Chapter 2

Systems Biology of Glioblastoma Multiforme

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Additional information is available at the end of the chapter

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1. Introduction

Gliomas encompass approximately 80% of primary brain malignancies [1]. The five-year survival rate is dependent on the subtype of glioma. According to the Central Brain Tumor Registry there are about 13,000 deaths and 18,000 new cases per year of primary brain cancer in the United States and the overall average annual age-adjusted incidence rate for 2006-2010 for primary brain and CNS tumors was 21.03 per 100,000 [2]. In this chapter, our main focus is glioblastoma (GBM), which is by far the most common and the most malignant of all primary brain tumor [2]. Often described as the most lethal or the most devastating brain tumors, gliomas continue to carry a very poor prognosis at all levels, quantity and quality of life. GBM almost exclusively recurs despite meticulous conventional therapies, including surgical resection, radiation, and chemotherapy and bevacizumab. Despite all advances, the survival rates continue to be low, with a median survival of approximately 15 months in patients with malignant gliomas [3].

The multiple hit theory of cancer speculates that the origin and progression of GBM is the product of complex series of molecular processes like activation of oncogenes and alterations in tumor suppression genes. However, the complexity of the molecular interactions in malignant gliomas imposes a great challenge that indeed has crucial implications on treatment. This cannot be achieved without a meticulous understanding of this multifaceted process and its molecular mechanism, and therefore dictates dissection at systems level. The Cancer Genome Atlas Research Network has sequenced the genome of GBM and deduced that the apparatus of tumor growth and recurrence is the result of complex epigenetic mechanisms and gene interactions [4]. The twenty first century has been referred to as the genomic millennium, thus in an era where genes dictate remedy a comprehensive understanding of the systems biology of gliomas may be a key to the cure. Our goal in this chapter is to touch on

the complexity of the molecular networks of GBM by presenting on an overview without delving into details. We will focus on how molecules and pathways are dysregulated in GBM rather than presenting detailed graphs of networks as the latter are readily easily found. To illustrate the clinical relevance of a systems approach to molecular networks, we dissect the case of the use of rapamycin in a GBM clinical trial and discuss the pathogenesis of its adverse effect in causing activation of AKT, an oncogene.

2. Classes of GBM

Malignant gliomas develop as part of a multistep process comprising chronological and collective genetic modifications resulting from core and environmental dynamics. Cowden, Turcot, Li-Fraumeni, neurofibromatosis type 1 and type 2, tuberous sclerosis, and familial schwannomatosis are among the predisposing syndromes for glioblastoma occurrence [15]. From a molecular perspective, malignant gliomas are greatly heterogeneous tumors [14]. In a nutshell, 4 transcriptional subclasses of GBM have been proposed: *classical*, *mesenchymal*, *proneural*, and *neural* [1]. The classical type glioblastoma typically exhibits chromosome 7 amplifications, chromosome 10 deletions, *EGFR* amplification, *EGFR* mutations (point and vIII mutations), and *Ink4a*/*ARF* locus deletion. The mesenchymal subclass shows a high frequency of *NF1* mutation/deletion and high expression of *CHI3L1*, *MET*, and genes involved in tumor necrosis factor and nuclear factor–κB pathways. Proneural type glioblastoma is characterized by changes of *PDGFRA* and mutations in *IDH1* and *TP53*; these are features common to lower-grade gliomas and secondary GBM. A characteristic feature of the neural subclass of GBM is the expression of neuronal markers.

