J Neurooncol (2011) 103:417–427
DOI 10.1007/s11060-010-0442-z

TOPIC REVIEW

PI3Kinase signaling in glioblastoma

M. M. Lino · A. Merlo

Received: 9 March 2010 / Accepted: 21 October 2010 / Published online: 10 November 2010

Abstract Glioblastoma (GBM) is the most common primary tumor of the CNS in the adult. It is characterized by exponential growth and diffuse invasiveness. Among many different genetic alterations in GBM, e.g., mutations of *PTEN*, *EGFR*, *p16/p19* and *p53* and their impact on aberrant signaling have been thoroughly characterized. A major barrier to develop a common therapeutic strategy is founded on the fact that each tumor has its individual genetic fingerprint. Nonetheless, the PI3K pathway may represent a common therapeutic target to most GBM due to its central position in the signaling cascade affecting proliferation, apoptosis and migration. The read-out of blocking PI3K alone or in combination with other cancer pathways should mainly focus, besides the cytostatic effect, on cell death induction since sublethal damage may induce selection of more malignant clones. Targeting more than one pathway instead of a single agent approach may be more promising to kill GBM cells.

Keywords GBM · PI3K · Apoptosis · Proliferation

Abbreviations

CNS	Central nervous system
GBM	Glioblastoma
PTEN	Phosphatase and tensin homologue
EGFR	Epidermal growth factor receptor
Moabs	Monoclonal antibodies
PKI	Protein kinase inhibitors
VEGF	Vascular endothelial growth factor
ATM	Ataxia telangectasia mutated
ATR	Ataxia teleangectasia rad3 related
DNA-PK	DNA-dependent serine/threonine protein kinase
CTMB	Carboxyl-terminal modulator protein
SNPs	Single nucleotide polymorphism
RTEL1	Regulator of telomere elongation helicase 1
CDKN2B	Cyclin-dependent kinase inhibitor 2B
MGMT	O6-methyl-guanine-DNA-methyl-transferase
MMR	Mismatch repair

M. M. Lino (✉) · A. Merlo
Laboratory of Molecular Neuro-Oncology and Departments of Neurosurgery, University Hospitals, Hebelstrasse 20, 4031 Basel, Switzerland
e-mail: maddalena.lino@unibas.ch

Introduction

Gliomas, originating from the predominant glial tissue in the CNS, are the most common primary tumors of the central nervous system (CNS) in adults [1]. The prevalent form is astrocytoma WHO grade IV or glioblastoma (GBM). In affected patients, median survival is less than 1 year [2, 3]. Gliomas consist of 3 different tissue types: astrocytomas (about 70%), oligodendrogliomas (10–30%) and ependymomas (less that 10%). Malignant astrocytomas include tumors of WHO grade II (low-grade malignancy), III (anaplastic stage) and IV (highly malignant form, also named GBM) [4]. GBM accounts for approximately 50% of all glial tumor types. They are characterized by rapid growth and diffuse invasiveness into the adjacent brain parenchyma. Only the nodular component of the disease can be controlled surgically. The infiltrative component of the tumor, however, is left to non-specific and cytotoxic chemo- and radiotherapy that may control tumor progression for a limited time window.

Molecular genetics of GBM

The stochastic and complex process of brain tumorigenesis involves activation of oncogenes and inactivation of tumor suppressor genes [5–7]. A large number of genetic alterations have been detected and catalogued in different brain tumors. Familial cancer syndromes, although rare, provided a first clue to understanding the role of specific genes, their associated pathways and to testing them in animal models.

The most common genetic alterations detected in gliomas are loss of heterozygosity at 10q, PTEN mutation [8], and EGFR amplification/overexpression, along with EGFRvIII expression [9, 10], p16/p14 co-deletion [11–13], p53 mutation [14, 15], MDM2 amplification [16], loss of 1p/19q [17], and telomerase re-activation [18]. Besides these classic mutations, a recent comprehensive analysis was able to confirm the known mutations and discovered still unknown genes mutated in GBM, although at low frequency. Interestingly, mutations in the active site of isocitrate dehydrogenase 1 (IDH1) were detected in 12% of GBM patients, mostly young patients with secondary GBMs [19].

