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Will Kinase Inhibitors Make it as Glioblastoma Drugs?

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Abstract

Kinase inhibitors have emerged as effective cancer therapeutics in a variety of human cancers. Glioblastoma (GBM), the most common malignant brain tumor in adults, represents a compelling disease for kinase inhibitor therapy because the majority of these tumors harbor genetic alterations that result in aberrant activation of growth factor signaling pathways. Attempts to target the Ras—Phosphatidylinositol 3-kinase (PI3K)—mammalian Target of Rapamycin (mTOR) axis in GBM with first generation receptor tyrosine kinase (RTK) inhibitors and rapalogs have been disappointing. However, there is reason for renewed optimism given the now very detailed knowledge of the cancer genome in GBM and a wealth of novel compounds entering the clinic, including next generation RTK inhibitors, class I PI3K inhibitors, mTOR kinase inhibitors (TORKinibs), and dual PI3(K)/mTOR inhibitors. This chapter reviews common genetic alterations in growth factor signaling pathways in GBM, their validation as therapeutic targets in this disease, and strategies for future clinical development of kinase inhibitors for high grade glioma.

1 Introduction

Gliomas represent a spectrum of primary brain tumors which are classified by the World Health Organization (WHO) into low grade and high grade tumors based on the degree of tumor cell proliferation, cellular atypia, and microvascular proliferation (Louis et al. 2007). The median survival for patients with GBM has remained below 2 years despite multimodality therapy, including surgery, radiation, chemotherapy (Stupp et al. 2005), and most recently the anti-VEGF antibody bevacizumab (Friedman et al. 2009; Kreisl et al. 2009a). The term “low-grade” glioma (WHO grade II) refers to a group of tumors with histopathologically less aggressive features. However, many patients with these tumors also succumb to their disease within 3–10 years due to tumor “transformation” to an anaplastic glioma (WHO grade III) or GBM (WHO grade IV). GBMs that have evolved from a clinically overt, low-grade precursor lesion are referred to as “secondary” GBMs in contrast to de novo or “primary” GBMs. Primary and secondary GBMs differ substantially in their molecular pathogenesis (Lai et al. 2011; Ohgaki and Kleihues 2007).

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The histopathological appearance of GBM is particularly diverse and has earned it the moniker “multi-forme” (multiformis [Latin]: many shapes) (Louis et al. 2007). This morphological heterogeneity of GBM is often viewed as a reflection of the exceptional genetic heterogeneity of this cancer. Recent genomic studies provide a perhaps more encouraging view of GBM with a finite number of highly recurrent gene copy number alterations (Beroukhim et al. 2009) and missense mutations (TCGA 2005; Parsons et al. 2008). Genome wide RNA expression profiling identifies distinct disease subgroups (Phillips et al. 2006) each of which is enriched for particular mutations (Verhaak et al. 2010).

One key result of the extensive profiling of human glioma samples (Beroukhim et al. 2007; Kotliarov et al. 2006; McLendon et al. 2008; Misra et al. 2005; Parsons et al. 2008) is the *re*-appreciation that nearly all human GBMs harbor genetic alterations in three “core” pathways, namely the RTK/RAS/PI3K signaling axis, the p53-ARF-MDM2/MDM4 pathway, and the RB-CDK4-INK4A pathway. Many of the genetic lesions most consistently found in human tumors have been shown to cooperate in glioma formation in mice (Chow et al. 2011; Reilly et al. 2000; Zheng et al. 2008; Zhu et al. 2005a) and represent the currently most “actionable” drug targets (Fig. 1). This chapter highlights genetic alterations in growth factor signaling pathways in GBM and discusses new directions to develop kinase inhibitors as glioma therapeutics. Our comments largely focus on the adult patient population as genetic alterations (Bax et al. 2010; Paugh et al. 2010) and considerations regarding clinical drug development differ considerably for pediatric glioma patients.

2 Mutations in Growth Factor Receptors

Receptor tyrosine kinases (RTKs) are proteins which transmit signals from the cell surface to the nucleus and participate in most fundamental aspects of cell growth, survival, differentiation, and metabolism. Signaling through RTKs is initiated by ligand binding and terminated by receptor internalization from the cell surface, dissociation of the receptor-ligand complex, receptor dephosphorylation, and degradation of the receptor protein (Lemmon and Schlessinger 2010). The RTK family of proteins includes the epidermal growth factor receptor family (EGFR, HER2, ERBB3, and ERBB4), the platelet-derived growth factor receptor family (PDGFR-α and PDGFR-β), the MET receptor tyrosine kinase, the Vascular-Endothelial Growth Factor Receptor family (VEGFR1/FLT1, VEGFR2/KDR/FLK1, and VEGFR3/FLT4), and others. Many human cancers harbor mutations in RTKs which relieve auto-inhibitory constraints on the kinase activity or impair the downregulation of the ligand-activated receptor protein (Blume-Jensen and Hunter 2001). Within the RTK family, mutations in the genes encoding EGFR, PDGFR-α, and MET are the most common in high grade gliomas (Table 1 and Fig. 2).

2.1 Epidermal Growth Factor Receptor (EGFR)

Genetic alterations that result in uncontrolled EGFR kinase activity were amongst the first to be associated with human cancer (Gschwind et al. 2004). A number of alterations involving the *EGFR* gene have been described in GBM. These include: (a) *EGFR* gene amplification in ~40% of primary GBMs (Libermann et al. 1985; Wong et al. 1987); extra gene copies reside on double-minutes and are easily detected by fluorescence-in situ hybridization (FISH) (Jansen et al. 2010); (b) In-frame deletions affecting the 5′ end of the *EGFR* gene (Malden et al. 1988; Yamazaki et al. 1988); these are found mostly, but not exclusively, in tumors with *EGFR* gene amplification. The most common EGFR variant IIII (or EGFRvIII) is a deletion of exons 2–7, resulting in an 801 amino acid in-frame deletion within the EGFR extracellular domain (Sugawa et al. 1990). The EGFRvIII mutant does not bind the ligands EGF or TGF-α, but is constitutively active (Ekstrand et al. 1994); (c) truncations affecting the C-terminus of the EGFR protein. These alterations are seen in 15–25% of high grade

gliomas with *EGFR* gene amplification (Ekstrand et al. 1992; Eley et al. 1998; Frederick et al. 2000). The EGFR C-terminus encodes receptor portions that are required for ligand-induced receptor internalization (Chen et al. 1989; Decker et al. 1992) and (d) missense mutations in the *EGFR* extracellular domain in about 10% of primary GBMs (Lee et al. 2006b).

Most EGFR alterations found in human GBM have been shown to represent gain-of-function events. Expression of a truncated EGF receptor lacking the extracellular ligand binding domain induces transformation of immortalized rodent fibroblasts (Haley et al. 1989). Expression of the EGFRvIII mutant enhances the tumorigenicity of GBM cells (Nishikawa et al. 1994) and is able to transform mouse NIH-3T3 fibroblasts in the absence of ligand (Batra et al. 1995). In mice, low grade oligodendrogliomas develop when the retroviral oncogene *v-erb-B*, encoding a truncated version of EGFR (Schatzman et al. 1986), is expressed under the control of the S100β promoter (Weiss et al. 2003). Expression of the EGFRvIII mutant is not sufficient to induce glioma formation in mice but cooperates with mutant *H-RAS* or inactivation of *CDKN2A* in glioma formation (Bachoo et al. 2002; Ding et al. 2003; Holland et al. 1998; Zhu et al. 2009). A C-terminal EGFR truncation mutant has been shown to confer anchorage-independent growth and tumorigenicity to NIH-3T3 cells (Wells et al. 1990; Masui et al. 1991). Several of the extracellular *EGFR* missense mutations (R108K, T263P, A289V, G598V) have been shown to transform NIH3T3 cells and confer tumorigenicity (Lee et al. 2006b). In contrast to the *EGFR* mutants described above, overexpression of wild-type EGFR does not transform mouse NIH3T3 in the absence of exogenous EGF (Di Fiore et al. 1987). In mice, overexpression of wild-type EGFR induces glioma formation only in the presence of *CDKN2A* deletion and, even then, with very low efficiency (Zhu et al. 2009).