3. Gliomagenesis

The multiple hit theory of cancer stipulates sequential molecular events leading to GBM. The following is a summary of current ideas. One of the first steps in tumoregenesis is loss of cell cycle control. The cell cycle checkpoint that has been of most interest is the G1-S phase. This important checkpoint is mainly controlled by p16INK4a/cyclin-dependent kinase (CDK)-4/RB (retinoblastoma) 1 pathway, which involves p16, CDK-4, cyclin D1, and RB1[16]. The CDK/cyclin D1 complex phosphorylates RB1 therefore releasing the E2F transcription factor, which in turn activates the genes, involved in the G1/S transition [17]. Subsequent steps in gliomagenesis include the overexpression of growth factors and their receptors. A diverse array of growth factors such as epidermal growth factor receptor (EGFR), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF, FGF-2), transforming growth factor (TGF)-alpha, and insulin-like growth factor (IGF)-1 are overly expressed in glioblastoma [18, 19]. Malignant gliomas are highly vascular tumors; the angiogenic molecule that has been most widely implicated in GBM is vascular endothelial growth factor (VEGF), an endothelial cell mitogenic [20].

Another key event contributing to gliomagenesis is the abolishment of apoptosis, or programmed cell death. Malignant glioma cells, not only divide uncontrollably, but also intentionally lose the ability to undergo apoptosis. *p53*, a key molecule involved in apoptosis, is often mutated during gliomagenesis [21]. An important process contributing to gliomagenesis is genetic instability, which refers to the property that random mutations are introduced in dividing cancer cells because of the loss of check points and the molecular machinery that ensures that the genome is copied faithfully during mitosis [22]. A clinical correlation to genetic instability is the Turcot syndrome [23].

4. Signaling pathways

A large number of signaling pathways exchange information to generate a large molecular network that controls the phenotypes of GBM. A detailed discussion is beyond the scope of this chapter. In this section, we will discuss how the RTK/PI3K/Akt, mTOR, Ras/MEK/MAPK, p53, ATM/Chk2, Rb, and stat3 pathways are affected to GBM. Additional pathways will be briefly discussed in the section on crosstalk.

4.1. RTK/PI3K/Akt pathway

This pathway regulates a range of cellular processes such as proliferation, growth, apoptosis, and cytoskeletal rearrangement. It involves receptor tyrosine kinases (RTKs), like EGFR, PDGFR, and VEGFR, as well as tumor suppressor protein phosphatase (PTEN), and protein kinases PI3K, AKT. Irregular activation of RTK/PI3K/AKT is commonly seen in malignant gliomas [24].

4.1.1. Receptor tyrosine kinases

RTKs relay extracellular signals to activation of intracellular networks through PI3K and AKT. *EGFR* gene amplification is the most widespread alteration present in GBM [25]. The most common is EGFR vIII, which relays ligand independent accumulative growth signals [26, 27]. Some studies have previously shown a correlation between aberrance of EGFR and aggressiveness of tumor and therefore shorter survival [28, 29]. Unfortunately, EGFR inhibitors such as Gefinitib and Erlotinib have not produced promising results in clinical trials of patients with GBM [30, 31]. Overexpression of PDGFR (especially PDGFR-α) and PDGF have been documented in astrocytic tumors irrespective of the grade [32], [33]. *PDGFRA* amplification and *IDH1* mutation are a characteristic of the proneural subtype of GBM implying a possible association of the proneural subtype and secondary GBM [4]. Anti-PDGFR therapy such as imatinib has not been promising either [34].

4.1.2. PI3K–PTEN-AKT signaling

AKT, a serine/threonine kinase that acts to regulate cell growth, proliferation, and apoptosis, is activated in about 80% of human GBMs [35]. PI3K belongs to the family of lipid kinases.

PI3K enzymes produce phosphatidylinositol-3,4,5-trisphosphate (PIP3), a lipid secondary messenger, which is found to be at high levels in cancer cells [36, 37]. Binding of PI3Ks to RTKs results in activation of AKT through PiP3 and PDK1 [38]. Dissecting the PI3K complex, it is composed of a catalytically active protein, p110α, encoded by *PIK3CA*, and a regulatory protein, p85α, encoded by *PIK3R1*. In primary GBM, *PIK3CA* mutations and amplification are seen in about 5% to 13% of cases [39]. Furthermore, *PIK3R1* mutations have been reported in about 10 % in GBM patients [4].