A specific molecular signature been detected so far for oligodendrogliomas [17, 20].

A recent paper from the TCGA (The Cancer Genome Atlas Network) based on gene expression-based molecular classification subdivides GBM into Classical, Mesenchymal and Proneural subtype. Each group shows a different aberration and gene expression, which may predict therapy efficacy. The Proneural subtype was associated with younger age, PDGFRA abnormalities, IDH1 and TP53 mutation and resistance to temozolomide and radiation therapy. The Classical GBM with EGFR abnormalities showed the best reaction to therapy, while the mesenchymal subtype, characterized by high expression of CHI3L1 and MET and NF1 mutation/deletion, reported only a partial response to treatment [21].

Recently, it was shown that high-grade glioma risk is associated with inherited variation in a region of 9p21 containing CDKN2B and a region of 20q13.3 tagged by two intronic SNPs in RTEL1 [22].

MGMT, a DNA repair enzyme, is associated with glioblastoma sensitivity to alkylating agents. Two different groups reported that patients with glioblastoma containing a methylated MGMT promoter benefited from temozolomide, in comparison with patients that did not have a methylated MGMT promoter [23–25]. Methylation of MGMT promoter has been proved to be an independent and stronger prognostic factor, better than age, stage and tumor grade, and predicting responsiveness of glioma to alkylating agents. The TCGA group found that the mutational spectra in the MMR genes paralleled MGMT methylation status and treatment consequences. MMR deficiency and MGMT methylation together may therefore influence the overall frequency and pattern of somatic point mutations in glioblastoma tumors [26].

Even though GBM share many of these alterations, each individual tumor has its own unique pattern of genetic changes that represents a considerable barrier to the development of therapeutic intervention [27]. The putative Achilles heel of GBM may not be a single major genetic alteration, but rather a secondary acquired imbalance in the aberrant signaling network that impinges on essential regulatory pathways.

In this review, we will describe the PI3Kinase network and its role in GBM.

EGFR and GBM

EGFR is the first member of the ErbB (Her) family of RTKs. The two main EGFR ligands are EGF and TGF-α (transforming growth factor-α), among other ligands like beta-cellulin, epiregulin, heparin binding EGF (HB-EGF), and amphiregulin. Ligand binding to EGFR induces receptor phosphorylation, which in turn activates a complex downstream signaling network [28]. Downstream signaling through PI3K-PKB [20], PI3K-Rac-Rho, Ras-Raf-Mek-Erk and Jak-STAT influence proliferation [29], migration [30], invasion, resistance to apoptosis [31, 32], and tumor neovascularization [33, 34] (Fig. 1). Overexpression of EGFR has been found in many different tumor types including GBM, and has been consistently found to be correlated with a poor outcome [12, 35]. Genetic alterations like overexpression, small deletions or mutations can lead to oncogenic upregulation of the receptor [36]. In GBM, activation of EGFR is present in 40–60% of tumors [37]. The most frequent activating mutation is the EGF mutant receptor vIII (EGFRvIII) [36]. Amplification of EGFR gene in GBM leads to downstream activation of PI3K/PKB/mTOR/rpS6. Interestingly, it has been shown that inhibition of EGFR signaling correlates with decrease p-mTOR and p-rpS6 in cells wild-type for PTEN. In contrast, inhibition of EGFR signaling fails to affect p-mTOR or p-rpS6 in cells mutant for PTEN. Recently, a PKB independent pathway linking EGFR to mTOR through PKC (protein kinase C) was described indicating an additional drug target [38].

