Hypothesis and theoretical model of the role of therapy-mediated cell loss in promoting recurrences in glioblastomas

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Hypothesis

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Abstract

A hypothesis and theoretical model incorporating the role of dynamically distinct cell compartments in governing growth and relapses in glioblastoma was published in 2010. Subsequent reports have given further insight into some of the mechanisms that might influence the functional properties of the model and its implication on the functional biology and therapeutic implications for this tumor, which usually has a dismal prognosis. This updated essay is an attempt to document some of the relevant developments in this regard. It also proposes an extension of the model by contemplating on the existence of an exploitable weakness or "Achilles Heel" in these tumors. Although the model has been developed with respect to glioblastomas, conceptually it might well be applicable to other tumors.

Keywords: Mechanism of tumor relapse, Gliomas, Glioblastomas, Cancer stem cells, Heterogeneity, Neoplastic plasticity

Introduction

Glioblastomas (GBs) are lethal tumors with dismal prognosis due to the inevitability of recurrences that follow therapy. This usually involved maximal surgical resection, radiotherapy, and concomitant and adjuvant chemotherapy with Temozolomide (Sathornsumetee et al., 2007). These recurrences are initiated by a sub-population of neoplastic tumor initiating cells with self-renewal properties referred to as cancer stem cells, and in the case of GBs - Glioma Cancer Stem Cells (GCSCs) (Lathia et al., 2015). These cells are also more resistant than other Glioma Cancer non-stem cells (GNSCs) to different therapies (Leibelt et al., 2016). Provoked primarily by several case reports (Goh et al., 2009; Zaki et al., 2004; Buxton et al., 1997), wherein some GBs rapidly disappear following corticosteroid treatment only to recur very rapidly in a multi-centric fashion, a hypothetical model of tumor recurrence has been

envisaged (Mallik, 2010). Other reports of corticosteroid-induced "vanishing glioblastomas with rapid recurrences" subsequently appeared (Hasegawah et al., 2009, D'Elia et al., 2013). It was then assumed that the triggering of surviving neoplastic cells following rapid neoplastic cell loss would also occur in other neoplasms as well as GBs.

My earlier hypothesis (Mallik, 2010) proposed that GBs comprise 2 dynamically distinct types of cell compartments, whose properties are regulated by distinct populations of cancer stem cells at their helms. These were named: i) Active Compartment, and (ii) Back-up compartment. It was hypothesized that the active compartment is responsible for contributing the major bulk of the tumor and driving its growth, while the back-up compartment remained relatively quiescent. Whenever any form of therapy depletes the active compartment, the back-up compartment is activated and this is transformed to a new active compartment (a "secondary" active compartment), and a new secondary back-up compartment is generated at the same time. The cycle continues with multiple rounds of therapy through development of tertiary and quaternary active and back-up compartments. The phenomenon through which the back-up compartment is triggered to convert to active compartments was called the Tumor Re-Initiation Phenomenon (TRIP).

The aim of this essay is document an update on the above multi-compartment model in the light of publications that have appeared subsequently, along with a review of older reports that may now be more relevant in this context. The literature discussed is not only confined to those related to glioblastomas, but to other malignant tumors, since many of the fundamental issues and mechanism involved may be similar.

Issues regarding dormancy in cancers and their relapses

Tumor recurrence is a critical problem in the management of cancers. The time during which a patient remains asymptomatic before relapse is when the cancer is considered dormant. This may range from months to several years (Yeh and Ramaswamy, 2016). Although conceptually the term tumor dormancy apparently indicates a relatively long period of time during which the patient remains clinically asymptomatic or according to several imaging modalities, the concept may be extended at least theoretically to tumors like GBs where this period is much shorter.

A number of mechanisms have been suggested as being responsible for keeping the tumor cells dormant. In GBs, the period of quiescence following therapy is short, but similar mechanisms are likely to be at play. These mechanisms or phenomena are broadly categorized under (a) angiogenic dormancy, (b) immunologic dormancy, and (c) cellular dormancy (Yeh and Ramaswamy, 2016, Aguire-Ghiso, 2007).