PTEN (phosphatase and tensin homologue, located on chromosome 10) is a tumor suppressor gene. PTEN mutations are associated with several types of cancer including GBM. Loss of heterozygosity of chromosome 10, which causes deletions or mutations of PTEN, is a common event in GBMs. PTEN negatively regulates the PI3K/AKT/PKB pathway by blocking AKT signaling via the reduction of intracellular levels of PIP3. Furthermore, lower PTEN activity induces activation of the RTKs/PI3K/AKT pathway. This is due to the negative inhibition accomplished by PTEN antagonizing PIK3 [40]. GBMs typically harbor diminished expression of PTEN through homozygous deletion or mutations of PTEN, which contributes the activation of the RTKs/PI3K/Akt pathway [4, 41, 42]. Mesenchymal and classical types of GBM exhibit loss of PTEN (www.cbtrus.org). It is noteworthy that GBM cells expressing EGFRvIII with an intact PTEN appear to have a higher response rate to EGFR inhibitors [35, 43].

4.2. mTOR

Signaling through mTOR is mediated by two independent complexes, mTORC1 and mTORC2. mTORC2 is activated by growth factors and ribosomes and in turn activates AKT among other kinases via phosphorylation [44]. mTORC1 controls cellular metabolism, biosynthesis, stress, and by several growth factors such as EGF and its receptor, EGFR [45]. In settings promoting cell growth, mTORC1 phosphorylates substrates to stimulate anabolic processes such as ribosome biogenesis, translation, and synthesis of lipids and nucleotides and to abolish catabolic processes such as autophagy [45]. Likewise, mTORC2 promotes cancer growth by stimulating glucose uptake via activation of AKT and activating serum/glucocorticoid regulated kinase (SGK), which contributes to proliferation and survival [46]. Inhibitors of mTOR, like rapamycin, sirolimus, temsirolimus, everolimus have not shown efficacy in GBM [47, 48]. In fact, inhibitors of mTOR lead to elevated expression and activity of growth factor receptors, which increases PI3K activity and RAS signaling. Below we discuss the effects of rapamycin on the mTOR pathway in detail.

4.3. Ras/MAPK pathway

The 3 components of the human Ras genes (Rat Sarcoma) are transmuting oncogenes and include: H-Ras, N-Ras, and K-Ras. Ras is a member of the G protein family, which basically means that it is activated by binding to guanosine triphosphate (GTP), and deactivated by binding to guanosine diphosphate (GDP) [49]. Ras serves to activate serine tyrosine kinases (STK) including Raf, MAPK (ERK1 and ERK2), PI3K, among other proteins that influence cell proliferation, differentiation, and survival [50]. Although the mutual activation of Ras and AKT in neural progenitors contributes to gliomagenesis in mouse models [51], Ras mutations

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are uncommon in human GBM [4]. Activated Raf phosphorylates and activates MAPK kinase (MAPKK), also called MEK, which in turn phosphorylates and activates MAPK [52, 53][54], which then moves to the nucleus to induce other transcription factors including Elk1, c-myc, Ets, STAT (signal transducers and activators of transcription), and PPARγ (peroxisome proliferator-activated receptor γ), which induce cell cycle progression and anti-apoptosis genes [50, 55].

NF-1, a tumor suppressor gene encoding neurofibromin, negatively regulates Ras and influences adenylate cyclase- and AKT-mTOR-mediated pathways [56]. NF-1 mutation and homozygous deletions are detected in 18% of GBM [4]. Mesenchymal type GBM appears to respond to concomitant chemo-radiation therapy and happens to commonly have inactivation of the NF-1 (37%), p53 (32%), and PTEN genes [57].