The role of PI3Kinase in the glioma signaling network

Members of the phosphatidylinositol 3-kinase (PI3K) family are lipid kinases involved in diverse signaling pathways that regulate proliferation, differentiation, migration, trafficking, and glucose homeostasis [39–42]. They contain a p110 catalytic subunit that heterodimerizes with five distinct regulatory subunits (p85α, p55α, p50α, p85β, and p55γ). The p110 catalytic subunit includes an N-terminal p85 binding

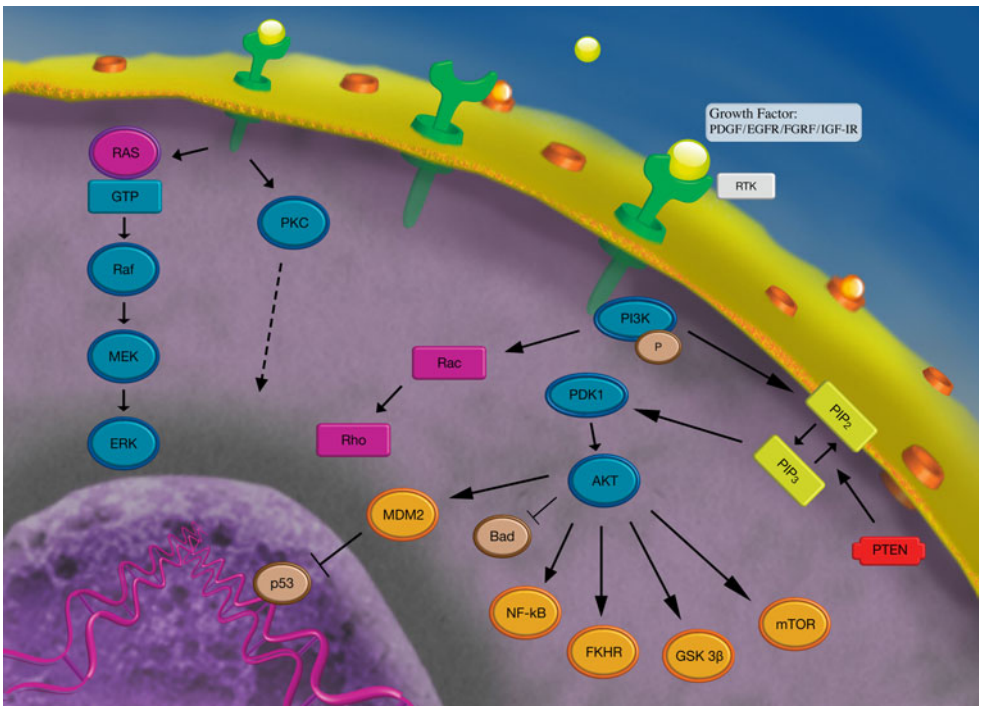

Fig. 1 The PI3K pathway activates different processes that regulate proliferation, apoptosis and migration. Schematic representation of the signaling pathway activated by receptor tyrosine kinase (RTK) and the PI3K downstream activation. Designed by www.lebensart003.com

domain, a Ras binding domain, a C2 domain, a phosphatidylinositol kinase homology (PIK) domain, and a C-terminal catalytic domain. The PIK and catalytic domains of p110 are homologous to other protein kinase domains including mTOR, ATM (ataxia telangectasia mutated), ATR (ataxia teleangectasia rad3 related) and DNA-PK (DNA-dependent serine/threonine protein kinase). Mutations within the p110 subunit of PI3K, that are mainly gain of function mutations, have been identified mostly in exon 9 (helical domain) and in exon 20 (kinase domain) [43, 44]. In mammals, 8 distinct PI3K have so far been described. They are divided into classes I–III according to their substrate specificity, regulation and structure. Class I PI3Ks contain two subgroups, IA (p110α, p110β and p110δ) and IB (p110γ), which are activated by growth factor receptor tyrosine kinase (RTKs) and by G-protein-coupled receptors (GPCRs), respectively [42]. Class II PI3Ks consist of a single p110-like catalytic subunit that regulates membrane trafficking and receptor internalization [41, 45]. Class III PI3Ks has been found to regulate mTOR activity in response to availability of amino acids for the control cell growth [46–48].

PI3K are activated by a wide range of upstream signals and phosphorylate the lipid phosphatidylinositol-4,5,-bisphosphate, generating phosphatidylinositol-3,4,5-trisphosphate (PIP3) [41, 42]. The protein serine/threonine kinase PKB (also known as AKT) is the principal PIP3 target. PKB is recruited to the membrane upon binding of PIP3 with subsequent phosphorylation by the mTOR-rictor kinase complex and by PDK1 (3-phosphoinositide-dependent kinase). Activation of PKB, in turn, phosphorylates many target proteins which regulate cell metabolism, cell cycle and cell survival [49, 50], protein synthesis [47], cell polarity, cell motility [51], and vesicle sorting [52] (Fig. 1).