A) *The angiogenic dormancy model*: A tumor whose size is greater than a couple of millimetres needs new blood vessels (neovascularization) through angiogenesis in order to provide its nutrient requirements. Thus the inability of small groups of tumor cells to grow new blood vessels could be the limiting factor preventing them from growing to clinically significant forms until they acquire this ability through the modulation of the interaction between the tumor and its micro-environment by way of altering the balance between pro-and anti-angiogenic factors (Naumov et al., 2009).

b) *The immunologic dormancy model*: It is well established that innate and adaptive immunity can check the initiation and growth of tumor. The model envisages the role of the immune system in cancer progression as a 3-stage process: (i) elimination - during this stage the immune cells eliminate the cancer cells, (ii) equilibrium - it can control progression of some cancer cells, but cannot eliminate all cells, (iii) escape - the cancer cells are non-longer susceptible to immune surveillance (Teng et al., 2008).

c) *The cellular dormancy model:* According to this model, irrespective of extraneous factors (angiogenic requirements or immune-surveillance), there are a number of intrinsic cellular factors that contribute to maintain a cell in a dormant state. Downregulation of the RAS-MAPK pathway and PI (3)K-AKT signaling are crucial in maintaining cellular dormancy through arrest in the G0-G1 phase of the cell cycle (Lu Z et al., 2008). Interestingly, although components of these pathways are frequently mutated in cancer, dormant cells can still downregulate these pathways. In contrast, upregulation of the p38 pathway contributes to the maintenance of dormancy under certain situations (Aguirre-Ghiso et al., 2003). Along with these pathways, certain intrinsic cellular factors, such as hypoxic stress, could also contribute towards the maintenance of cellular dormancy. Hypoxia might contribute towards dormancy in GCSCs by its mediation through by protein phosphatase 2A (Hofstetter et al., 2016).

Relapses: Extrapolating on the above models that contribute to the maintenance of tumor dormancy, it seems likely that incidents, such as a pro-angiogenic switch and/or acquirement of the ability to escape immune surveillance or reactivation of downregulated RAS-MAPK signaling (or AKT signalling), leads to tumor relapse. Many of the cues for such reactivation could come from the microenvironment mediated through molecules that include the integrins, focal adhesion kinases, and cell surface receptors that reactivate the RAS-MAPK and AKT signaling pathways (Ghajar et al., 2013, Weaver et al., 1997, Dey Guha et al., 2011). Clearly the maintenance of tumor dormancy and the factors governing its relapse is a complex interplay of molecular factors and events.

Role of therapy in modulating tumor relapse

While the purpose of surgical or non-surgical anti-neoplastic therapy is to eliminate the tumor, or at least stall its progress sufficiently to improve survival, it is highly likely that any form of therapy that leads to depletion of the neoplastic cell population might trigger bio-molecular changes in the surviving cells, which then contribute towards relapse. The hypothesis becomes relevant clinically in the context of relapses that occur relatively quickly or where changes may be shown in a dormant cell population after therapy. One of the earliest reports questioning the role of surgery in modulating the dynamics of surviving breast cancer cells following surgery was by Baum et al. (2005). This was further elucidated upon by Retsky et al. (2010). This Harvard group has continuously and consistently challenged the continuous growth paradigm in breast cancers, and has suggested the effects of surgery in promoting the process of relapse. On the other hand, the development of the current hypothesis and multi-compartmental model that conceives of the role of therapy in tumor recurrence in glioblastoma came from a number of reports of certain glioblastomas that quickly disappear following treatment with the corticosteroid, dexamethasone (Goh et al., 2009; Zaki et al., 2004; Buxton et al., 1997, Hasegawah et al., 2009, D'Elia et al., 2013). Parenthetically speaking, dexamethasone is used in these patients with the intention of reducing the intra-cranial tension, and not as an anti-neoplastic agent. However, GBs do

respond dramatically in their rapid disappearance as shown by imaging, only to return rapidly in a multi-centric manner. This indicates that the process of rapid cell loss has the potential of triggering a very strong relapse response. On the other hand, various forms of non-surgical anti-neoplastic therapies (chemotherapy or radiotherapy) lead to the enhancement of a population of resistant cells with stem cell properties that act as the seedbeds of recurrences in glioblastomas and lung cancers (Bao et al., 2006, Kang et al., 2007, Hamilton et al., 2013, Lathia et al., 2015).