4.4. The p53 pathway

The *p53* gene, labeled as the "guardian of the genome", is located at chromosome 17q13.1 and encodes a protein that takes action against miscellaneous cellular stresses to regulate the corresponding genes that provoke programmed cell death or apoptosis, cell differentiation, senescence, DNA repair, and neovascularization [58]. The p53 pathway is the most frequently mutated pathway in human cancer and is essentially disrupted in roughly 80% of high-grade gliomas. p53, activated in response to DNA damage, induces transcription of genes such as p21Waf1/Cip1 that arrest the cell cycle progression at the G1 phase [59].

An important regulator of the p53 pathway is MDM2, an E3 ubiquitin ligase that negatively modulates p53 through transcriptional inhibition by direct binding as well as by degradation through its E3 ligase activity [60] [61]. On the other hand, the transcription of the MDM2 gene is induced by wild-type p53 [62]. This creates an autoregulatory feedback loop which controls the function of both the expression of MDM2 and the activity of p53. Another regulator of the p53 pathway is the tumor suppressor protein ARF (p14ARF), which controls p53 transcriptional activities by binding to MDM2 and consequently hindering its E3 ubiquitin ligase activity [63, 64]; conversely p14ARF expression is negatively regulated by p53 [59]. Both low grade and high grade gliomas exhibit inactivation or mutation of p14ARF [65]; homozygous deletion of p16INK4a/p14ARF/p15INK4b locus is one of the common mutations in GBMs [66]. Remarkably, mouse models revealed that co-deletion of ARF and INK4a increased accordingly with tumor progression from low- to high-grade gliomas [67]. This suggests that ARF and INK4a mutations are important steps in gliomagenesis.

4.5. ATM/Chk2 pathway

Disruption of the ATM/Chk2 pathway increases the speed of growth and development of glioma [68]; it also contributes to resistance to radiation therapy by helping the malignant cell activate a group of sensor kinases including ATM, ATR, and DNA-dependent protein kinase [69]. The latter phosphorylates multiple downstream mediators such as checkpoint kinases Chk1 and Chk2 that lead to cell-cycle checkpoint initiation and/or apoptosis [70]. Chk2, encoded at chromosome 22q12.1, acts as a tumor suppressor as it regulates p53-dependent

apoptosis [71]. Chk2 mutations have in general been rarely reported; however single copy loss of the chromosomal region containing Chk2 has been reported in gliomas [4].

4.6. Rb pathway

The retinoblastoma gene, Rb, is implicated in progression from low grade to higher grade astrocytoma [72], and it is inactivated in GBM [73]. The Rb pathway suppresses cell cycle entry and progression and curbs the p53 pathway by binding and inhibiting transcription factors of the E2F family. Of note, Rb controls progression from G1 to S-phase of the cell cycle [16]. Rb is regulated by the complex of cyclin-dependent kinases (CDKs); in the G1 phase, Rb is normally inactivated by Cyclin D/CDK4/CDK6- induced phosphorylation, causing its release from E2F and consequent cell cycle progression into the S phase. CDKN2B, a CDK inhibitor, which is commonly inactivated in GBM, forms a complex with CDK4 or CDK6, thus preventing the activation of CDKs. The outcome of this inhibition is prevention of cell growth. In addition to the inactivation of CDKN2B, amplification of CDK4 and CDK6 is also common in GBM, demonstrating that both CDK4 and CDK6 have a fundamental function in gliomagenesis and progression [74]. The CDKN2A (p16INK4a) protein binds to CDK4 and inhibits the CDK4/cyclin D1 complex, consequently inhibiting cell cycle transition from G1 to S phase [73]. This implies that any alteration of Rb, CDK4, or CDKN2A causes aberrant dysregulation of the G1-S phase transition. Complete loss of Rb, homozygous deletion or mutation of CDKN2A, CDK4 amplification, CDKN2B (p15INK4b) homozygous deletion, CDKN2C (p18INK4c) homozygous deletion, CCND2 (cyclin D2) amplification, and CDK6 amplification are observed in almost 80% of GBM [75-77].

