



Published in final edited form as:

Metab Brain Dis. 2013 September ; 28(3): 355–366. doi:10.1007/s11011-013-9401-7.

Targeting Oncogenic ALK and MET: A Promising Therapeutic Strategy for Glioblastoma

Gerald C Wallace IV¹, Yaenette N Dixon-Mah¹, W Alex Vandergrift III¹, Swapan K Ray², Catherine P Haar¹, Amber M Mittendorf¹, Sunil J Patel¹, Naren L Banik¹, Pierre Giglio¹, and Arabinda Das^{1,*}

¹Department of Neurosciences (Divisions of Neurology and Neurosurgery) & MUSC Brain & Spine Tumor Program Medical University of South Carolina, Charleston, SC 29425, USA

²Department of Pathology, Microbiology and Immunology, University of South Carolina School of Medicine, Columbia, SC 29208, USA

Abstract

Glioblastoma is the most common aggressive, highly glycolytic, and lethal brain tumor. In fact, it is among the most commonly diagnosed lethal malignancies, with thousands of new cases reported in the United States each year. Glioblastoma's lethality is derived from a number of factors including highly active pro-mitotic and pro-metastatic pathways. Two factors increasingly associated with the intracellular signaling and transcriptional machinery required for such changes are anaplastic lymphoma kinase (ALK) and the hepatocyte growth factor receptor (HGFR or, more commonly MET). Both receptors are members of the receptor tyrosine kinase (RTK) family, which has itself gained much attention for its role in modulating mitosis, migration, and survival in cancer cells. ALK was first described as a vital oncogene in lymphoma studies, but it has since been connected to many carcinomas, including non-small cell lung cancer and glioblastoma. As the receptor for HGF, MET has also been highly characterized and regulates numerous developmental and wound healing events which, when upregulated in cancer, can promote tumor progression. The wealth of information gathered over the last 30 years regarding these RTKs suggests three downstream cascades that depend upon activation of STAT3, Ras, and AKT. This review outlines the significance of ALK and MET as they relate to glioblastoma, explores the significance of STAT3, Ras, and AKT downstream of ALK/MET, and touches on the potential for new chemotherapeutics targeting ALK and MET to improve glioblastoma patient prognosis.

Keywords

Glioblastoma; ALK; MET; AKT

*Correspondence should be directed to Arabinda Das, Department of Neurosciences (Divisions of Neurology and Neurosurgery) & MUSC Brain & Spine Tumor Program, Medical University of South Carolina, 96 Jonathan Lucas Street, Charleston, SC 29425; Arabinda Das (dasa@musc.edu).

1. Background

Glioblastoma is one of the most aggressive, highly glycolytic, and deadly brain tumors. Approximately 9,000 new cases are diagnosed each year in the United States according to the Central Brain Tumor Registry of the United States (www.cbtrus.org). Among these lethal CNS neoplasms, glioblastoma (WHO classification of Grade IV) is the most common (Wen & Kesari 2008). The rarity of glioblastoma diagnosis is contrasted by its extreme lethality; patients have a median life expectancy following diagnosis and aggressive treatment of just 14 months (Wen & Kesari 2008).

Pathologically, glioblastoma presents as a highly heterogeneous tumor in regard to histological morphology and genetic profile. Intratumoral giant cells, sporadic necrotic loci, and a high mitotic index are all pathological hallmarks of glioblastoma (Benito *et al.* 2010). In addition, glioblastoma tumors present intense metabolic needs that are fed by increased neo-vascularization often directly induced by such tumors (Benito *et al.* 2010). Despite increased angiogenic potential in glioblastoma, CNS tumors can develop necrotic or apoptotic cores if mitosis outpaces neo-vascularization of the tumor. These areas can be identified both by observation of necrotic cells and surrounding pseudopalisading tumor cell formations. Ultimately, these pro-survival angiogenic features elaborate the tenacity of glioblastoma tumors.

Current standard of care therapy, which includes combination radiation and temozolomide (TMZ) treatment, extends survival on average to 14.6 months (Stupp *et al.* 2005). Despite such extreme glioblastoma related lethality, long-term life expectancy increases with each year a patient survives with the disease (Polley *et al.* 2011). This would seem to suggest that a therapy capable of extending a patient's life expectancy in the immediate future also significantly increases long-term survival. However, Polley and others cautioned that such survival findings as theirs were based on data from patients who had enrolled in clinical trials and thus were predisposed to have a better prognosis than patients with more severe (*i.e.* study ineligible) cases of glioblastoma (Polley *et al.* 2011). Certainly, this does not diminish the validity of current and new therapies that extend life for glioblastoma patients, but it highlights that glioblastoma remains incurably lethal despite aggressive funding and research. Regardless of whether or not a patient survives one, two, or even five years following a diagnosis of glioblastoma, the disease itself presents with numerous debilitating neurological deficits which are only exacerbated by standard of care therapies.

The remainder of this review focuses on two components of glioblastoma related tumorigenesis that may represent significant regulatory targets for glioblastoma: anaplastic lymphoma kinase (ALK) and the hepatocyte growth factor (HGF) receptor (HGFR or MET). It will explore early clinical observations related to these factors, examine the functional significance of their dysregulation in glioblastoma, and finish by discussing current and future therapies that may correct or inhibit ALK and MET functionalities in glioblastoma.