The role of mutant EGFR for tumor maintenance needs to be defined more extensively, but current data suggests that at least a subset of *EGFR* mutant gliomas require EGFR signals for maintenance of the malignant phenotype (Eller et al. 2002; Martens et al. 2008; Sarkaria et al. 2007).

2.2 Platelet-Derived Growth Factor Receptor (PDGFR)

Platelet-derived growth factor (PDGF) is a potent mitogen for glia-derived cells (Richardson et al. 1988) and consists of five dimeric isoforms. These include homodimers of A-, B-, C-, and D-polypeptide chains (i.e., PDGF-AA, -BB, -CC, and –DD) and a PDGF-AB heterodimer. PDGF dimers bind to the RTKs PDGFR-α and PDGFR-β and activate the receptors by inducing receptor dimerization. Different types of receptor dimers are induced by different ligands: A-, B-, and C-chains of PDGF bind to PDGFR-α, whereas B-and D-chains bind to PDGFR-β. PDGF ligands and receptors are frequently co-expressed in human gliomas, perhaps reflecting the presence of an autocrine signaling loop (Hermanson et al. 1992; Lokker et al. 2002).

Mutations in genes encoding PDGF ligands or receptors have been found in a variety of human cancers, including Dermatofibrosarcoma Protuberans (*PDGFB*), Chronic Myelomonocytic Leukemia (*PDGFR-*β), and Gastrointestinal Stromal Tumors (*PDGFR-*α). Many of these cancers respond to PDGFR kinase inhibitors such as imatinib (Ostman and Heldin 2007). Kumabe et al. first reported amplification of the *PDGFR-*α gene locus in 1/9 (11%) human gliomas. Interestingly, the one case with *PDGFR-*α gene amplification also harbored an in-frame deletion of exons 8 and 9 of *PDGFR-*α (Kumabe et al. 1992). Subsequent studies reported *PDGFR-*α gene amplification in 8–11% of primary human GBMs (Beroukhim et al. 2009; Fleming et al. 1992). Functional characterization of the *PDGFR-*α-Δ8,9 mutant by Peter Dirk's laboratory showed that the 243 base pairs deletion results in a constitutively active receptor with greater transforming and tumorigenic ability

than wild-type PDGFR-α (Clarke and Dirks 2003). Other *PDGFR-α* mutations reported in GBM include a two basepair deletion in exon 23, resulting in a truncation of the C-terminus of the receptor (Rand et al. 2005) and an oncogenic gene fusion between the 5′ end of the *kinase insert domain receptor* (*KDR*) and the kinase domain and 3′ portion of *PDGFR-α* (Ozawa et al. 2010) (Table 1).

The contribution of PDGF signaling toward glioma formation in mice has been well documented. Injection of a PDGF-B chain-encoding retrovirus into the brain of newborn C57B16 mice induced brain tumor formation in 40% of animals (Uhrbom et al. 1998). This result has been confirmed by somatic cell type-specific gene transfer experiments in which targeted expression of PDGF-B in nestin-expressing neural progenitors or GFAP-expressing astrocytes induced gliomas in 60 and 40% of mice, respectively (Dai et al. 2001). Retroviral infection of adult white matter progenitor cells similarly resulted in glioma formation (Assanah et al. 2006) and expression of doxycycline-regulated PDGF-B in the spinal cord produced mixed oligoastrocytomas (Hitoshi et al. 2008).

2.3 MET Tyrosine Kinase Receptor

The MET tyrosine kinase is the cell surface receptor for hepatocyte growth factor (HGF). Aberrant activation of MET in human cancers results from amplification of the *MET* gene (e.g., gastric/esophageal carcinoma, medulloblastoma), missense mutations in the *MET* gene (e.g., papillary renal cancer), and transcriptional upregulation of MET and its ligand HGF (Comoglio et al. 2008; Koochekpour et al. 1997). While missense mutations in *MET* are rare in human GBM, focal amplification of the *MET* gene occurs in about 5% of primary human GBMs (Beroukhim et al. 2009). Amplification of MET has been linked with increased sensitivity to MET kinase inhibition in a panel of human cancer cell lines (McDermott et al. 2007) and radiographic regression of a *MET*-amplified GBM was recently reported in a patient treated with the MET/ALK kinase inhibitor crizotinib (PF-02341066) (Chi et al. 2011).

3 Mutations in the Ras-Raf Axis

The family of RAS GTPases (HRAS, NRAS, and KRAS) are proteins which cycle between a GTP-bound active form and a GDP-bound inactive form. Guanine nucleotide exchange factors (GEFs) promote formation of GTP-bound RAS, whereas GTPase-activating proteins (GAPs) stimulate the hydrolysis of GTP on RAS. The first critical effector of Ras to be identified in mammalian cells was the RAF-MEK-ERK pathway. Serine/threonine kinases of the RAF family (C-Raf or Raf-1, A-Raf, and B-Raf) bind to RAS-GTP and then relocalize to the plasma membrane where they are phosphorylated. Once activated, they phosphorylate and activate mitogen-activated protein kinase kinase (MAPKK or MEK) that, in turn, phosphorylates and activates extracellular signal-regulated kinases 1 and 2 (ERK1/2) (Castellano and Downward 2011; Shaw and Cantley 2006). The RAS-RAF axis is activated in glioma through several mechanisms, including mutations in *NRAS* and *KRAS*, inactivating mutations in the neurofibromatosis gene (*NF1*), and mutations involving *BRAF* and *CRAF* (Table 1 and Fig. 2).

3.1 Mutations in KRAS, NRAS, HRAS

Mutations in *KRAS*, and *NRAS* are rare in human gliomas and particularly rare in WHO grade III and IV gliomas in adult patients. Sequencing of 94 high grade gliomas for mutations in *NRAS* (entire coding region), *KRAS* (entire coding region) and the first exon of *HRAS* identified 2 cases (2/94 = 2%) with *G12D-NRAS* mutation (Knobbe et al. 2004). Another study examined 93 gliomas for “hotspot” mutations in *HRAS* (exon 2/3), *KRAS* (exon 2/3), and *NRAS* (exon 2/3) and reported one *NRAS* mutation (G10E-*NRAS*) (Jeuken

et al. 2007). Mutations in *KRAS* have been reported in 5–10% of pediatric gliomas (Cin et al. 2011; Forshew et al. 2009; Janzarik et al. 2007; Maltzman et al. 1997; Schiffman et al. 2010; Sharma et al. 2005). Based on the effects of mutant *RAS* on glioma formation and maintenance in genetically engineered mouse models (de Vries et al. 2010; Ding et al. 2001; Holland et al. 2000; Holmen and Williams 2005; Marumoto et al. 2009; Uhrbom et al. 2002), Ras mutations are likely relevant for the biology and drug response of the (rare) gliomas in which they are found.