4.7. STAT3

STAT (Signal transducers and activators of transcription) complexes are a family of cytoplasmic proteins that have SH2 (Src Homology-2) domains functioning as transcription factors that control cellular responses to cytokines and growth factors by signal transduction from the plasma membrane to the nucleus [78]. Target genes are then transcribed and contribute to proliferation, invasion, and apoptosis. STAT3 is an example of the STAT family proteins; it is rendered active by EGF and is overexpressed in GBM [79]. STAT3 also plays a role in the development of neural stem cells and astrocytes [80]. Targeting STAT3 may influence glioma cell motility, resistance to temozolomide, as well as clinical outcome [81-83].

5. Crosstalk

A distinguished characteristic of signaling networks in GBM is the presence of crosstalk, or communication between subnetworks, which interact to promote gliomagenesis and all the phenotypes of GBM. For example, there is evidence of mutual cross talk between cells inactivation of either Ras/Raf/MAPK or PI3K/AKT/mTOR triggering activation of the other [39]. Other examples are the interactions between Ras and stem cell factor (SCF)/c-kit signaling, mTOR, and MAP kinase pathways and the interactions between PI3Kand STAT3 pathways

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and NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells, in gliomas [84] [85]. In this section, we examine selected networks that play a role in glioma stem cells (GSC) and cell motility.

5.1. GSC

GSC have the particular ability to auto-renew and initiate gliomagenesis, express neural stem cell markers, and differentiate into multiple phenotypes such as neuronal, astrocytic, and oligodendroglial. Sonic hedgehog homolog (SHH) and Notch are overly expressed in GSC and therefore aberrantly regulate neural progenitor cells [86]. SHH is an important mitogen for medulloblastoma precursor cells. The SHH pathway also contributes to glioma formation as it is activated in GSCs. The SHH pathway is also closely related to the cell cycle as it inactivates Rb and causes over-expression of cell cycle regulators such as N-myc. PDGF signaling in neural stem cells is required for oligodendrogenesis, and amplification of this signal causes an abnormal proliferation of neural stem cells and the formation of large glioma-like lesions [87].

Notch (Notch1-4 in mammals) is a family of transmembrane receptors that control intercellular signaling [88]. They are transmembrane proteins that bind to notch and reveal the receptor to proteolytic activation. Notch is cleaved by presenilin 1 which generates a Notch1 intracellular domain (NICD), a nuclear transcriptional activator. Notch activation induces expression of downstream target genes, such as p53, and promotes neural stem cell growth [89]. BMPs are growth factors that act through binding to cell-surface receptor kinases (BMPRs); the effectors of BMPRs are the Smad proteins, which play a major role in bone and cartilage formation. The overall activity of BMPs is regulation of transcription. BMP ligands exhaust the GSC population by inducing the differentiation of GSCs into astroglial and neuron-like cells. Treating GSCs with BMPs *in vivo* delays tumor growth and diminishes tumor invasion [90].

miRNA is a small non-coding RNA that post-transcriptionally downregulates gene expression. Several studies have identified aberrant miRNA (microRNA-21, miR-326, microRNA-34a) expression in gliomas, and linked some of them to GSC maintenance and growth [91, 92]. Finally, Tumor Necrosis Factor alpha-Induced Protein (TNFAIP) 3 regulates both the NF-κB pathway as well as GSC self-renewal, growth, and apoptotic resistance [93].

5.2. Brain invasion and motility

Brain invasion is a hallmark of gliomas. Tumor cell migration requires highly coordinated steps of dissociation of existing cellular adhesions, remodeling of the actin cytoskeleton to project lamellipodium extensions, formation of new adhesions, and tail detachment along with proteolytic processing and secretion of extracellular matrix proteins along the trajectory. This complex phenotype requires crosstalk between networks that control the extracellular matrix, growth factors, cdc42, GTPases, actin polymerization, PAK, src, cadherins, PIP3, integrins, and myosin (see [96] for details).