Therefore, the signaling components PI3K/PKB/mTOR are central regulators of cell proliferation, growth, differentiation, and survival. Moreover, PI3K regulates migration and invasion, mainly by the Rho family members Cdc42, Rac and Rho [53, 54]. These small GTPases dynamically remodel the actin cytoskeleton and give rise to filopodia, lamellipodia and stress fibers [55, 56].

PI3K dysregulation is observed in a variety of tumors [44, 57–60] including GBM [44, 59], but also in other non-neoplastic human disorders [61]. For example, attenuated PI3K signaling downstream of the insulin receptor significantly contributes to the type-2 diabetes phenotype [62]. In human cancers, p110 of PI3K is frequently amplified [44]. This observation has been confirmed by another study that described PI3K p110 mutations in 15% of glioma samples, and 21% in pediatric and 17% in adult brain tumor samples [63]. In addition, elements of the PI3K signaling pathway are also frequently mutated, such as PTEN [64, 65] and the carboxyl-terminal modulator protein (CTMB) [66, 67]. Transgenic and knockout mouse models have confirmed the role of the PI3K-PBK pathway in tumorigenesis [68]. The TCGA group reported novel in frame deletions in the adaptor domain of PIK3CA. Interestingly in PI3KCA wild-type patients were found mutation in PIK3R1. These mutations clustered always around amino acid residues that disrupt C2-iSH2 interaction reducing the inhibitory effect of p85α on p110α [26].

PTEN (*Phosphatase and tensin homologue*) tumor suppressor gene encodes a phosphatase that catalyzes the dephosphorylation of phosphatidylinositol 3,4,5 triphosphate (PIP3), negatively regulating the activity of PI3K [8]. The PI3K/PTEN/PKB-pathway is of critical importance for angiogenesis, cell proliferation and survival [31, 50]. Homozygous *Pten*-knockout mice are embryonically lethal while heterozygous animals are viable but develop various tumors [69]. Loss of function mutations of PTEN are frequent in GBM and activate PKB in a similar way as mutations in PIK3CA [70]. Epigenetic gene silencing by promoter methylation also inactivates PTEN [71]. In animal models, haploinsufficiency was sufficient to promote tumorigenesis for certain tumor types, and progressive reduction of PTEN resulted in increasingly aggressive tumors [72, 73]. Transfection of PTEN resulted in reduced proliferation and induction of cell cycle arrest at G0/G1, accompanied by inactivation of PKB phosphorylation at Ser-473 [74]. In addition, exogenous PTEN expression induces astrocytic differentiation in the presence of the ECM [74] while neural stem cells self-renewal is negatively regulated by modulating GO-G1 cell cycle entry [75, 76]. PTEN expression sensitizes GBM cells to radiation, but not to chemotherapeutic drugs [77]. Epigenetic and genetic inactivation of *PTEN* is associated with shorter survival in GBM patients [8, 12, 78, 79].

PI3Kinase mouse model

P85β knockout mice develop hypoinsulinemia and hypoglycemia [80, 81]. Loss of all isoforms of *PIK3R1* (including p50α and p55 α) resulted in perinatal lethality and caused a decrease in the expression and activity of class IA PI3K catalytic subunits. Heterozygous disruption of *PIK3R1* improved insulin signaling and glucose homeostasis [82].

Mice carrying homozygous deletions for either p110α or p110β were found to be embryonic lethal [83, 84]. Inhibitors of p110α blocked insulin-stimulated phosphorylation of PKB, while inhibitors of p110β had no effect on insulin-stimulated phosphorylation. These results suggested that p110α has a key role in the PI3K-dependent insulin signaling [62]. Direct tumorigenic effect was proved in conditional and prostate-specific PTEN mutation: ablation of p110 blocked PTEN$^{-/-}$-induced tumor formation [85].