2. ALK & MET in Glioblastoma

2.1 Significance of ALK & MET to cancer

Cancer in general is precipitated by aberrant expression of pro-mitotic and pro-metastatic genes resulting in potentially lethal proliferation and invasion by affected cells. However, the very mitotic and metastatic factors that are the hallmarks of many adult cancers are normally expressed or up-regulated at various points during embryonic development and wound healing. With this in mind, recent research has worked to identify pathways selectively expressed in cancers, including glioblastoma, in order to better differentiate cancer cells from normal cells and also to aid in the design of novel, specific chemotherapies.

Much work has focused on the role of receptor tyrosine kinases (RTK) and their role in the initiation and progression of glioblastoma. This is unsurprising considering nearly 40-60% of all glioblastomas express increased levels of epidermal growth factor receptor (EGFR), an angiogenic agent (Houillier *et al.* 2006, Ohgaki *et al.* 2004, Shinjima *et al.* 2003, Szerlip *et al.* 2012). In these cases, and in those where EGFR expression may be unaltered, other pro-survival, proliferative, and/or vasculogenic pathways are deregulated, including: the PTEN/Akt/mTOR pathway, the TP53/MDM2/ARF pathway, and INK4a/RB1 pathway. The latter pathways are discussed in greater detail elsewhere (Ohgaki & Kleihues 2007, 2008), but the significance of PTEN, AKT, and mTOR will be discussed further. As fellow members of the RTK family, ALK and MET have gained extensive experimental attention over the last few decades.

2.1.1 ALK—ALK and its ligand, pleiotrophin (PTN), are highly expressed during embryonic development of the nervous system and are normally limited in adult tissues (Grzelinski *et al.* 2009). Aberrantly expressed ALK was first determined to be a contributing oncogenic factor in non-Hodgkin's lymphoma (Morris *et al.* 1994). Since the initial discovery of oncogenic ALK translocations in lymphoma, various ALK mutations have been described in multiple cancer lines including glioblastoma (Fujimoto *et al.* 1996, Pulford *et al.* 1997, Pulford *et al.* 2004a, Pulford *et al.* 2004b, Dirks *et al.* 2002). ALK and PTN are expressed at significantly higher levels in high grade brain tumors (glioblastoma and anaplastic oligodendrogliomas) when compared to normal brain tissue and low grade tumors (Stylianou *et al.* 2009). The binding of PTN to its extracellular domain on ALK mediates growth stimulation and antiapoptotic pathways.

Previously, the PTN gene has been targeted, resulting in suppression of tumor angiogenesis, growth, and metastases in a variety of tumors, including glioblastoma (Grzelinski *et al.* 2009). Additionally, there was decreased growth and increased apoptosis of glioblastoma xenografts in athymic nude mice with ribozyme-mediated targeting of ALK (Grzelinski *et al.* 2009). A recent presentation at the November 2011 Conference in San Francisco, “Molecular Targets and Cancer Therapeutics” suggested that ALK/MET activity was present in over half of the patient tumors analyzed (http://mct.aacrjournals.org/cgi/content/short/10/11_MeetingAbstracts/A42?rss=1).

Another possible reason for the widespread connection between ALK and cancer may lie in the generalized transcriptional and protein level expression of ALK during embryonic development (Vernersson *et al.* 2006). Since ALK expression is partially responsible for developmental control of cellular proliferation, it may be that increased activation of ALK plays a part in tumor stem cell like proliferation in glioblastoma. In mice, increased expression of ALK in the developing CNS and PNS versus other tissues further highlights the likely importance of this RTK in CNS tumors like glioblastoma (Vernersson *et al.* 2006).

Pro-oncogenic ALK mutations may occur through a gain-of-function effect (i.e. constitutively expressed ALK) as suggested by two cases of mutation-related disruption of CNS development (de Pontual *et al.* 2011). In their paper, de Pontual and others make a strong case for ALK mediated abdominal and medullar tumor formation in two infants born with ALK germline translocation mutations. In both cases, *de novo* germline mutations were associated with both PNS and CNS tumor formation and, ultimately, death. Experimental observations also lend support to the theory of ALK mediated tumorigenesis in glioblastoma. Stylianou and others had developed both activating and inactivating antibodies aimed at the PLT binding domain on ALK that increased or decreased (respectively) the oncogenic activity of the ALK receptor in U87MG cells *in vitro* and *in vivo* (Stylianou *et al.* 2009).

2.1.2 MET—HGF and its target receptor, MET, are two factors that are generally detectable only in epithelial or mesenchymal cells during development and wound healing (Di Renzo *et al.* 1991, Sonnenberg *et al.* 1993). Both immune response and injury repair mechanisms depend heavily on HGF/MET activation (Michalopoulos & DeFrances 1997, Jin *et al.* 2003). Together, HGF and MET are essential to normal murine development and result in embryonic lethality when absent (Schmidt *et al.* 1995, Uehara *et al.* 1995, Bladt *et al.* 1995, Michalopoulos & DeFrances 1997, Jin *et al.* 2003). An excellent history of HGF/MET discovery and functional characterization can be found in Birchmeier and colleagues' review article (Birchmeier *et al.* 2003).