3.2 Mutations in NF1

Neurofibromin, the protein product of the neurofibromatosis gene (*NF1*) gene, is a $p21^{ras}$-GTPase activating protein (RasGAP) which critically regulates Ras signal output (Buday and Downward 2008; Shaw and Cantley 2006). Somatic missense mutations in the NF1 gene were first reported in astrocytoma many years ago (Li et al. 1992; Thiel et al. 1995). More complete sequencing of the *NF1* coding sequence in a larger number of tumors has uncovered missense mutations in *NF1* in ~15% of GBM patients (McLendon et al. 2008; Parsons et al. 2008). Other mechanisms of *NF1* silencing in GBM include heterozygous or homozygous *NF1* copy loss and posttranslational modifications that result in destabilization of the NF1 protein (McGillicuddy et al. 2009). There is strong evidence for a role of NF1 in gliomagenesis. Patients with neurofibromatosis type I, a genetic disorder caused by germline mutations in *NF1*, are at increased risk to develop high grade gliomas (Listernick et al. 1999; Rodriguez et al. 2008) and these gliomas often show inactivation of the second NF1 allele (Gutmann et al. 2003). Mice with targeted disruption of the *NF1* locus develop astrocytosis and *NF1* inactivation cooperates with *TP53* inactivation and *PTEN* inactivation to produce high grade gliomas with rapid onset and high penetrance (Alcantara Llaguno et al. 2009; Bajenaru et al. 2003; Reilly et al. 2000; Zhu et al. 2005a, b).

3.3 Mutations in BRAF

The frequency and type of *BRAF* alterations in glioma varies substantially between adults and children and between distinct glioma subtypes. *V600E-BRAF* or *V600M-BRAF* mutations are rare in WHO grade III/IV gliomas in adults, ranging from 0 to 3% in most studies (Basto et al. 2005; El-Habr et al. 2010; Hagemann et al. 2009; Jeuken et al. 2007; Knobbe et al. 2004; Schindler et al. 2011). The frequency of *V600E-BRAF* mutations is substantially higher in pediatric gliomas. One series reported *V600E-BRAF* in 23% (7/31) of WHO grade II–IV pediatric astrocytomas (Schiffman et al. 2010); another series observed *V600E-BRAF* mutations in 9% (4/42) of WHO grade III/IV pediatric gliomas (Schindler et al. 2011). *V600E BRAF* mutations are particularly common in the two glioma subtypes *pleomorphic xanthoastrocytoma* (60–70%) and *gangliogliomas* (20–60%) (Dougherty et al. 2010; MacConaill et al. 2009; Schindler et al. 2011).

The majority (50–70%) of *pilocytic astrocytomas (PAs)*, the most common central nervous system tumor in children, show low-level copy gain at the *BRAF* gene locus at 7q34 (Bar et al. 2008; Deshmukh et al. 2008; Pfister et al. 2008). Detailed characterization of this genomic alteration in 44 human PAs by Peter Collins' group uncovered a tandem duplication that produces fusion proteins between *KIAA1549* and *BRAF* in 66% (29/44) of pilocytic astrocytomas. The most common event fuses *KIAA1549* exon 16 and *BRAF* exon 9. KIAA1549-BRAF fusions generate proteins that lack the BRAF autoregulatory domain and exhibit enhanced BRAF kinase activity (Jones et al. 2008). Further examination of PAs without 7q34 duplication identified alternative mechanisms for activation of the RAF-MEK axis. These included *NF1* inactivation (family history of neurofibromatosis type I) (3/44 cases), *V600E BRAF* mutation (2/44 cases), a tandem duplication at 3p25 fusing *SLIT-ROBO Rho GTPase Activating Protein 3* (*SRGAP3*) and *CRAF*/*RAF1* (1/44 cases), and a 3 bp insertion at codon 598 in *BRAF* (1/44 cases). The *SRGAP3-RAF1* fusion protein and the

p.A598_T599insT BRAF mutant both increased the kinase activity of RAF1 and BRAF, respectively. Altogether, 82% (36/44) of pilocytic astrocytomas in this series showed mutational activation of the RAS/RAF axis (Jones et al. 2008, 2009).

Profiling of human pilocytic astrocytomas by other groups confirmed near-universal activation of the RAS/RAF/MEK signaling axis in this disease through *KIAA1549-BRAF* fusions (~70%), *V600E BRAF* mutations (5–10%), *BRAF* codon 598 insertions (1–3%), *NF1* inactivation (1–3%), *KRAS* mutations (1–3%), the *SRGAP3-RAF1* fusion (1–3%), and a recently described fusion involving *BRAF* and *Family with sequence similarity 131, member B* (*FAM131B)* (2%) (Cin et al. 2011; Eisenhardt et al. 2010; Forshew et al. 2009; Jacob et al. 2009; Pfister et al. 2008; Schindler et al. 2011; Sievert et al. 2009; Yu et al. 2009). Functional studies support a role of glioma-related *BRAF* mutants and activated Raf-1 in transformation and gliomagenesis, in particular in combination with *CDKN2A* inactivation (Gronych et al. 2011; Jones et al. 2008, 2009; Lyustikman et al. 2008; Robinson et al. 2010, 2011).

4 Mutations in PI3K and PTEN

Phosphatidyloinositide 3-kinases (PI3K) belong to a family of lipid kinases that phosphorylate the 3-hydroxyl group of the inositol ring of phosphatidylinositol (PtdIns), PtdIns4P, and PtdIns (4, 5)P_2. PI3Ks have been assigned to different classes based on substrate preference and structural features. Class I PI(3)Ks are activated by receptor tyrosine kinases and G-protein coupled receptors and use PtdIns(4, 5)P_2 as substrate to generate phosphoinositide 3,4,5 trisphosphate (PIP_3). Other classes are class II and class II PI3Ks (Fig. 3a) and the family of PI3K-related protein kinases (PIKKs) which include mammalian Target of Rapamycin (mTOR), ATM, ATR, DNA-PK, and hSMG1 (Vanhaesebroeck et al. 2010).

PI(3)Ks are composed of a catalytic subunit and a regulatory subunit (Fig. 3b). Catalytic subunits include p110α (encoded by *PIK3CA*), p110β (encoded by *PIK3CB*), p110γ (encoded by *PIK3CG*), and p110δ (encoded by *PIK3CD*). Regulatory subunits include p85α, p55α, and p50α (all encoded by *PIK3R1*), p85β (encoded by *PIK3R2*), p55γ (encoded by *PIK3R3*), p101 and p87. p110 subunits share a five-domain structure, which includes an *N*-terminal adaptor binding domain (ABD domain), a Ras binding domain (RBD domain), a C2 (Protein kinase C homology-2) domain, a helical domain, and a catalytic domain. All p85 isoforms (p85α, p55α, p50α, p85β, p55γ) have two Src homology domains 2 (SH2) domains and an intervening domain (iSH2) that binds to the adapter binding domain in p110 (Vanhaesebroeck et al. 2010). P85 isoforms provide at least three functions to p110 proteins: (i) they stabilize the intrinsically unstable p110 protein, (ii) they recruit p110 proteins to pTyr residues in receptor and adaptor molecules (through the SH2 domains of p85 isoforms) upon activation, and (iii) they restrain the kinase activity of p110 proteins in their un-activated state.