The phenomenon should be seen differently from the paradigm of resistance to anti-neoplastic therapy as espoused traditionally, and as a distinct mechanism that tries to formulate a "cause-and-effect" relationship between neoplastic cell loss and dynamic alterations in surviving cells that might positively contribute towards a relapse.

Heterogeneity in cancer and cancer stem cells, and cancer cell plasticity

The existence of phenotypic and functional heterogeneity among neoplastic cells is well established. This heterogeneity is driven by genetic and epigenetic changes in these cells, amplified by presence of genetic instability (Singh et al., 2015). With the emergence and subsequently the increasing popularity and acceptance of the "Cancer Stem Cell (CSC) model", the phenomenon of heterogeneity amongst neoplastic cells can be more comprehensively explained. CSCs are a distinct functional subtype of neoplastic cells capable of self-renewal and differentiation, thereby creating a functionally heterogeneous population of descendants made up of amplifying cells and differentiated cells (Lathia et al., 2015). Piccirello et al. (2009) was one of the earliest studies convincingly demonstrating the presence of at least 2 dynamically distinct types of GCSCs in a single tumor. Indeed, their findings were used as an argument in favor of the multi-compartmental model in question that is under review here. On the other hand, the phenomenon of cancer cell plasticity can enhance the heterogeneity amongst the stem cells and their more differentiated progeny. Plasticity is a phenomenon through which cells can transit not only from the stem cell to the more differentiated state, but unexpectedly from the more differentiated to the stem cell state both in non-neoplastic and neoplastic conditions (Chaffer et al., 2011). Micro-environmental cues as well as intrinsic factors govern these transitions. Berezovsky et al. (2014) have shown the role of SOX2 as a molecule that promotes the dedifferentiation of cell to stem cells in GBs.

Apart from the occurrence of dynamically distinct compartments, the multi-compartment model hypothesis requires the presence of cells that divide slowly in a tumor such as GB, which is otherwise synonymous with explosive growth. Paradoxical as it may sound, such cells do exist in glioblastomas. Using a novel technique with carboxyfluorescein succinimidyl ester (CFSE), Dellyrole et al. (2012) identified and isolated a slowly cycling sub-population of cells. Indeed in the earlier mentioned report by Piccirello et al. (2009), the 2 GCSC subtypes had significant differences in their proliferative and self-renewal activities, thus conforming to the basic requirements of the model. Apart from the context of the model, the heterogeneity in CSCs makes therapeutic targeting of these cells difficult and almost certainly ineffective. Slow cycling cells are found in other tumors, posing some of the most daunting challenges in developing effective anti-neoplastic therapy (Chen et al., 2016).

The different compartments are regulated by dynamically different stem cells that may have the same parent at some point in their evolution, acquiring different phenotypic characters through asymmetric cell division. For example, the 2 pools of GCSCs described by Piccirello et al. (2009) had the same parental lineage, with a possibility that during the course of tumor evolution, they diverged phenotypically through asymmetric cell division - a major mechanisms by which heterogeneity arises in neoplastic cells (Pine and Liu, 2014). From a conceptual angle, this heterogeneity should be addressed as intra-tumor heterogeneity rather than the differences between different tumors, as exemplified by the different well-established molecular sub-types of GBs (Verhaak et al., 2010).

Quiescence and activation among non-neoplastic cells

For the multi-compartmental model to be feasible, there should be evidence of the presence of dormant/quiescent cells that respond to environmental cues by entering cell division from a growth-arrested state.