The first characterization of MET came from the identification of the human oncogene, *tpo-met* (often referred to as c-Met) (Cooper *et al.* 1984). Since then, MET and HGF expression have been correlated with glioblastoma prognosis (Arrieta *et al.* 2002). In Liu *et al.*, it was found that patients who expressed a higher level of c-Met had a significantly shorter progression free survival time of 6.1 months, versus 11.5 months in those patients who expressed a lower level of the oncogene (Liu *et al.* 2011). It has also been suggested that autocrine MET tumors (i.e. tumor HGF targeting tumor MET) rather than paracrine MET tumors are more responsive to MET inhibitor therapy (Xie *et al.* 2012). Given that ligand independent activating mutations of ALK are known to contribute to CNS tumor formation, it is somewhat intriguing that ligand dependent activation of MET should drive MET mediated glioblastoma growth.

In recent years, activity of the epidermal growth factor receptor (EGFR) mediated PI3K/AKT/mTOR signal transduction cascade has gained significant attention. EGFR is the primary amplified RTK related to Ras/PI3K activation among glioblastoma tumors (2008, 2008, 2008, 2008, Verhaak *et al.* 2010). Amplifications of EGFR, which is itself a

member of the RTK family, appear in 36% of glioblastoma samples with 1/3 of those cases exhibiting a specific EGFRvIII mutation (Wong *et al.* 1992, Rao *et al.* 2010, Rao *et al.* 2008). In part due to the known amplification of chromosome 7 (the chromosome on which MET is located) among glioblastoma tumors, it has been suggested that amplification of EGFR in glioblastoma could be related to aberrant MET expression (2008, Xie *et al.* 2012). Surprisingly, glioblastoma tumors expressing MET mutations only represent ~5% of the total population (2008). However, the significant downstream effects of MET activation make it a promising target in glioblastoma treatment. The role of MET in regulating glioblastoma stem cells (GSCs) has also been a target of recent discussion. Joo and colleagues isolated glioblastoma cells that expressed a high level of MET and which were found to be located in perivascular regions (Joo *et al.* 2012). These cells were found to be tumorigenic and resistant to radiation, and the disruption of their signaling to GSCs was proven, both *in vivo* and *in vitro*, to significantly reduce the proliferative and invasive capabilities of glioblastoma (Joo *et al.* 2012).

In short, MET activation is essential to development, engaged following injury to assist with wound repair, and expressed widely in both developing and adult organisms. All of these characteristics allow for oncogenic transformation of normal cells to carcinomas through MET mutations. However, MET and ALK modifications broadly affect normal cellular processes (as do many RTKs) via numerous pathways. Many of these processes are only activated in highly mitotic cells, such as those found in developing embryos or in adult stem cell populations, and as such, play a significant role in cell survival and motility. Therefore, a careful review of these downstream effects is paramount to understanding the implications for ALK/MET-related oncogenesis and ALK/MET anti-cancer therapies.

2.2 Downstream effectors: STAT3, Ras, AKT

Ligand activation of RTKs triggers phosphorylation of intracellular tyrosine residues and a cascade of pro-survival and pro-mitotic effectors. Likewise, constitutive phosphorylation of the intracellular domains of RTKs also affects pro-oncogenic cascade induction. Not surprisingly, activation of ALK and MET (either ligand dependent or independent) also triggers these cascades. For most RTKs, including ALK and MET, there are three points of intracellular signal diversion which may mediate the transition from a normal cell to cancer cell (Figure 1). STAT (signal transducer and activation of transcription; STAT3, in particular), AKT (also known as protein kinase B), and Ras ('rat sarcoma'; named from the first identified gene transcript in rat sarcomas) downstream signaling appear to be at once independently and interactively responsible for the oncogenic activities of both ALK and MET (Boufaied *et al.* 2010, Zhang *et al.* 2002, Chiarle *et al.* 2005, Porter & Vaillancourt 1998, De Luca *et al.* 2012). Though these three downstream components are highly dependent upon transduction factors such as Grb2, Gab1 and JAK, which respond more directly to RTK tyrosine phosphorylation, they represent intracellular turning points that individually promote oncogenic transition and tumor growth (Birchmeier *et al.* 2003). Interactions among these cascades do occur, and understanding those interactions therefore becomes vital to understanding the significance of ALK/MET oncogenic activities. To begin with, it is important to understand the individual effects of STAT3, Ras, and AKT.

2.2.1 STAT3 transcriptional activator—STAT3 is one of the master transcriptional regulators responsible for a majority of downstream signaling events most commonly associated with aggressive carcinomas and poor patient prognosis (Cooper *et al.* 2012). Activation of ALK or MET directly stimulates STAT3 phosphorylation (Zhang *et al.* 2002, Zamo *et al.* 2002). Following phosphorylation, STAT proteins translocate to the nucleus where they act as transcriptional promoters for various genes, which themselves can promote cell survival (Aaronson & Horvath 2002) and anchorage independent growth (Zhang *et al.* 2002, Schaper *et al.* 1997). In ALK positive tumors, ALK-STAT3 activation promotes tumor cell outgrowth and directly prevents apoptosis, in part, by increasing Bcl-x(L) expression (Zamo *et al.* 2002).