PI3K were first linked to cancer through the study of oncogenic viruses. Their critical role in the pathogenesis of human cancer was fully recognized following the discovery that many human tumors harbor mutations in genes whose gene products regulate levels of the lipid molecule PIP_3 (Shaw and Cantley 2006; Vogt et al. 2009). These include the phosphatase and tensin homolog deleted on chromosome 10 (*PTEN*) (Li et al. 1997; Steck et al. 1997), the regulatory subunit p85 (*PIK3R1*) (Philp et al. 2001), and the catalytic subunit p110α (*PIK3CA*) (Samuels et al. 2004), and *PIK3R2* (Cheung et al. 2011). Almost 100 mutations have been found throughout the *PIK3CA* coding region. The Ras binding domain (RBD) appears to be spared from mutations and mutation “hot-spots” include the p85-binding domain, the helical domain, and the kinase domain (Vanhaesebroeck et al. 2010; Vogt et al.

2009). Many studies have documented the biochemical effects and transforming ability of *PTEN*-inactivation (Salmena et al. 2008) and various *PIK3CA* (Vogt et al. 2009) and *PIK3R1* mutations (Huang et al. 2007; Jaiswal et al. 2009; Philp et al. 2001; Sun et al. 2010; Wu et al. 2009). Mutations have not been found in genes encoding other PI3K catalytic subunits (*PIK3CB*, *PIK3CG*, *PIK3CD*). In contrast to p110α, however, which only shows transforming ability it its mutant form, overexpression of wild-type p110β, p110γ, and p110δ can transform chicken fibroblasts (Kang et al. 2006) and these PI3K family members may represent critical oncogenic units in certain genetic contexts (Berenjeno and Vanhaesebroeck 2009). In GBM, mutations in the PI(3)K-mTOR axis are most frequently found in the genes encoding the catalytic and regulatory subunit of PI(3)K (*PIK3CA* and *PIK3R1*, respectively) and in *PTEN* (Table 1 and Fig. 2).

4.1 Mutations in PIK3CA

The initial discovery of *PIK3CA* mutations in cancer reported a mutation prevalence of 26.7% (4/15) for GBM (Samuels et al. 2004). Subsequent studies in larger numbers of tumors suggest that these mutations occur less frequently in primary GBMs. Sequening of exons 9 and 20 of *PIK3CA* in a diverse panel of primary brain tumors found mutations in 5/105 (5%) GBMs, 3/21 (14%) anaplastic oligodendrogliomas (WHO grade III), 1/31 (3%) anaplastic astrocytomas (WHO grade III), 0/24 (0%) WHO grade II astrocytomas, 4/78 (5%) medulloblastomas, and 0/26 (0%) ependymomas (Broderick et al. 2004). Other studies screened the entire *PIK3CA* coding region and reported mutations in 0/30 (0%) (Mueller et al. 2005) and 5/70 (7%) GBMs (Hartmann et al. 2005). Direct sequencing of a large part of the *PIK3CA* coding sequence (exons 1, 2, 4, 5, 7, 9, 12, 13, 18, 20) in 38 primary human GBMs identified mutations in 3/14 (21%) pediatric and 4/24 (17%) adult GBMs (Gallia et al. 2006). Another study focused on exons 9 and 20 of *PIK3CA* and reported mutations in 5/107 (5%) de novo GBMs and in 1/32 (3%) secondary GBMs (Kita et al. 2007). Differences between studies in the reported frequency of *PIK3CA* mutations are likely due to a combination of factors, including the sensitivity of the mutation detection assay (single strand conformational polymorphism versus direct bidirectional sequencing), gene coverage (mutation hot-spots versus entire coding sequence), glioma subtype, sample size, and inclusion of cell lines.

Amplification of the *PIK3CA* gene locus was first reported as oncogenic event in ovarian cancer (Shayesteh et al. 1999) and later in other cancers. No evidence for PIK3CA gene copy gain (>5-fold) or RNA overexpression was found in an analysis of 145 primary brain tumors (including 50 GBMs and 35 WHO grade III gliomas) (Broderick et al. 2004). Using a slighty less stringent cutoff for *PIK3CA* gene copy gain (>3-fold), Kita et al. reported *PIK3CA* amplifications in 14/107 (13%) de novo and 3/32 (9%) secondary GBMs (Kita et al. 2007).

4.2 Mutations in PIK3R1

A truncating mutation in *PIK3R1* was first reported in a GBM in 2004 (Mizoguchi et al. 2004). More recent studies reported mutations in *PIK3R1* in~10% of GBMs (McLendon et al. 2008; Parsons et al. 2008). These mutations clustered in the N-terminal SH2-domain and the inter-SH2 domain of the PIK3R1 gene, regions that interact with the C2 and helical domains of p110α. Detailed characterization of selected p85 mutants showed that they were unable to negatively regulate p110α, p110β, and p110δ activity, despite retaining their ability to bind and stabilize them (Huang et al. 2007; Jaiswal et al. 2009; Wu et al. 2009). Peter Vogt's group expressed the full panel of GBM p85 mutants (G376R, E439del, KS459delN, D560Y, DKRMNS560del, N564K, R574fs, T576del, W583del) in chicken fibroblasts. All mutants induced a transformed phenotype, retained the ability to interact with p110α, stabilized the endogenous p110α protein, and stimulated phosphorylation of

Akt and 4EBP-1. Efficiencies of transformation varied between mutants and did not clearly correlate with their biochemical effects on Akt and 4EBP1 (Sun et al. 2010).

4.3 Mutations in PTEN

Allelic loss of chromosome 10 has long been known to be common in high grade human glioma (James et al. 1988). After the identification of PTEN as tumor suppressor on chromosome 10 (Li et al. 1997; Steck et al. 1997), missense mutations in *PTEN* were reported in 17/63 (27%) of GBMs (Liu et al. 1997). Examination of the entire *PTEN* coding sequence in 331 gliomas reported *PTEN* missense mutations in 20/142 (14%) GBMs; homozygous *PTEN* deletions were observed in 7/142 (5%) GBMs (Duerr et al. 1998). Another study reported *PTEN* point mutations and homozygous deletions in 32/110 (29%) and 11/110 (10%) of GBMs (Smith et al. 2001). Later studies reported *PTEN* mutations in 14 (Hartmann et al. 2005) to 24% (Ohgaki et al. 2004). PTEN expression is silenced in additional GBMs through promoter methylation, micro-RNAs, and posttranslational modifications.

Genetically engineered mouse models (GEMMs) support a prominent tumor suppressor function of PTEN during glioma progression. Loss of PTEN in various cell types within the brain does not result in tumorigenesis (Fraser et al. 2004; Groszer et al. 2001; Kwon et al. 2001). Inactivation of *PTEN* in glioma-prone mice, in which oncogenic ^{V12}*H-Ras* is expressed from the GFAP promoter, greatly accelerated malignant glioma progression (Wei et al. 2006). In the *NF1/p*53 astrocytoma model, haploinsufficiency of *PTEN* accelerated formation of WHO grade III astrocytomas, whereas loss of *PTEN* heterozygosity coincided with progression into WHO grade IV tumors (Kwon et al. 2008). Combined deletion of *PTEN* and *TP53* in neural stem cells of the subventricular zone is sufficient to induce glioma formation (Jacques et al. 2010; Zheng et al. 2008). Combined inactivation of PTEN and TP53 in mature astrocytes in the adult brain induced high grade gliomas that showed striking similarity to human GBMs in terms of secondary gene copy number alterations and genome wide RNA expression changes (Chow et al. 2011).