Furthermore, some GBM exhibit enhanced motility at 5% ambient oxygen, which is higher than the typical 0.3-1% concentrations observed in cancer hypoxia. This result supports an increased propensity for invasion. The phenotype of increased motility in low ambient oxygen

conditions is mediated by phosphorylation of src, which in turn phosphorylates NWASP, Neural Wiskott-Aldrich Syndrome Protein (see [97] for details).

6. Effects of rapamycin on AKT

In the preceding section we have highlighted the complexity of the molecular interactions in GBM and the large number of subnetworks that communicate to generate the phenotypes. Because rapamycin inhibits the mTOR complex, it was considered a hopeful prospect for pharmaceutical therapy. However, in a clinical trial using rapamycin in the treatment of PTEN-deficient GBM, researchers encountered a paradoxical increase in AKT signaling, which was unexpected and undesirable as the latter promotes oncogenic processes [98]. The exact mechanisms for this finding are not yet known. Although many scientists postulate about a simple loss of negative feedback, there may be more than what meets the eye. To illustrate this point, we will delineate a well-characterized pathway in GBM molecular biology and discuss how intersecting activation and inhibitory pathways can lead to paradoxical downstream effects.

As reviewed in Howell et al. and Huang and Manning, mTORC1 acts ultimately as a negative regulator of AKT through various mechanisms [99-101]. First, mTORC1 directly phosphorylates IRS (insulin receptor substrate), which is thought to hinder the scaffolding ability of PI3K to activate AKT. Additionally, mTORC1 acts through its downstream effector S6K1 (S6 kinase 1), which also phosphorylates IRS at specific serine residues and reduces downstream AKT activation [102-105]. Zhang et al. in 2003 and 2007 showed that mTORC1 activation leads to repression of PDGFR A and B transcription, which inhibits PDGF signaling to AKT and blocks proper transmission of signals from other growth factors [106, 107]. AKT also acts as an activator of mTORC1; but this interaction is irrelevant to our discussion of mTORC1 inhibitors because direct inhibitors of mTORC1 are not influenced by AKT.

Since mTORC1 inhibits AKT (Figure 1, t = 0), bringing down mTORC1 via rapamycin (Figure 1, t = 1) would subsequently lead to an increase in AKT (Figure 1, t = 2).

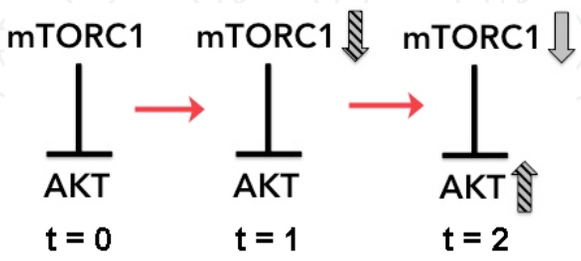

Figure 1. Cartoon depicting the negative effects of mTORC1 on AKT activation and its response to perturbations. Blocked arrows indicate repression/deactivation. The arrow pointing down indicates repression of mTORC1 activity at time = 1. The arrow pointing up indicates the response of the network by increasing the activity of AKT at time = 2.

At first sight this explanation is logical, but when we look deeper into the networks we discover additional factors to this relationship that can provide alternate explanations for the clinical

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trial's findings. The simple diagram of Figure 1a does not appear to be the appropriate explanation.

Subsequent studies have found that at low concentrations, rapamycin treatment leads to an increase in AKT activity; however, at high, super-physiological concentrations rapamycin causes a decrease in AKT activity [108]. Interestingly, at high concentrations of rapamycin, both mTORC1 and mTORC2 are inhibited. Hence, we need to consider the effects of mTORC2 on AKT. In fact, mTORC2 phosphorylates AKT on S473 (serine 473), which activates AKT at the plasma membrane [112]. The observation, that inhibiting both mTORC1 and mTORC2 caused a decrease in AKT activity, indicates that mTORC1 is a weak inhibitor of AKT as compared to mTORC2 as an activator (see Figure 2 for details).

