT. The use of TGF β inhibitors in GBM during RT may achieve the objective of increased tumor cell kill while blocking the deleterious consequences of TGF β that contributes to angiogenesis, immune suppression, and tumor invasion.

Summary

Recalling the seed and soil hypothesis put forward more than a century ago, the molecular definition and function of the microenvironment is indeed a rich soil in which tumors flourish. Surgical resection of GBM, like pulling weeds does little if the soil is still hospitable to scattered seeds. Rehabilitating the soil can be an effective means of limiting growth, perhaps even to the extent of abolishing clinical evidence of tumors. Furthermore it has been recognized that therapy itself may alter the microenvironment, which in the case of RT could be used to therapeutic advantage to further differentiate the tumor bed from normal tissue. The combination of RT and biological targets specific to the irradiated field may provide a particular advantage in GBM since successful treatment of brain tumors will entail a greater imperative to limit toxicity and protect normal brain function.

Abbreviations

GBM	glioblastoma
RT	radiotherapy
IR	ionizing radiation
TGFβ	transforming growth factor β

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