5 Experience with First-Generation RTK Inhibitors

The success with first-generation EGFR kinase inhibitors (gefitinib, erlotinib) in lung cancer and the high frequency of oncogenic *EGFR* alterations in GBM (~40%) raised expectations that these agents will show activity against GBM. This expectation has largely not been fulfilled. While most studies reported individual patients with tumor regressions in response to erlotinib or gefitinib, the frequency of these response is substantially lower (<5%) than the frequency of oncogenic EGFR alterations in GBM (Mellinghoff et al. 2011). The experience with imatinib, an ATP-site competitive inhibitor of the PDGFR, KIT and ABL-kinases has been similarly disappointing. A Phase II study of imatinib in 112 patients with recurrent gliomas reported a partial response for only 5 patients (Raymond et al. 2008). The radiographic response rates for the subgroup of GBM patients was 3/51 (6%) in this study. In a Phase I/II study of the North American Brain Tumor Consortium 3/57 (5%) GBM patients showed partial radiographic responses and no responses were observed for other histologic subgroups (Wen et al. 2006).

In contrast to the landmark clinical trials with imatinib in chronic myeloid leukemia (Druker et al. 2001) and with the HER2 antibody trastuzumab in breast cancer (Slamon et al. 2001), clinical trials with first-generation EGFR and PDGFR kinase inhibitors in GBM were not enriched for patients whose tumors harbored mutations in *EGFR* and *PDGFR-α*, respectively. This lack of molecular preselection likely contributed to the disappointing results in the clinic. Studies to identify determinants of EGFR kinase inhibitor response in GBM have associated tumor regressions with the presence of oncogenic EGFR alterations

(*EGFR* gene amplification or EGFRvIII) and expression of the PTEN tumor suppressor protein or low p-AKT levels (Haas-Kogan et al. 2005; Mellinghoff et al. 2005). Other studies have questioned the relationship between oncogenic EGFR and EGFR kinase inhibitor response in GBM (van den Bent et al. 2009). This discrepancy may, at least in part, be attributable to differences in the determination of EGFRvIII status in tumor tissue.

Several lines of evidence support that PTEN inactivation mediates EGFR kinase inhibitor resistance: (1) PTEN restoration sensitizes *EGFR* amplified/*PTEN* deleted cancer cells to cell death induction by EGFR kinase inhibitors (Bianco et al. 2003); (2) PTEN knockdown is sufficient to render *EGFR* mutant cancer cells resistant to cell death induction by EGFR kinase inhibitors (Vivanco et al. 2010); and (3) PTEN emerged as most consistent resistance factor from a shRNA library screen performed in breast cancer cells to identify mechanisms of resistance to trastuzumab (which targets the EGFR co-receptor HER2) (Berns et al. 2007), and (4) GBMs with intact PTEN expression showed enhanced responsiveness to combination therapy of erlotinib plus temozolomide (Prados et al. 2009).

How does PTEN status influence EGFR kinase inhibitor response? By dephosphorylation the second messenger PIP3, PTEN plays an important role in signal termination downstream of many RTKs. Even if one RTK is effectively inhibited, PTEN loss may allow the accumulation of sufficient PIP3 to activate PIP3 effector molecules, such as the serine-threonine kinase Akt. In other words, PTEN deficient cells may be able to better compensate for single RTK inhibition than cells with intact PTEN. This concept of redundant RTK activation is supported by the biochemical evidence for RTK coactivation in primary GBM tumor samples and the experimental observation that inhibition of multiple RTK, but not a single RTK, induces cell death in certain GBM lines (Stommel et al. 2007). We recently made the surprising discovery that PTEN inactivation raises EGFR protein levels and EGFR kinase activity by interfering with the CBL-mediated downregulation of the activated EGF receptor. Of note, PTEN knockdown did not confer “absolute” EGFR kinase inhibitor resistance but instead right-shifted the cell-death response to EGFR kinase inhibition toward drug concentrations which are difficult to achieve in the central nervous system with currently available EGFR kinase inhibitors (Vivanco et al. 2010). Further studies are needed to determine whether more complete EGFR kinase inhibition, simultaneous inhibition of multiple RTKs, or both are required to overcome EGFR kinase inhibitor resistance in GBM.

In many ways, the current experience with EGFR and PDGFR inhibitors is reminiscent of the experience in melanoma where the disappointing clinical activity of RAF and MEK inhibitors questioned the role of mutant *BRAF* for the maintenance of these tumors (Smalley and Sondak 2010). The vast majority of clinical trials with RTK inhibitors in neurooncology did not include tumor biopsies during treatment and it is hence unknown to what extent a negative clinical trial result might be attributable to poor drug penetration into the brain tumor. The available data indeed suggests that first-generation RTK inhibitors, given at standard daily doses, result in only weak (if any) pathway inhibition in GBM. Fresh operative samples from three GBM patients who received erlotinib or gefitinib prior to surgery and for whom a frozen pretreatment sample was available showed inconsistent drug effects on phosphorylation of EGFR, Erk, and Akt (Lassman et al. 2005). A more extensive analysis of multiple candidate EGFR effector molecules (p-AKT, p-GSK-3α/β, p-NFκB p65, p-STAT3, p-ERK1/2, p-MEK1, p-p38MAPK, p-p90RSK, p-p70S6 Kinase, p-S6 ribosomal Protein, p-PDGFR-B, and p-SRC) in GBM patients treated with gefitinib similarly showed poor EGFR pathway inhibition (Hegi et al. 2011).

Conclusions regarding target inhibition in GBM have to be viewed as preliminary because tumors with the most informative genotype(s) and strong basal pathway activation were generally underpresented in these studies and because of difficulties to assemble a

sufficiently large number of drug-naive “control” tumor samples. Genotype-enriched clinical trials with a surgical arm cohort (Cloughesy et al. 2008) could address this important issue and may be instrumental to “weed out” compounds that fail to achieve sufficient kinase inhibition in GBM tumor tissue and therefore do not warrant further clinical testing in GBM.

6 Clinical Experience with Rapalogs

The PI(3)K pathway represents a very active area of drug development in cancer. Strategies to target members of this pathway are of particular interest in GBM because the majority of these tumors harbor mutations in one or more genes that regulate this pathway (McLendon et al. 2008), including *EGFR*, *PDGFRA*, *MET*, *PTEN*, *NF1*, *PIK3CA* and *PIK3R1* (Fig. 1). Biochemical evidence for frequent PI(3)K pathway activation in GBM is provided by immunohistochemical studies which showed phosphorylation of the downstream pathway member S6 ribosomal protein in the majority of these tumors (Choe et al. 2003). The optimal strategy to target the PI(3)K pathway in glioma and other human cancers is currently unclear. Options include inhibitors of PI(3)K, the serine-threonine kinase Akt, the mammalian target of rapamycin (mTOR), and a combination thereof (Workman et al. 2010).

The first member of the PI(3)K pathway for which a clinical grade inhibitor became available was mTOR which exists as a member of two distinct protein complexes called complex I (mTORC1) and complex 2 (mTORC2). mTORC1 responds to a variety of stimuli (growth factors, changes in amino acid availability, energy status, oxygen levels, DNA damage) and promotes protein translation by phosphorylating p70 ribosomal S6 kinase (S6K) (Threonine 389) and eukaryotic initiation factor 4E-binding protein (4EBP) (Threonine 37/46). mTORC2 functions are incompletely understood and include phosphorylation of Akt (Serine 473), serum glucocorticoid-induced kinase (SGK), and specific Protein Kinase C isoforms (Mendoza et al. 2011; Sengupta et al. 2010). The natural product rapamycin inhibits mTORC1 functions allosterically by binding with high affinity to the immunophilin FK506-binding protein-12 (FKBP12).