Phosphorylated STAT3 can be found following prolonged (>24hr) activation of the ALK ligand binding site, but the level of phospho-STAT3 engendered by ligand-independent activation of ALK was exponentially higher. This discovery may point to a ligand independent role for ligand-independent oncogenesis constitutive activation of STAT3 by ALK and possibly MET. Recently, STAT3 mediated activation of the insulin like growth factor 1 (IGF-1) autocrine loop has enthralled clinicians and researchers (Gariboldi *et al.* 2010). Glioblastoma cell line experiments have identified a role for IGF directed pro-metastatic changes and temozolomide resistance (Hsieh *et al.* 2011). Phospho-STAT3 suppresses IGFBP-5, which normally suppresses IGF-1, with resultant increases in IGF-1 leading to autocrine stimulation of antiapoptotic transcription factors such as hypoxia inducible factor (HIF)-1 α . In turn, HIF-1 α stimulates pro-survival and pro-metastatic effectors such as: toll like receptor 9, VEGF, IGF, and indirectly, Bcl-2 (Sinha *et al.* 2011, Gariboldi *et al.* 2010).

Finally, embryonic stem cell studies have determined a necessary role of STAT3 activation for preserving pluripotency among stem cell populations (Matsuda *et al.* 1999). In glioblastoma, stem cell populations are postulated to mediate drug and radiotherapy resistance. STAT3 directly binds to and promotes transcription of the *Cdc25A* gene, which controls cell cycle progression from G1 to S phase (Barre *et al.* 2005). In cytokine receptor activation, STAT3 activation also contributes to transcription of *c-myc* and *Pim-1* genes to further contribute to both mitosis and survival (Shirogane *et al.* 1999). Finally, STAT3 also blocks p21 transcriptional activities that would otherwise impede cell cycle progression (Zhang *et al.* 2002). Thus the significance of STAT3 phosphorylation in tumor cells, including glioblastoma, likely depends on four key features: 1) activation of pro-survival pathways, 2) promotion of cell cycle progression, 3) induction of metastatic marker expression, and 4) by potentiating the development of stem-like cells in tumors. Figure 2 provides a summary of the discussed effects of STAT3 phosphorylation by ALK/MET.

2.2.2 Ras: dual oncogenic pathways—Ras, embedded in the cellular membrane proximally to ALK or MET, is quickly activated following receptor tyrosine phosphorylation (De Luca *et al.* 2012). Once activated, Ras may stimulate PI3K-mediated AKT activity as well as the RAF/MEK/ERK pathway which ultimately promotes activation of cell cycle progression factors (De Luca *et al.* 2012). A general diagram is found in Figure 3. Recently, it was determined that Ras mediated tumorigenesis in epidermal cells first stimulates elevated Rac1 activity (Samuel *et al.* 2011). Since Rac1 activity is dependent

upon GTP, Ras mediated activation of downstream MEK and ERK is likewise dependent upon Rac1-GTP binding activities. Ultimately, interactions between Ras and Rac1 eventually result in increased β -catenin activity, which then leads to E-cadherin production (Gherardi *et al.* 2012). E-cadherin is thus capable of stabilizing cancer cells for migration and metastasis.

The Ras/MEK/ERK pathway has been clearly mapped out largely due to its involvement in numerous cancers. Still, it is known that Ras activation which stimulates ERK, and subsequently Elk-1 transcription factor activation, directly contributes to HGF production and autocrine stimulation of MET (Goudar *et al.* 2005). In the case of cytokine stimulation of Ras/ERK pathways, a feedback loop which prevents excessive STAT activation through an intracellular Ras expression modulator (RasGAP), simultaneously appears to further activate Ras/ERK mediated cell survival activity (Chi *et al.* 2012, Chin *et al.* 2012, Cooper *et al.* 2012, Del Vecchio *et al.* 2012). The interplay between Ras and other members of the RTK downstream cascade remains underexplored. For instance, what activation level of Ras or its associate cascades identified by AKT and/or STAT3 activation, is necessary for oncogenic transformation? A fascinating study that outlined the genetic creation of astrocytomas by inserting constitutively active Ras and AKT genes into the glial fibrillary acidic protein (GFAP) sequence may help to understand this question. Holland and colleagues discovered that in this genetic induction model of glioblastoma, both Ras and AKT together were necessary for oncogenesis and glioblastoma tumor formation (Holland *et al.* 2000). Separately, the roles for Ras and AKT are fairly well known, but the interdependency between the two pathways is less so.

These findings highlight the potential for AKT or Ras specific inhibitors as well as more generalized RTK inhibitors. They also highlight the significance of the third RTK-mediated oncogenic pathway pivoting on AKT phosphorylation.

2.2.3 AKT in oncogenesis—Following discovery in the late 1970's, a virus derived oncogene, AKT8, was used to induce tumorigenesis *in vivo* (Staal & Hartley 1988). Humans express three isoforms: AKT1, AKT2, and AKT3, which are implicated in DNA repair mechanisms, growth and insulin metabolism respectively (Chen *et al.* 2001, Garofalo *et al.* 2003, Easton *et al.* 2005).