Disease targets and ligands

Glioblastomas may develop de novo (primary GBMs) or through progression from low grade to high-grade astrocytomas (secondary GBMs) [27]. Radiotherapy has been shown to prolong the median survival of GBM patients [86] and is the standard adjuvant therapy for high-grade GBM, nowadays often combined with the radiosensitizer temozolomide [87]. However, GBM can relapse close to the targeted resection margins or within 2 cm of the resection cavity. Other therapies such as seed-based brachytherapy [88] or radiosurgery have limited additional therapeutic value. Novel loco-regional treatments using peptide-toxin- or radiolabeled peptide-conjugates are being evaluated [89].

To substantially improve prognosis, more specific therapies are being developed against a number of new critical molecular targets: growth factor receptor inhibitors (gefitinib and erlotinib) [90–92], matrix metalloproteinase inhibitors (marimastat, metastat and prinimastat) [93] and blockers of angiogenesis [94–98]. We will now focus on the role of PI3K in GBM, discuss the effects of PI3K inhibitors on glioma cell survival and proliferation, and perspectives of upstream and downstream interference.

Targeting PI3K and downstream pathway in GBM

Different RTK inhibitors have been developed and studied in different cancer types including GBM, targeting EGFR, PI3K, mTOR and PKB.

Targeting EGFR

Two different types of EGFR inhibitors have been developed: monoclonal antibodies (MoAbs) and small molecule inhibitors of EGFR tyrosine kinase activity, competing for the ATP binding site. The mechanism of receptor inhibition differs between the two types of drugs. MoAbs interfere with EGFR activation by blocking the extracellular ligand-binding domain. Protein kinase inhibitors (PKI) block the intracellular tyrosine kinase-mediated signaling pathways. Many different MoAbs have been developed with different affinity, specificity and negative regulatory effect. Cetuximab (IMC-225, Erbitux^{TM}; ImClone systems, Princeton, NJ, USA), a MoAb which binds EGFR with higher affinity than the natural ligands, has fairly recently been FDA-approved for treatment of patients with EGFR-positive metastatic colorectal cancer, and since December 2005 by Swissmedic for the treatment of patients with squamous cell carcinoma of the head and neck in combination with radiotherapy. Regarding GBM, the hR3 (TheraCIM; CIMYM Biosciences, ON, Canada), a human high affinity MoAb to EGFR that is now in phase I/II clinical trial (NCT00369252), has shown a partial response in a GBM study. The use of mAb 806 (Ludwig Institute, Victoria, Australia) that targets mutant EGFRvIII on glioma cell lines and mouse xenografts overexpressing EGFRvIII led to a dose-dependent growth inhibition [99]. Mab-806 is now in preclinical trial in an orthotopic murine glioma

model, using EGFRvIII-positive U87MG cells [100]. Moreover, a combinatorial study, which uses mAb 806 and mAb 528, showed additive antitumor activity in human tumor xenografts [99]. The use of Cetuximab in tumor cell lines and a xenograft model induces apoptosis and inhibits angiogenesis [101, 102]. The small molecule inhibitors of EGFR are less specific than the MoAb and therefore the clinical effect was found to be less predictable. Nevertheless, these drugs have a low molecular weight, allowing better tumor penetration and can be administered orally. Gefitinib (ZD1839; AstraZeneca, Wilmington, DE, USA) and Erlotinib (OSI-774, CP-358, 774, Tarceva; OSI Pharmaceuticals, in collaboration with Genentech and Roche Pharmaceuticals) have been FDA-approved for different cancer types and new clinical trials in GBM patients are ongoing. Gefitinib showed only a limited activity in GBM patients [103] and colorectal cancer [104]. It did not improve survival as mono-therapy in patients with non-small cell lung cancer stage III nor when following chemotherapy and radiation therapy [105]. AEE-788 (LymphoSign), a potent EGFR and VEGF2 inhibitor, is currently in Phase I clinical trial (NCT00116376) for GBM. Erlotinib appears to be more effective against malignant glioma than gefinitib when comparing the radiographic response rate, but none of them have a clear impact on survival.