The potent antiproliferative activity of rapamycin against PTEN-deficient tumor models (Neshat et al. 2001; Podsypanina et al. 2001) motivated a clinical trial with single-agent rapamycin for patients with PTEN-deficient, recurrent GBM. Screening for PTEN protein expression was performed by immunohistochemistry on the specimen from the initial tumor resection and rapamycin was given for 1–2 weeks prior to resection of recurrent tumor. Drug levels of rapamycin were measured in blood and tumor tissue and inhibition of mTOR was evaluated with phosphosite-specific antibodies against the S6K substrate S6 Ribosomal Protein and 40 kDa proline-rich AKT substrate (PRAS40). Inhibition of tumor cell proliferation by rapamycin correlated with mTOR pathway inhibition. About half the tumors showed increased phosphorylation of the Akt substrate PRAS40 during rapamycin treatment and PRAS40 hyperphosphorylation was associated with poor clinical outcome (Cloughesy et al. 2008). Akt activation during treatment with rapalogs has been observed in other cancers and has been attributed to de-inhibition of a negative feeback loop between the mTORC1 substrate S6K1 and adaptor protein insulin receptor substrate (IRS-1) (O’Reilly et al. 2006; Sun et al. 2005). Reactivation of the PI3-K pathway, and also the MAPK pathway (Carracedo et al. 2008), may contribute to the overall disappointing clinical activity of rapalogs in high grade glioma (Chang et al. 2005; Cloughesy et al. 2008; Galanis et al. 2005) and other human cancers (Dancey 2010).

Since PTEN-loss has been associated with EGFR kinase inhibitor resistance and mTOR represents an important effector protein downstream of PI(3)K, combined blockade of EGFR andmTOR might overcome this resistance. In GBM cell lines and other preclinical

models, rapamycin indeed showed synergism with the EGFR kinase inhibitor erlotinib (Buck et al. 2006; Wang et al. 2006). However, the combination of rapalogs and first-generation EGFR kinase inhibitors in patients with recurrent GBM was complicated by toxicity requiring substantial dose reductions and failed to show compelling clinical activity (Kreisl et al. 2009b; Reardon et al. 2010).

7 New Approaches to Target the PI(3)K-mTOR Axis

The observed PI(3)K pathway activation in response to rapalogs suggests that inhibitors of PI(3)K (e.g., XL147, GDC-0941, GSK1059615, ZSTK474, PX866) or dual PI(3)K/mTOR inhibitors (e.g., XL765, SF1126, BEZ235, GSK1059615) might accomplish superior pathway blockade and biological activity. Many such inhibitors have been synthesized and have shown broad antiproliferative activity in preclinical GBM models (Fan et al. 2006; Guillard et al. 2009; Shuttleworth et al. 2011). The majority of first generation PI(3)K inhibitors entering the clinic block all members of the class I PI(3) K family and appear to be surprisingly well tolerated (Shuttleworth et al. 2011) despite the central role of individual class I PI(3)Ks in glucose metabolism and immune function (Okkenhaug et al. 2002; Sasaki et al. 2000). Determination of their clinical activity in GBM is eagerly awaited.

Whether more selective inhibition of individual class I PI(3)Ks will widen the therapeutic window of these agents without compromising their activity (Foukas et al. 2010), is an open question. A screen of six GBM cell lines with a panel of chemotypically diverse and isoform-selective inhibitors of the PI(3)K family demonstrated that inhibitors of p110α or p110β were able to inhibit phosphorylation of Akt, but only p110α inhibitors induced proliferative arrest. The PI3K isoforms p110δ and p110γ were not expressed in these cells and inhibitors of p110β and p110δ had no effects on proliferation (Fan et al. 2006). More recent studies identified a critical role for p110β in certain PTEN-deficient malignancies (Jia et al. 2008; Wee et al. 2008). Further work is needed to determine which PI3K isoforms are most critical for tumor maintenance in GBM as many of these tumors harbor multiple lesions within the RTK/Ras/PI(3)-signaling axis (e.g., EGFR mutation and PTEN loss).

The only modest activity of rapalogs against many human cancers may, at least in part, be due to the fact that these drugs do not effectively block many functions of mTORC2 and mTORC1. This shortcoming could be overcome by a new class of *TOR* kinase domain inhibitors (also called TORKinibs). These “second-generation” mTOR inhibitors have shown compelling antiproliferative activity in a range of preclinical cancer models and have also advanced to clinical testing in GBM and other cancer types (Feldman and Shokat 2010; Liu et al. 2009a).

8 Leveraging the Cancer Genome Atlas for Clinical Drug Development

The clinical experience with kinase inhibitors as cancer therapeutics suggests that these drugs are most effective in patients whose tumors harbor gain-of-function mutations in the targeted kinase. Examples include *BCR-ABL* mutant leukemia, *KIT*- or *PDGFRA*-mutant sarcoma, *EGFR* or *ALK* mutant lung cancer, and *BRAF* or *KIT* mutant melanoma (Sawyers 2009). While the presence of an oncogenic mutation clearly does not guarantee response to the corresponding kinase inhibitor, the relationship between tumor genotype and drug response is compelling enough that mutational profiling is now routinely performed in the clinic for several human cancers (e.g., lung cancer, melanoma, colorectal cancer) and results incorporated into treatment decisions (Gerber and Minna 2010). A link between tumor genotype and drug response still remains to be proven for GBM, but many centers have begun to prospectively profile gliomas for the most commonly found alterations (e.g., *EGFR* gene amplification, *MGMT* methylation, *IDH1/2* mutations, *1p19q* deletion) (Jansen et al. 2010; Riemenschneider et al. 2010). The extent of profiling is currently driven by

institutional expertise, assay cost, and tissue availability but is likely to increase in the near future as newer profiling platforms yield robust results from formalin-fixed and paraffin-embedded clinical specimen.

While the impact of tumor profiling on disease classification and treatment in GBM remains to be firmly established, currently its greatest utility may be for clinical drug development. A logistic obstacle toward genotype-focused clinical development in GBM is the low absolute number of patients with tumors of a particular tumor genotype. As discussed above, many presumed "driver" mutations in GBM (e.g., *PDGFRA*, *PIK3R1*, *PIK3CA*, *BRAF*, *MET)* occur in only a small subset of patients and a substantial number of these patients will be ineligible for clinical trial participation due to disease-related morbidity. By determining the distribution of mutations within a large number of patients with primary GBMs, TCGA (2005) has provided valuable information for clinical trial planning. For example, mutations in *EGFR* and *PDGFRA* are rarely found in the same tumor (Fig. 4) and one could envision using prospective genotyping information to assign patients to clinical trials targeting either of these lesions. Genotype-enriched trials for other presumed "driver" mutations, such as *MET* gene amplification, *PIK3CA* mutations, or *PIK3R1* mutations will require screening of a substantially larger number of patients as these mutations are even less common. Based on the distribution of mutations throughout the coding sequence of various pathway members (Fig. 2), perhaps a combination of phosphoproteomic "pathway activation" measurements (Solit and Mellinghoff 2010) and selected genotyping for mutation hotspots (Jansen et al. 2010) may represent the most cost-effective screening approach until predictive biomarkers have been properly validated.