While the role of AKT in gliomas has been well elaborated previously (McDowell *et al.* 2011), it will be useful to briefly review the main mechanisms of AKT activity and downstream effects. AKT phosphorylation by RTKs begins with the phosphorylation of phosphatidylinositol (4,5)-bisphosphate (PIP2) to phosphatidylinositol (3,4,5)-triphosphate (PIP3) by phosphatidylinositol 3-Kinase (PI3K), which is itself directly activated following RTK tyrosine phosphorylation (Vignot *et al.* 2005, Larue & Bellacosa 2005). The transition from PIP2 to PIP3 is directly controlled by phosphatase and tensin homologue (PTEN). PTEN thus acts as a master switch for AKT activity intracellularly, a fact which makes the use of U87MG, SNB-19, and U373MG (the most popular cell lines for glioblastoma *in vitro* studies which differentially express PTEN activities) highly valuable to understanding AKT phosphorylation effects in tumorigenesis (McDowell *et al.* 2011). In general, PTEN

deletions are found in ~30% of gliomas making it an enticing target for future study (Li *et al.* 1997, Pulford *et al.* 1997, Ward *et al.* 1997).

AKT inhibits TSC2 mediated inhibition of the mammalian target of rapamycin, or mTOR, functionality (McDowell *et al.* 2011). When AKT activation leads to mTOR activation, translation of cell cycle progression markers as well as activation of pro-survival cascades favor tumor growth and progression (for a thorough review, see (Faivre *et al.* 2006) & (McDowell *et al.* 2011)). Rapamycin is known to inhibit cancer cell cycle progression and enhance sensitivity to radiation by blocking mTOR-mediated translational modifications of oncogenic gene products (Anandharaj *et al.* 2011). Experiments targeting mTOR suggest that blocking PTEN activity sensitizes cells to mTOR inhibition and prevents IGF-mediated pro-survival initiation (Vivanco & Sawyers 2002, Hagerstrand *et al.* 2010). Rapamycin mediated inhibition of survivin (a microtubule stabilizing protein associated with cell survival, mitosis, and metastasis) also inhibits AKT phosphorylation, significantly reducing viability of cancer cell lines (Anandharaj *et al.* 2011, Weiss *et al.* 2012). This suggests mTOR (and presumably AKT) controls expression of AKT phosphorylation mediators. Currently, everolimus (Novartis) is an orally available mTOR inhibitor with a high lipophilic index allowing penetration of the blood-brain barrier and with combination gefitinib, offer a clinically active combination in recurrent glioblastoma where EGFR and PI3K/AKT are co-activated (Kreisl *et al.* 2009). Additionally, survivin is of particular interest to cancer identification since only highly mitotically active cells and human tumors generally express this protein. High expression levels of survivin throughout development point back to a stem cell like phenotype that is increasingly important to glioblastoma study.

AKT blocks activation of a number of other factors as well. Indirectly, AKT inhibits p53 by enabling MDM2 phosphorylation (McDowell *et al.* 2011, Birchmeier *et al.* 2003). As a control mechanism for proliferation and anti-apoptotic survival, p53 is of great interest to glioblastoma studies (Bajbouj *et al.* 2012). Additionally, AKT mediated inhibition of FOXO transcription of *BIM* and *FASL* genes directly contributes to cell survival (Nakamura *et al.* 2000). Further stabilization of cellular survival comes in the form of anti-phospho-BAD activities as well as caspase inhibition by phospho-AKT (Birchmeier *et al.* 2003, McDowell *et al.* 2011). Finally, AKT mediated inhibition of Gsk3 β , which forms a complex with other proteins to promote β -catenin degradation, results in increased production of E-cadherin which in turn promotes motility and metastasis (Gherardi *et al.* 2012). This is a particularly interesting effect as Ras activation also modulates β -catenin expression to promote E-cadherin production (Gherardi *et al.* 2012). Finally, AKT mediates activation of NF κ B, which in turn mediates transcription of numerous pro-survival factors (Syed *et al.* 2008). However, whether NF κ B activation is essential to AKT related glioblastoma progression is unclear (Zeng *et al.* 2002). An overview of the discussed AKT pathway can be found in Figure 4.

3. ALK & MET in Relation to Current Therapies

TL-4601, a farnesylated dibenzodiazepine which inhibits the Ras/MEK signaling cascade, has been evaluated in a phase II clinical trial (Mason *et al.* 2011). Unfortunately, TL-4601 failed to significantly affect tumor progression at the doses given in patients and only

transiently suppressed Ras-ERK MAPK signaling compared to the more marked reductions seen in laboratory studies (Campbell *et al.* 2010, Boufaied *et al.* 2010). Crizotinib (PF-02341066) is an orally available ATP-competitive selective inhibitor of ALK and MET tyrosine kinases that inhibits tyrosine phosphorylation on these receptors at nanomolar concentrations (McDermott *et al.* 2008, Christensen *et al.* 2007). Pharmacokinetic-pharmacodynamic studies have indicated a near complete inhibition of MET activation in mouse glioblastoma tumors; indeed, near total inhibition may be required for successful targeting of MET (Yamazaki *et al.* 2008). Despite limited widespread use, crizotinib has been presented to positively affect non-small cell lung carcinoma metastasized to the brain of at least one patient (Costa *et al.* 2011). Additionally, this patient presented with fewer contraindications while on crizotinib versus previous treatments.