Llorens-Bobadilla et al., 2015), through single-cell RNA sequencing of acutely isolated neural stem cells (NSCs) from the adult sub-ventricular zone (SVZ), isolated and identified lineage-primed NSCs that interestingly reside along a continuum of co-existing states between dormancy and activation; these states differ in their molecular properties and activity. Thus heterogeneity is present even in the dormant non-neoplastic NSC population. Most importantly from amongst the dormant NSC population, a primed quiescent group of cells emerges in response to brain ischemia, mediated by interferon-ɣ signaling (Llorens-Bobadilla et al., 2015). As described below, such cytokine-related signalling mechanisms may also be heavily involved in activating quiescent neoplastic cells. Similarly Rodgers et al. (2014) have shown using muscle stem cells that the quiescent phase of the cycle has 2 distinct functional phases, G0 and a G_{alert}. The transition of the cells between these 2 phases in response to injury requires mTORC1 activity and functions through c-Met and HGF1. Moreover neural precursor cells can proliferate and migrate to the sites of brain infarct following a stroke (Dibajnia and Morsehead, 2013). These examples indicate the place of injury in initiating proliferative responses in otherwise quiescent populations of cells. Similar mechanisms of injury-induced activation of quiescent neoplastic cells are highly likely, as will be elucidated below

Relationship between cell death and proliferation, with emphasis on apoptotic mechanisms in stimulating cell proliferation

A key element of the hypothetical model is the activation of dormant cells by some stimuli that arise as a result of neoplastic cell depletion. Indeed, apoptotic activity is known to stimulate proliferation under certain circumstances. Haynie and Bryant (1977) showed that irradiation-induced cell death in *Drosophila* wing imaginal discs leads to a compensatory proliferation which results in the formation of an adult wing of nearly normal size. However, it was later found that apoptosis-induced proliferation in *Drosophila* has different mechanisms depending on the incipient conditions. In proliferating tissues, apoptosis in the imaginal disc leads to dying cells stimulating compensatory proliferation through secretion of the Wingless (Wg) and Decapentaplegic (Dpp), the *initiator* caspase Dronc controlling this process. However, effector caspases do not have any such effect; in contrast, in differentiating eye

tissues the *effector* Caspases trigger the Hedgehog (Hh) signalling pathway to induce compensatory proliferation. Interestingly, effector caspases in photoreceptor neurons stimulate Hh signalling, which in turn triggers cell cycle re-entry of cells that had previously exited it (Fan and Bergmann, 2014). Dpp is the *Drosophila* homologue of the vertebrate Bone Morphometric proteins (BMPs), whereas the vertebrate counterpart of Wingless is Wnt. A relevant point is that both BMP proteins and Wnt signaling are important in cancer stem cell behavior (Gong et al., 2012, Piccirillo et al., 2006). Indeed, apoptosis-induced proliferation has also been seen in neoplastic cells. Huang et al. (2011) elegantly showed how dying tumor cells promote proliferation in surrounding neoplastic cells; when 4T1 murine breast cancer cells are seeded with dying tumor cells after radiotherapy, they grew much faster than when seeded alone. This phenomenon was regulated by caspase-3, thereby depicting a close link between apoptosis and proliferation, which occurs through a defined chain of molecular events. Caspase-3 leads to activation of calcium-independent phospholipase A2 (iPLA2), which then downstream activates arachidonic acid (AA), in turn activating prostaglandin E2 (PGE2). The Caspase3-iPLA2-AA-PGE2 axis leads to a pro-proliferative response (Huang et al., 2011). The role of caspases released from dying cells in stimulating proliferation is evident in pancreatic ductal adenocarcinomas (Cheng et al., 2015) and melanomas (Donato et al., 2014). In particular, Mao et al. (2013) uncovered the role of caspase 3 released from dying endothelial cells as a crucial promoter of angiogenesis in GBs. Angiogenesis again is seen as a crucial prerequisite in tumor relapse.