9 Future Perspective

The clinical development of PI(3)Kinhibitors for GBM would be greatly accelerated by enrichment of clinical trials for patients whose tumors that are more likely to respond. While several ATP-site competitive pan-class I PI(3)K inhibitors have shown antiproliferative activity against a broad panel of human GBM cell lines (Fan et al. 2006; Guillard et al. 2009; Koul et al. 2010; Liu et al. 2009b; Prasad et al. 2011), it is not clear whether any disease subgroup might exhibit a degree of PI(3)K pathway "addiction" associated with tumor cell death and tumor regressions in other cancers. In breast cancer, for example, such PI(3)K pathway addiction appears to exist for tumors with *PIK3CA* mutation and *HER2* gene amplification as evidenced by cell death induction in response to pan-class I PI(3)K inhibitors (Ihle et al. 2009; Junttila et al. 2009; Mallon et al. 2010; O'Brien et al. 2010; Serra et al. 2008), Akt inhibitors (She et al. 2008), and the emerging class of mTOR kinase domain inhibitors (Weigelt et al. 2011). Lung cancer cell lines harboring *EGFR* kinase mutations, on the other hand, do not appear to depend on PI(3)K signals for survival (Faber et al. 2009). More extensive testing of novel compounds in genetically faithful glioma models (Hambardzumyan et al. 2011) and cell lines (Lee et al. 2006a) will provide the foundation to formulate hypotheses for genotype-enriched clinical trials.

Different dosing schedules (e.g., intermittent or "pulsatile" dosing) (Shah et al. 2008) and isoform-specific (e.g., PI3K) or mutant-specific (e.g., BRAF) compounds may increase the therapeutic window of individual kinase inhibitors. Ultimately, however, a combination of agents may be required to achieve clinically meaningful tumor regressions. Such therapies may include: (i) a combination of multiple selective RTK inhibitors, (ii) the simultaneous inhibition of multiple members within the same signaling pathway (e.g., PI3K and mTOR), (iii) a multipronged attack on key oncoproteins through distinct pharmacological approaches (e.g., RTK antibody plus RTK kinase inhibitor or HSP90 inhibitor plus RTK inhibitor), or (iv) a combination of agents based on synthetic lethal relationships between signaling

pathways (Kaelin 2005). These strategies warrant rigorous testing in genetically faithful preclinical models.

One example for a rational combination therapy is the combination of autophagy inhibitors with PI(3)K-mTOR inhibitors. Autophagy is a process of "self-degradation" of cellular components which can provide substrates for energy production during periods of low extracellular nutrients. mTORC1 suppresses autophagy by blocking autophagosome initiation via phosphorylation of ATG13 and ULK1 (Zoncu et al. 2011). Conversely, inhibition of mTORC1 by rapamycin (Takeuchi et al. 2005) or dual pan-class I PI(3)K/mTOR inhibitors (Guillard et al. 2009; Liu et al. 2009b) induces autophagy in GBMcells. Induction of autophagy may represent an important survival mechanism during mTOR inhibitor therapy and explain why inhibitors of PI3K and mTOR induce growth arrest without cell death. Consistent with that model, recent studies show that inhibitors of autophagy can synergize with PI3K-mTOR inhibitors to induce apoptosis (Fan et al. 2010; Xu et al. 2011), representing an opportunity for mechanism-based combination therapy.

GBM has long been known as a disease with mutations in growth factor signaling pathways. The full extent of these alterations and their relationship to each other has emerged more clearly through The Cancer Genome Atlas. Despite discouraging clinical results with first-generation RTK inhibitors and rapalogs, there is considerable optimism that new RTK inhibitors, "second-generation" mTOR inhibitors, and compounds inhibiting class I PI(3)Ks will be better suited to effectively shut down critical signaling nodes in GBM. As the number of clinical grade inhibitors continues to grow, it will become increasingly important to develop an approach to clinical drug development which fully leverages our knowledge of the GBM cancer genome, lessons from kinase inhibitor therapy in other human cancers, and the ability to extract robust molecular information from small amounts of routinely collected human tumor tissue.

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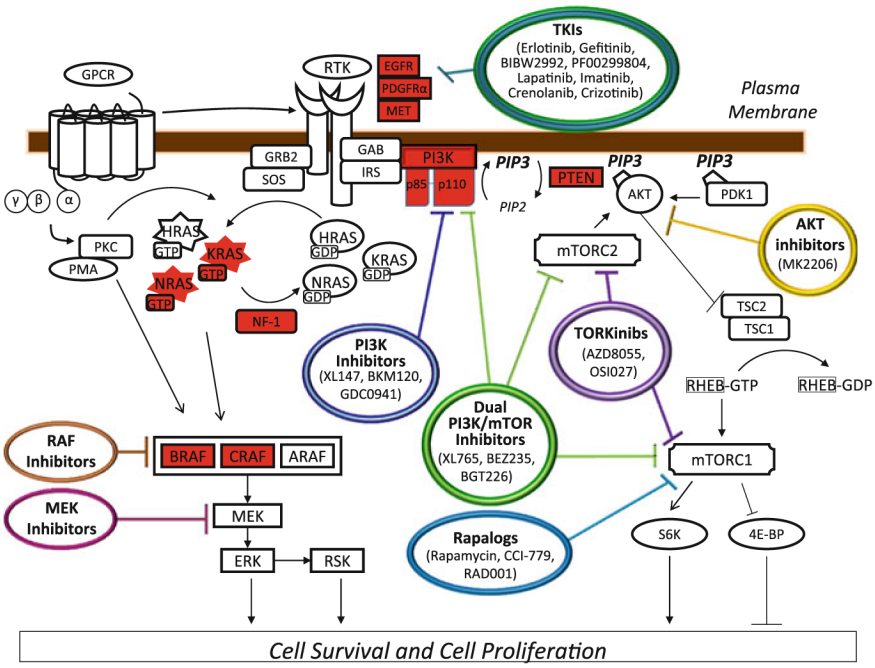

Fig. 1.

The Ras-PI(3)K-mTOR pathway in GBM. Pathway members highlighted in *red* are mutated in human GBM tumor samples. Pathway inhibitors that have been or will be explored as therapeutics for GBM are indicated

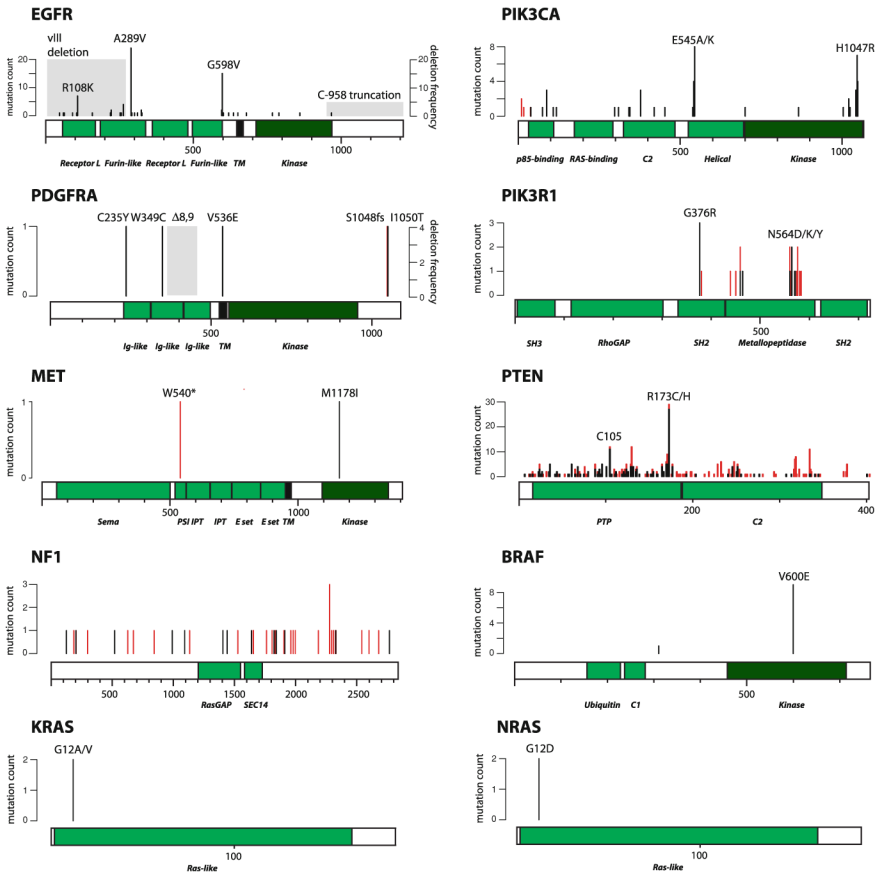

Fig. 2.