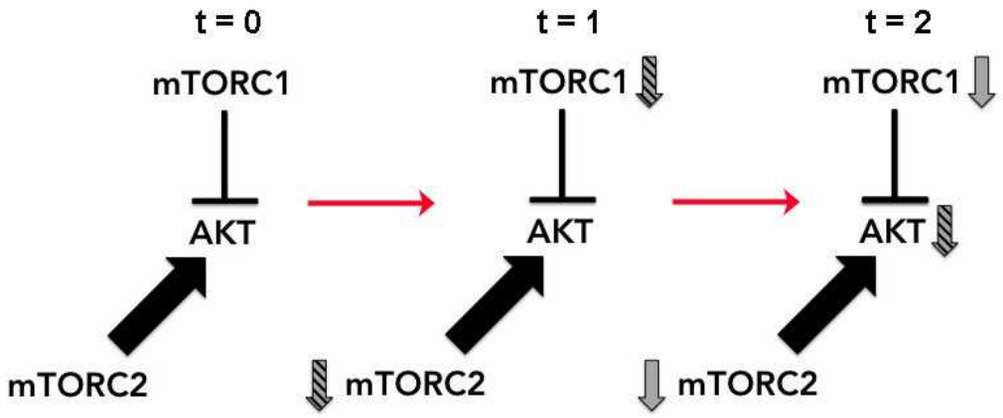

Figure 2. Cartoon depicting relative effects of mTORC1 and mTORC2 on AKT and the response of the network to high concentrations of rapamycin. Blocked and regular arrows indicate repression/deactivation and activation, respectively. The thickness of the arrows reflects the level of repression or activation. If mTORC2 is a stronger activator of AKT than mTORC1 is a repressor (time = 0), treating the cells with high concentrations of rapamycin, which inhibits both mTORC1 and mTORC2 (time = 1), causes a decrease in AKT activity (time = 2).

If we delve deeper into these pathways, we learn of a negative loop between AKT, TSC2 (tuberous sclerosis complex 2), and mTORC2 (See Figure 3) [101]. AKT directly inhibits the activity of the TSC2 complex by phosphorylating TSC2 [109-111]. Furthermore, Huang and Manning provide evidence for the subsequent arm of the loop where the TSC2 complex activates mTORC2 in a manner independent of mTORC1 [100]. These relationships together comprise the negative loop illustrated in Figure 3.

We assume that the physiological levels of rapamycin used in the clinical trial inhibit the activity of mTORC1 without any effects on mTORC2; let us now study the reaction of the network in the presence of the AKT/TSC2/mTORC2 negative loop (see Figure 4). Theoretically, if mTORC1 levels go down (Figure 4a), AKT activity should initially increase (Figure 4b). However, higher AKT activity would lead to augmented inhibition of the TSC2 complex (Figure 4c). The lower levels of TSC2 complex would then reduce the activation of mTORC2 (Figure 4c), which in turn feeds back to influence AKT. The ultimate result on AKT depends on the dynamics and the strengths of the connections the negative loop. At this stage, two possibilities arise as follows.

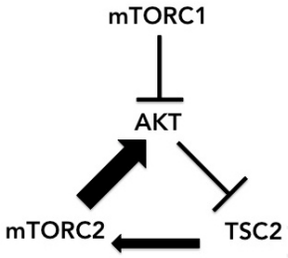

Figure 3. Cartoon depicting negative loop between AKT, mTORC2 and the TSC2 complex. Blocked and regular arrows indicate repression/deactivation and activation, respectively.