Relevant molecular mechanisms in the activation of quiescent cells - role of distally acting molecules

Neoplastic cells in GBs migrate long distances beyond the gross (resectable) boundaries of the tumor. However, after surgery and adjuvant therapy, most recurrences occur very close to the original margins of resection (Hou et al., 2006). These areas may be rich in niche factors that favor tumor cell repopulation. Therefore mechanisms may exist that attract the reinitiating cells of a tumor to these sites, thereby necessitating the existence of molecules and pathways to allow such a phenomenon to occur, i.e. by exerting their influence over a significant distance. A strong candidate for this is chemokine stromal-derived factor (SDF)-1/CXCL12 and G-protein-coupled 7-span transmembrane receptor, CXCR4 axis (SDF1-CXCR4 axis).

Imitola et al. (2004) have shown that human neural stem cells (NSCs) migrate long distances towards a site of CNS injury, such as an infarction, using the SDF1-CXCR4 axis. Astrocytes and endothelia at these injured sites upregulate SDF1 secretion. CXCR4, the cognate receptor for SDF1 is expressed by NSCs. When exposed to SDF1, quiescent NSCs have enhanced proliferation and promote chain migration through the activation of different molecular pathways; these include a rapid and sustained activation of p38MAPK, phosphorylation of ribosomal S6 kinase, and phosphorylation of c-jun and paxillin.

The SDF1-CXCR4 axis may be modulated by a number of molecules, e.g. thrombin, fibrin, fibronectin, complements and hyaluronic acid, which further substantiates its role as a modulator of stem cell response to injury (reviewed by Chaterjee et al., 2014).

CXCR4 is also expressed in cancer stem cells, including glioma stem cells (Ehetesham et al., 2013). And Lee et al. (2013) found that the SDF1-CXCR4 axis is essential in maintaining stemness properties of glioma cancer stem cells. Thus disruption of this axis has the therapeutic potential in acting not only as an anti-CSC manoeuver, but also as an inhibitor of the reactivation response among quiescent cancer

cells. Indeed, preclinical data supporting the possible use of anti-CXCL12 in therapy has been conjectured (Debnath et al., 2013). The role of the SDF1-CXCL12 axis in promoting tumor cell survival, invasion and their effect of the niche is well known. In light of the SDF1-CXCR4 axis being involved in promoting injury-induced cell proliferation, it seems that inhibiting its mediating action in injury-induced proliferation could be very significant in preventing tumor recurrence. This strategy should be effective in increasing the efficacy of other anti-neoplastic measures. There are other molecules that could also act at distant targets and be similarly involved. Eprhrin and semaphorins are 2 candidates whose function in GB biology has been established, and their potential as therapeutic targets looks promising (Nakada et al., 2011, Bagci et al., 2009).

Extension of the hypothetical model to conceptualize an Achilles' heel

The biggest problem with almost all forms of anti-neoplastic therapy is the ability of a population of neoplastic cells to evade the assault and survive. These resistant cells re-initiate the tumor, leading to relapses and the cycle continues. The main reason behind the poor prognosis in GBs is the ability of these therapy-resistant cells to rapidly re-initiate a tumorigenic process. Many cancers have shown improved prognosis following the advent of newer therapeutic protocols, although in GB patients the figures remain dismal. The neoplastic cells seem to be able to improvise their resistance mechanisms against each form of therapy, but also relapse rapidly with very fast proliferating cells. The key might lie in preventing the reactivation of the less dormant cells, since - at least in some situations - keeping a cell dormant for a suitably long period of time may promote apoptosis (Topham and Taylor, 2013).
In the context of the above predicament, we can only hope that some form of Achilles' heel is present in these tumors, which can be effectively exploited in therapy. One such possibility is the "Oncogene Addiction" phenomenon, according to which a cancer cell depends on one pathway or an oncogene to perpetuate itself, in spite of - at least theoretically - having multiple options to by-pass the molecule or pathway compromised by therapy. This addiction is exploited to treat some tumors very effectively, as exemplified by the use of Gefitinib (Iressa) in treating gastrointestinal stromal tumors and chronic myeloid leukemias (Torti et al., 2011). Instead of using the approach of "getting lucky" in discovering an Achilles' heel, it might be pragmatic to envisage such an area of weakness and direct our investigations to determine whether it exists. Extension of the Multi-compartmental Tumor Re-initiation Phenomenon hypothesis with the presence of a simple set of molecules that might regulate the effect of the active compartment on the back-up compartment is such an attempt. It simply involves the possible *production* of 2 molecules called "A" and "B" by the active compartment, which affects the back-up compartment in the following ways (Fig. 1):