4. Future Directions for ALK & MET Targeted Therapies

4.1 Challenges to ALK/MET manipulation

Numerous bioactive agents have been developed in recent years to inhibit HGF/MET activity with the effect of blocking brain tumor progression (Welsh *et al.* 2009, Lal *et al.* 2009, Guessous *et al.* 2010, Crosswell *et al.* 2009, Cecchi *et al.* 2010). In all cases, inhibition of these RTKs reduces mitotic and metastatic profiles for numerous cancer cells. However, the very benefits imparted by RTK inhibitors, may pose significant risks to patients by blocking normal wound repair mechanisms (Ward *et al.* 1997). For example, STAT3 activation, as found downstream of ALK or MET activation, is involved in normal wound healing of the lung during infections (Pechkovsky *et al.* 2012). Combining ALK/MET inhibitors with current chemotherapies which diminish immune function would necessitate close observation to prevent severe pulmonary infections and scarring. Additionally, since ALK activity is integral to smooth muscle wound healing pathways (Ward *et al.* 1997), administration of ALK inhibitors may affect post-operative healing following surgical resections and could increase vascular pathology in glioblastoma patients with poor cardiovascular health.

Unfortunately, RTK inhibitors (like Bevacizumab) which target the RTK VEGF receptor, appear to exacerbate treatment by promoting both ischemic and hemorrhagic stroke in treated patients (Fraum *et al.* 2011). Cediranib was hailed as a potent anti-angiogenic agent that targeted the VEGF receptor and effectively halted new vessel growth in glioblastoma (Batchelor *et al.* 2010). One challenge of some anti-angiogenic inhibitors, like cediranib, is that it does not target ALK or MET. Thus, it is unclear how strong of a role ALK or MET plays in angiogenesis. Similarly, rapamycin, which blocks mTOR dependent protein synthesis, was expected to interfere with AKT mediated oncogenesis, but actually resulted in increased phospho-AKT levels and drug resistant tumor cell survival (Fan *et al.* 2010). Until trials are undertaken to explore the effectiveness of ALK/MET inhibition in human glioblastoma cases, it will be impossible to predict contraindications and complications.

4.2 Multi-drug therapy

The anti-proliferative potential for ALK/MET related inhibitors are exciting, yet their potential for inducing tumor regression is unknown. It is likely that any such drugs will

excel at inhibiting tumor growth as well as metastatic potential. Still, blocking STAT3, AKT, and Ras downstream cascades would reduce pro-survival markers as discussed earlier. For instance, rapamycin co-treatment with a PTEN inhibitor blocks IGF mediated cell survival (Faivre et al. 2006, Anandharaj et al. 2011). Since PTEN activity is required for AKT activation, perhaps co-treatment of rapamycin with an ALK or MET inhibitor would enhance the activities of both drugs.

A few studies have undertaken to show similar effects with TMZ. Co-treatment of tumors with TMZ and AMG-102 (an antibody against HGF) significantly enhances *in vitro* apoptosis and *in vivo* tumor regression in animal models (Jun *et al.* 2007). Whether such effects will be mirrored in tumors expressing activating mutations of ALK or MET receptors remains to be seen. Nevertheless, the significance of extracellular repression of MET activation combined with TMZ cannot be understated. Blocking effectors downstream of ALK and MET in combination with more generalized ALK and MET inhibitors may also enhance therapy. For instance, administration of rapamycin in conjunction with gefitinib, an EGFR inhibitor, greatly increases the effectiveness of gefitinib while also overcoming the pro-survival AKT phosphorylation inducible by rapamycin alone (Fan et al. 2010, Goudar et al. 2005). The complex interactions of the broadly activating effectors in the RTK intracellular cascade both enhance the potential for ALK and MET inhibitors and also highlight the limitations of mono-therapies targeting these receptors. Indeed, the complexities of glioblastoma itself indicate the need for a complex approach to treatment.

4.3 Targeting metabolic pathways by ALK/MET inhibitors

Many known oncogenic signaling pathways involved in gliomagenesis have strong influences on tumor cell metabolism and promote the switch from oxidative phosphorylation to aerobic glycolysis for ATP generation (Wolf, et al). Glioblastoma cells, which have a higher ratio of lactate to pyruvate, require glutamate dehydrogenase (GDH) to survive impairments of glucose metabolism or Akt signaling. Akt indirectly regulates GDH through its effects on glucose metabolism where the inhibition of Akt signaling facilitates glycolysis and increases GDH activity and the over expression of Akt suppresses it (Wolf, et al). Therefore, future studies should investigate the impact of inhibiting specific enzymes in the metabolic network as a whole to include fatty acids, nucleotides, and amino acid metabolism by ALK/c-Met inhibitors. Results obtained from these studies will help us to understand the role of glucose metabolism, glutamine metabolism, and oncogenic signaling in glioblastoma cells. Exploiting compensatory pathways of glutamine metabolism can improve the efficacy of glioblastoma treatments that impair glucose utilization.

5. Conclusions

For too long, patients have endured the aggressively lethal tumors of glioblastoma with little to no hope of survival. Finding new therapies to block the mitotic, metastatic, and pro-survival pathways activated in glioblastoma is therefore vital. ALK and MET activated pathways, among other RTKs, present promising targets for such inhibition. By inactivating pro-survival and proliferative transcriptional and translational activities induced by ALK and MET activation, new therapies stand a stronger chance of blocking tumor growth and progression in glioblastoma patients. However, the three-part cascade of STAT3, AKT, and

Ras activation also presents numerous difficulties in modulating treatments. The interplay among cascade components both pre- and post-transcriptionally, as well as the intrinsically self-propagating nature of those components will require careful manipulation. Thus, multi-target therapies utilizing standard chemotherapeutics, like TMZ, and novel drugs must be combined with ALK/MET inhibitors in future studies. Future studies should investigate the impact of ALK/MET inhibitors on metabolic network as a whole including fatty acid, nucleotide and amino acid metabolism.