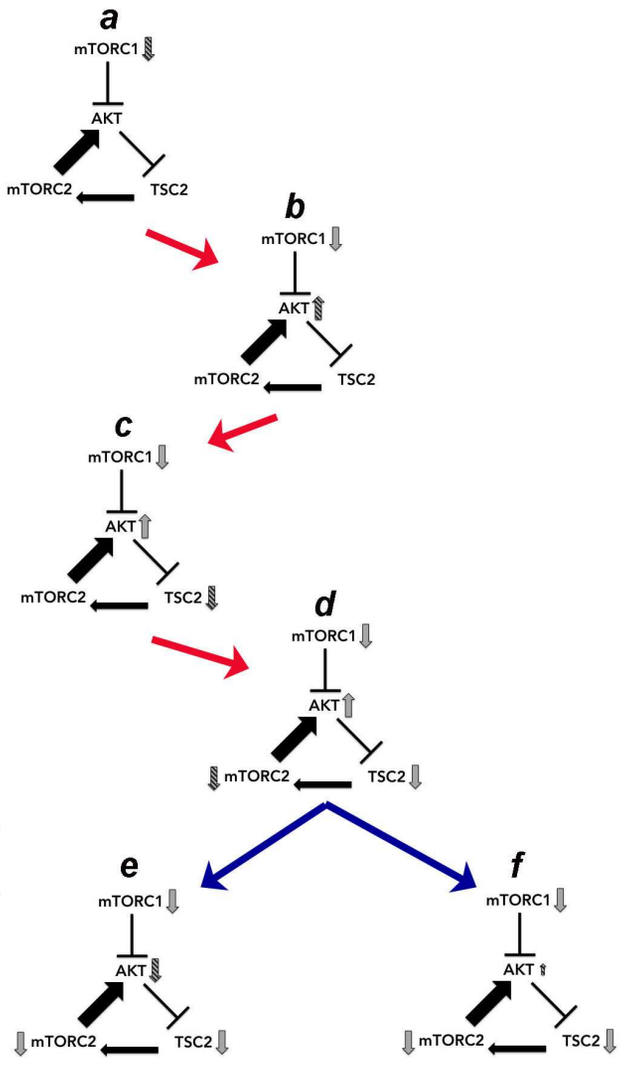

Figure 4. Cartoon depicting the reaction of the network to rapamycin in the presence of the negative loop. Blocked and regular arrows indicate repression/deactivation and activation, respectively. Arrows pointing up or down indicate perturbations causing increased or decreased activity, respectively.

Possibility A: If both AKT's inhibitory effect on TSC2 and TSC2's activation effect on mTORC2 are strong, then an increase in AKT will lead to a significant decrease in mTORC2. Because the

latter is a stronger activator than mTORC1 is a repressor (Figure 3), this causes an ultimate *decrease* in AKT activity (Figure 4e).

Possibility B: The negative loop would cause a decrease in mTORC2 activity in any case. This could attenuate but may not reverse the increase in AKT (Figure 4f).

This exercise highlights the profound effects of the presence of a negative loop in the simple network. The results of the clinical trial where rapamycin leads to an increase in AKT activity would be consistent with the explanation of possibility B. However, possibility A cannot be excluded, since other regulatory loops likely influence this pathway as well. In creating treatments and therapeutic strategies in GBM, it is imperative to gain a complete picture of the complexity of intersecting pathways since inhibition can lead to paradoxical, sometimes detrimental results.

7. Conclusion

The molecular networks of GBM include a large number of molecules and interactions, as well as multiple subnetworks and crosstalk. These large networks appear to have the ability to not only bypass therapeutic blockade, but to react to therapeutic modalities by activation of oncogenic subnetworks. We are not surprised that little progress has been made against these deadly and intelligent tumors. Success requires a clear understanding of these large networks as well as predictions of their dynamical (time-dependent) behavior in response to perturbations (*ie.* therapeutic interventions). Fortunately, recent advancements in genomics and mathematical biology bring us closer to attaining these goals.

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