Molecule A has 2 effects
a) it inhibits proliferation of the back-up compartment and maintains its quiescence.
b) it has a pro-apoptotic effect on the back-up compartment, but is unable to trigger it because of the presence of B

Molecule B has one effect
a) it protects the back-up compartment from the pro-apoptotic effect of A

Analysis of situations before and after therapy

a) Before therapy: substance A produced by the active compartment maintains quiescence of the back-up compartment and tries to trigger apoptosis of the cells in it. However, substance B produced at the same time prevents the pro-apoptotic effect of A and thus the back-up compartment survives, although in a quiescent state.

b) After therapy: The active compartment is to a great extent removed, which depletes both A and B. Therefore the back-up compartment gets activated because of the lack of A. The absence of B becomes irrelevant in the absence of A. Thus tumor re-initiation response is brought about.

c) *How can this be exploited?*: If such a phenomenon exists, then the tumor should be treated by methods that use anti-B methods as adjuncts to ensure apoptosis of cells in the back-up compartment. This might be used to make GBs more sensitive to therapy.

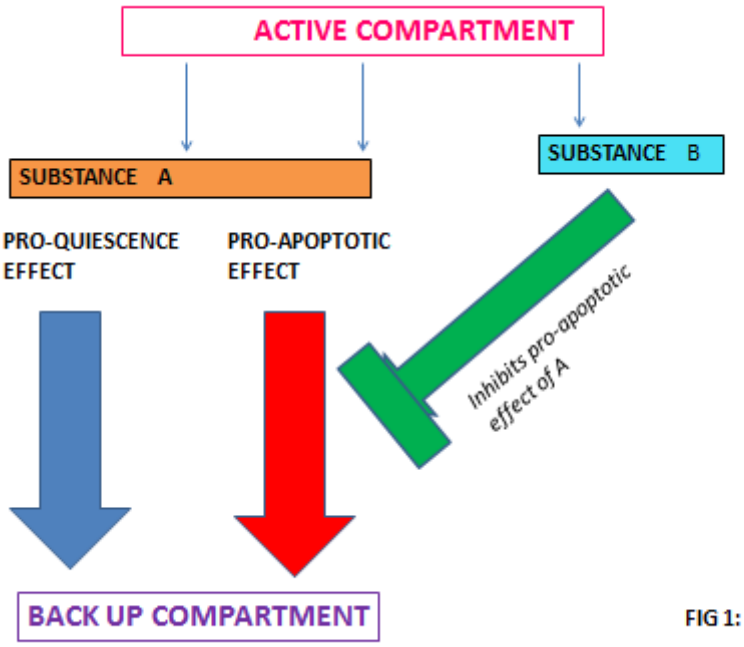

Figure 1. The active compartment regulates the back-up compartment through the production of two molecules A and B. The molecule A inhibits the proliferation of the back-up compartment and maintains it quiescence. It also has a pro-apoptotic effect on the back-up compartment which is inhibited by the substance B.

At present this model is purely theoretical, but we know that the rules of engagement between neoplastic cells are a complicated mix of cellular and extracellular factors. In particular, the niche elements, and indeed many molecules, are known whose biological effects differ depending on the surroundings. Let us take the example of Ephrin signalling; although its role has been established as a

molecule that promotes the progress of GBs, Ephrin signaling is responsible for regulating the size of the brain during development through actions that involve apoptosis in neural progenitors (Depaepe et al., 2005).