Finally, the varied presentation of tumors among patients also calls for further developments in targeted chemotherapy for glioblastoma tumors. Early efforts to target the most commonly altered genes (PDGFR, VEGF, EGFR, etc.) have met with frustratingly little success despite widespread presentation among patient tumors. While ALK/MET aberrations are far less common than EGFR mutations, it may be that these receptors play a larger role in mediating the effects of more commonly mutated RTK receptor activities in glioblastoma. RTKs in general remain promising therapeutic targets, but recent clinical trial setbacks and poorly understood RTK-RTK intracellular signaling dynamics will necessitate increased bench-side and bedside study.

Acknowledgments

Completion of this project was made possible by funding from the National Institutes of Health (NIH) and National Institute of Neurological Disorders and Stroke (NINDS): (NS31622, NS-38146, NS-57811, and NS-41088), the State of South Carolina Spinal Cord Injury Research Project (SCSCIRF), Pfizer, Inc., and the Jerry Zucker Fund for Brain Tumor Research of the MUSC Foundation.

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Highlights

- Glioblastoma is a devastating, deadly prognosis for thousands each year.
- ALK & MET pathways are integral to tumor survival, growth, and metastasis.
- STAT3, Ras, and AKT are 3 key downstream effectors of ALK & MET.
- New drugs targeting ALK & MET offer great promise for glioblastoma patients.

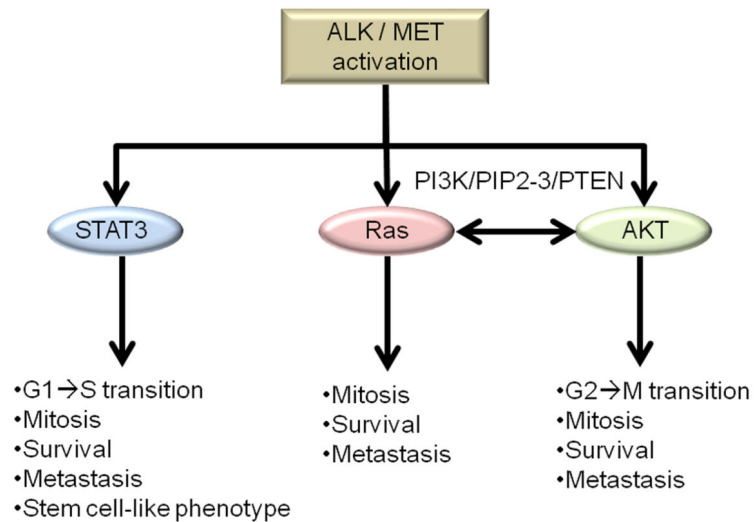


Figure 1. Downstream effectors: STAT3, RAS, AKT

ALK/MET mediated phosphorylating activation of STAT3, Ras, and AKT result in expression of numerous distinct, yet interconnected pro-survival, pro-mitotic, and pro-metastatic pathways. While STAT3 acts directly as a transcription factor, Ras and AKT cascades begin at the membrane and are connected by the PI3K/PIP_x/PTEN modality.

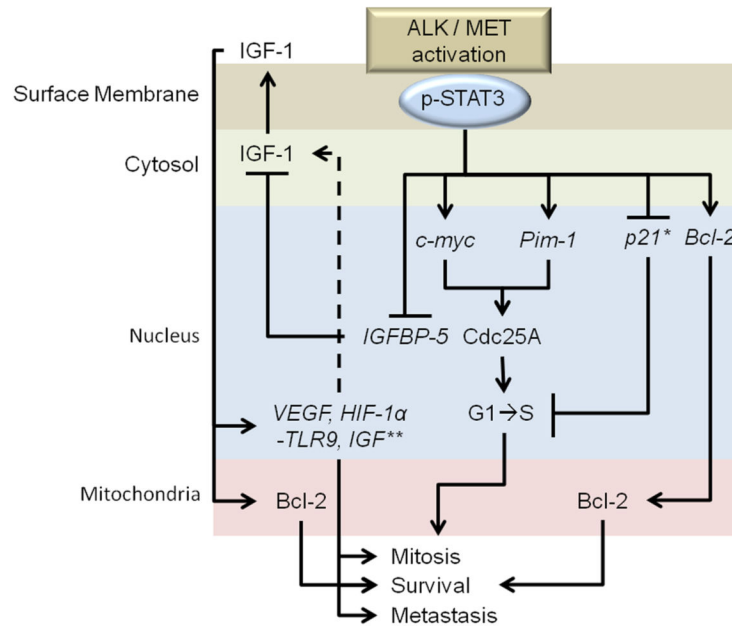


Figure 2. STAT3 transcriptional activation

Phospho-STAT3 dimerizes with other STAT proteins upon translocation to the nucleus where it potentiates transcription of: *c-myc*, *Pim-1*, *p21*, and *IGFBP-5*. Among other transcripts, production of *Cdc25A* by *c-myc* and *Pim-1* directly contributes to the G1 to S phase transition. p-STAT3 inhibits *IGFBP-5* to promote an IGF-1 autocrine loop. The IGF-1 autocrine loop thus contributes to production of cell growth signaling and contributes to increased: VEGF, HIF-1 α , TLR9, IGF. *p21 activities are numerous, but here it suppresses mitosis and survival protein production; **IGF initiates numerous other pro-mitotic/survival/metastatic cascades