Mutations in the Ras-PI(3)K-mTOR axis in WHO grade IV astrocytoma. Shown are all confirmed somatic or previously reported mutations that have been listed in COSMIC v54 under astrocytoma grade IV. The *y*-axis indicates the number of times a particular mutant has been observed in a primary tumor sample, xenograft tumor, or cell line. Missense mutations are shown in *black*; nonsense and frameshift mutations as well as small in-frame insertions and deletions are shown in *red*. Recurrent deletions/truncations in the coding regions of *EGFR* and *PDGFRA* are indicated in shaded *gray* and their estimated frequency is shown as percent of all GBMs (*right y*-axis)

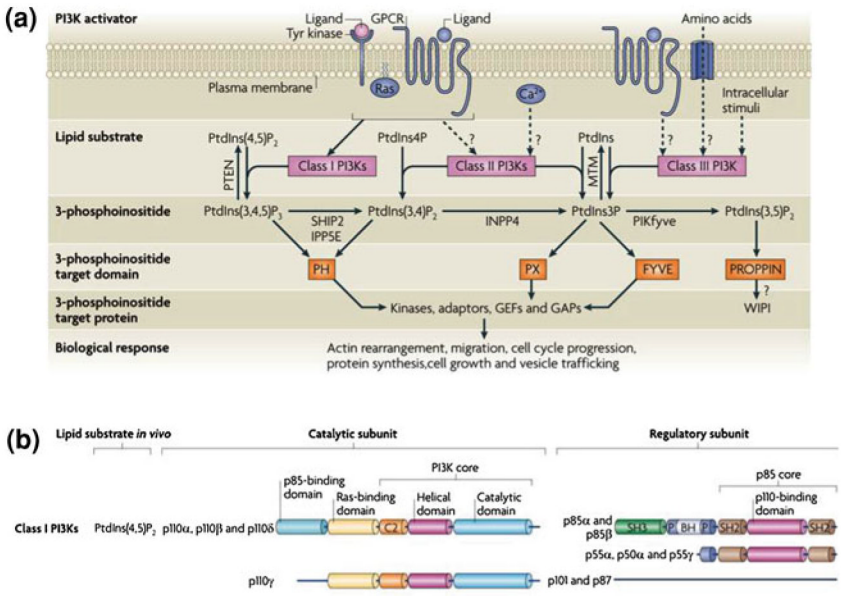

Fig. 3.

The 3-phosphoinositide lipid network. (**a**) Following activation by upstream agonists, phosphoinositide 3-kinases (PI3Ks) generate phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P3), PtdIns-3,4-bisphosphate (PtdIns(3,4)P2) and PtdIns-3-phosphate (PtdIns3P). These lipids interact with lipid binding domains in PI3K effector proteins and change their localization and/or activity. Lipid phosphatases degrade or interconvert 3-phosphoinositides. These include lipid phosphatases for PtdIns(3,4,5)P3, such as phosphatase and tensin homologue deleted on chromosome 10 (PTEN), inositol polyphosphate-5-phosphatase E (IPP5E), and SH2 domain-containing inositol 5-phosphatase type 2 (SHIP2). GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor; GPCR, G protein-coupled receptor. (**b**) Classification and domain structure of mammalian Class I PI3Ks. All PI3K catalytic subunits have a PI3K core structure consisting of a C2 domain, a helical domain and a catalytic domain. Class I PI3Ks exist in complex with a regulatory subunit, either a p85 isoform (for p110*α*, p110*β* and p110*δ*) or p101 or p87 (for p110*γ*). All p85 isoforms have two Src homology 2 (SH2) domains and are encoded by either PIK3R1 (which encodes p85*α*, p55*α* and p50*α*), PIK3R2 (which encodes p85*β*) and PIK3R3 (which encodes p55*γ*). Figure modified from (Vanhaesebroeck et al. 2010)

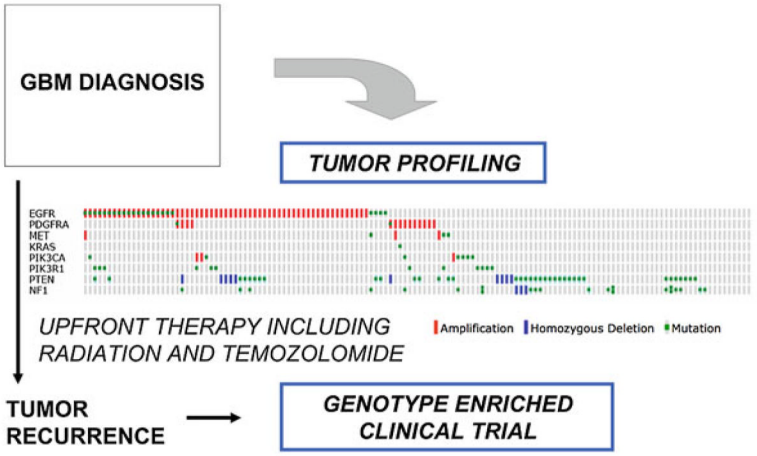

Fig. 4.

Potential strategy for genotype-enriched clinical trials in GBM. Tumor tissue collected during the initial surgery ("GBM Diagnosis") is profiled to identify candidate "driver" mutations and clinical trial participation at the time of tumor recurrence (i.e., following standard "Upfront Therapy") is guided by the molecular profiling results. Shown is the distribution of mutations in 138 GBMs which were profiled by the TCGA (2005) and for whom complete Sanger Sequencing and Gene Copy Number data is available

Table 1

Mutations in Growth Factor Signaling Pathways in GBM (adult patients)

Gene	Alteration	Frequency in GBM %
EGFR	Amplification	35–40
	EGFR-variant III (EGFRvIII)	~20
	Nonsynonymous Mutations	10–15
	other in-frame deletions/truncations	5–10
PDGFRA	Amplification	5–15
	delta-8,9 truncation	2–5
	Nonsynonymous Mutations	<2
MET	Amplification	2–5
	Nonsynonymous Mutations	<2
KRAS	Nonsynonymous Mutations	<2
NRAS	Nonsynonymous Mutations	<2
NF1	Homozygous Deletion	2–5
	Hemizygous Deletion	5–10
	Nonsynonymous Mutations	~15
BRAF	V600E BRAF	<2 (2–5 in PA)
	KIAA1549-BRAF fusions	n.d. (60–70 in PA)
	p.A598_T599insT BRAF	n.d. (1–3 in PA)
	FAM131B-BRAF fusion	n.d. (1–3 in PA)
CRAF	SRGAP3-CRAF fusion	n.d. (1–3 in PA)
PIK3CA	Nonsynonymous Mutations	5–10
	Amplification	~2
PIK3R1	Nonsynonymous Mutations	5–10
PTEN	Homozygous Deletion	5–10
	Hemizygous Deletion	~70
	Nonsynonymous Mutations	15–25

Please see text for references

n.d not detected, *PA* Pilocytic Astrocytoma