Discussion

The continuously dismal prognosis in GBs necessitates "out-of-the-box" thinking, i.e. we need to propose some refreshingly new ideas. Since rapid relapses are the major reasons behind the poor prognosis in GBs, it is important to direct our energy towards measures which could inhibit the surviving cell compartment and particularly the ones that initiate these relapses, the GCSCs.

One of the pre-requisites to test the model is to identify neoplastic cells that are members of the so-called dynamic compartments and their degree of differentiation through well-established markers. Such technology remains unavailable, although with rapid advances in sequencing methods it might be possible to gather a clearer picture of the dynamic heterogeneity amongst neoplastic populations. Llorens Bordella et al. (2015) alluded to earlier have shown the use of single-cell sequencing in defining dynamically distinct sub-populations of cells in quiescent NSCs, and is a harbinger of things that gives hope for a better understanding in this context.

The other issue that must be addressed with reference to this model is to question the role of necrosis in possibly activating the back-up compartment. Cell necrosis due to hypoxia and genetic instability is one of the defining characters of GBs. What role does this have in determining the activation state of the so-called back-up compartment? It is possible that tumors develop a kind of equilibrium between this intrinsic necrosis and cell proliferation, such that it becomes upset by therapy. In fact, surgery in GBs attempts to remove most of the tumor mass, and subsequent to radiation or chemotherapy thereafter is supposed to assist; nevertheless recurrences occur close to the original tumor margins (Hou et al., 2006). Thus it is possible that the cells with the properties of the back-up compartments are present away from the tumor in a relatively quiescent state and are reactivated once the main tumor mass is depleted, mainly through molecules like SDF1 acting over a distance. So locally released molecules following an apoptosis or distally acting cytokines can have a triggering function in such scenarios.

A difficult quagmire to negotiate is to integrate the fundamental philosophy of the model into decision-making regarding therapeutic intervention. If the assumption of neoplastic cell loss-mediated activation of quiescent tumor initiating cells is true, then does it imply that we withhold or delay therapy? This would, of course, lead to the tumor progressing rapidly. Indeed, current therapeutic protocols do enhance survival of GB patients over those who are left entirely untreated. The answer might lie in devising methods of developing some protocol for "periodization" of therapy (the timing intervals between treatments). Leder et al. (2014), after taking into consideration the heterogeneity and instability in differentiation states, used an iterative approach of combined theoretical and experimental strategy to predict the effectiveness of different radiation *schedules* in PDGF driven GBs in mice. A proper understanding of the dynamic properties of neoplastic cells should help streamline such

approaches wherein both the intensity of the therapy and its schedule can be precisely controlled depending on our ability to understand the triggers involved in tumor re-initiation.

Apart from therapeutic implication, factors that promote quiescence may have an adverse effect on prognosis. For example, Protein Phosphatase 2 promotes dormancy in GB, in hypoxic conditions through HIF1-α. However, paradoxically, overexpression of PP2A, a molecule that promotes dormancy in GB, is associated with poor prognosis (Hofstetter, 2012). It seems that, in GBs, maintenance of dormancy and overall survival may be a complicated issue

Thus, the dynamically distinct multi-compartmental model appears to be a tenable one in the light of publications since its inception, especially with regard to intra-tumoral heterogeneity, neoplastic plasticity, and findings related to apoptosis-induced cell proliferation and injury-stimulated molecules (e.g. SDF1) that might act on quiescent cells. On the other hand, we are still limited by the absence of well-established and robust methods that could map these cells and make it easier to study their properties. The effect of cellular quiescence on prognosis and therapy throws up multiple questions that require answers.

This update is also an extension of the original model, incorporating some hypothetical molecules in an attempt to seek the Achilles' heel of glioblastomas. There is no doubt that, if there is one tumor we need to find quickly, it is this one.

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