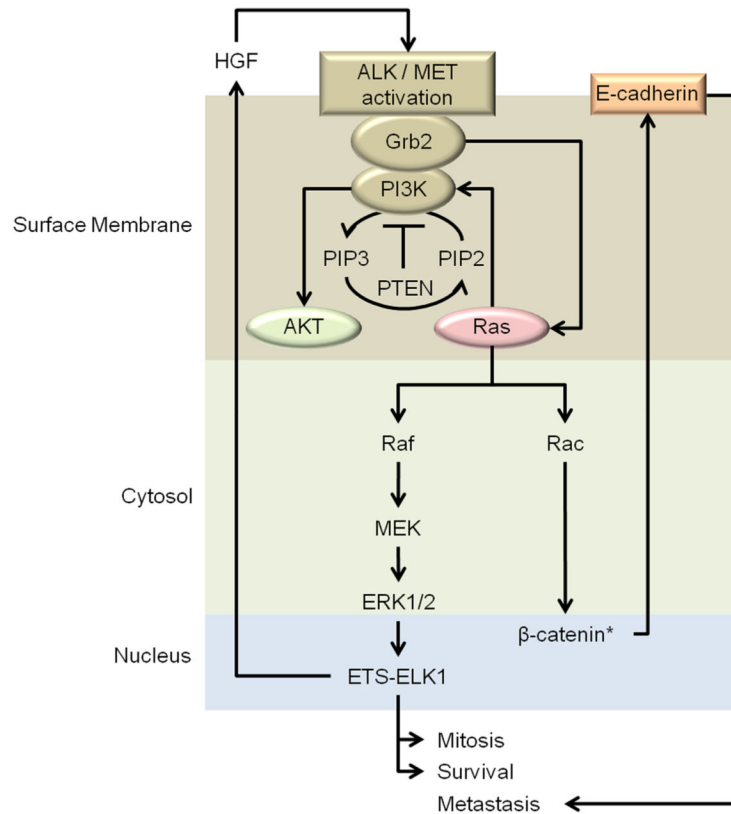


Figure 3. Ras: dual oncogenic pathways

Ras activation further potentiates AKT activation by stimulating PI3K mediated phosphorylation of PIP2 to PIP3; this process is inhibited in the presence of functional PTEN. Ras/Rac activities work with AKT to further stabilize β -catenin activity and thus promote the pro-metastatic adherin product, E-cadherin. Ras/Raf activity triggers activation of the Raf/MEK/ERK cascade which culminates in, among others, increased transcriptional activity of the ETS-ELK-1 complex to further activate pro-mitotic and pro-survival pathways. Ras mediated ELK-1 activation results in an autocrine HGF/MET feedback loop which may further stabilize cell survival and metastatic potential. *Represents another point of interaction with AKT

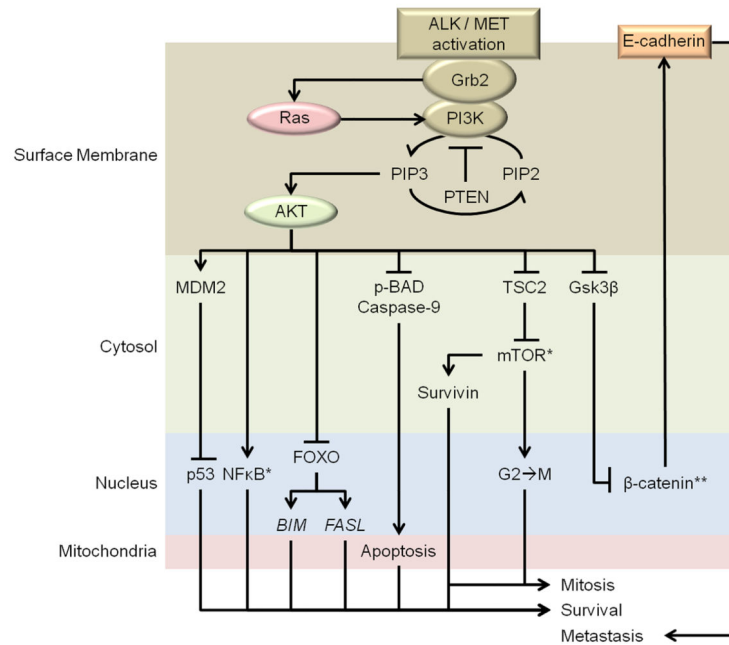


Figure 4. AKT in oncogenesis

ALK/MET activation results in activation of Ras and AKT. AKT activation inhibits various tonic inhibitors of mitotic and survival cascades including: p53 (downstream of AKT-MDM2 activation), FOXO (promotes transcription of pro-apoptotic genes like *BIM* and *FASL*), Caspase-9, Bad, TSC2 (inactivates pro-translational properties of mTOR), and Gsk3 β (an integral component of the complex responsible for β -catenin degradation). AKT also activates pro-survival gene transcription through NF κ B and, via mTOR disinhibition, potentiates expression of pro-mitotic factors like Survivin, which is highly expressed in glioblastoma. *NF κ B & mTOR direct numerous other pro-survival activities not shown here; **Represents another point of interaction with Ras.