The limited effect of these PKI against EGFR raised the question whether drug efficiency could not be improved by a combinatorial strategy. In a pre-clinical GBM cell line model blocking EGFR activity with the protein kinase inhibitory drug AEE788, we found a 10–20% reduction of cell proliferation but only a limited pro-apoptotic effect in the minority of GBM cell lines [106]. Gleevec (PKI of the PDGF receptor developed by Novartis) that inhibits Bcr-Abl and also has an activity against PDGFR and c-kit combined with AEE788 (Novartis) and RAD001 (an analog of rapamycin that inhibits mTOR developed by Novartis) was only marginally effective. However, the combination of AEE788 and patupilone (a cell cycle inhibitor blocking polymerization of microtubuli, developed by Novartis) synergistically induced apoptosis in 50% of GBM cell lines [106]. A synergistic effect was also found when combining erlotinib with the PI-103 that led to an increased proliferation block when compared with monotherapy or a combination of erlotinib and rapamycin. Such combinations may be effective against EGFR-driven PTEN mutant tumors [107]. EGFRvIII expression in GBM promotes DNA-PKcs (DNA dependent protein kinas catalytic subunit) and DBS (DNA double-strand breaks) repair most probably through the augmented PI3K/Akt1 signaling. This mechanism suggests that blocking the DBS and DNA-PKcs pathway together with an EGFR inhibition could lead to better results [108]. Using the EGFR inhibitor gefinitib together with an HMG-CoA reductase induces another interesting synergism, irrespective of EGFRvIII and PTEN status [109].

PI3K inhibitors

Several compounds inhibiting PI3K have been developed, e.g., wortmannin [110], Ly294002, staurosporine, quercetin, demethoxyviridin and PI-103 among others. Wortmannin and demethoxyviridin are potent, irreversible, but non-selective inhibitors of all PI3K. LY-294002 and quercetin are reversible and potent PI3K inhibitors, but non-selective and also bind other members of the PI3K family [111]. Most protein kinase inhibitors block access of ATP to the ATP-binding pocket by distinct stoichiometric interactions [112]. Wortmannin has shown activity against tumor xenografts from melanoma [113], breast [114], colon [114], ovarian [114], and pancreatic cancer [115]. Treating EGFRvIII glioma cell lines with wortmannin significantly suppressed cell growth to a similar extent as EGFR inhibitors [116]. Wortmannin has also been shown to be an effective radiosensitizer of glioma cells [117], and displayed, when tested on a panel of GBM cell lines, a slight pro-apoptotic effect [106]. LY-294002 efficacy has been studied in glioma cell lines. It significantly reduced the level of PtdIns (3,4,5)P_3, inactivated phospho-PKB, and blocked cell proliferation in a dose-dependent manner [118]. Moreover, Ly294002 blocks p53 induction by inhibiting stabilization of p53. Nevertheless, this down-regulatory effect on p53 by PI3K inhibitors (attenuating p53-dependent cell death) [119] may not be so relevant for many cancers, since the p53 pathway is inactivated in most cancers.

A new series of PI3K inhibitors, which selectively target different PI3K isoforms, were synthesized. The small molecule inhibitor PI-103 showed unique activity against genetically different GBM cell lines. PI-103 selectively blocked p110α and mTOR complex at nanomolar concentration [120]. Only down-regulation of p110α specifically blocked glioma proliferation in vitro, but not p110β [120]. PI-103 was efficient, irrespective of the genetic status of cell lines (PTEN, p53 and EGFR). In vivo data show that PI-103 was effective and non-toxic in glioma xenograft models [120, 121]. Since mTOR inhibition may lead to PI3K activation, theoretically, blocking mTOR combined with a PI3K inhibitor may induce cell death. PI3K inhibitors like Ly294002 enhance apoptosis triggered by TRAIL or cytotoxics (vincristine, doxorubicine, etoposide, etc.) [122]. Recently, an interesting paper showed that HOXA9 transcription was activated in GBM leading to decreased apoptosis and increased proliferation. Transcriptional activation of HOXA cluster was blocked by PI3K inhibitor through an epigenetic mechanism involving histone H3k27 tri-methylation [123].

PI3K downstream inhibition

PKB inhibition

The serine/threonine kinase PKB is one of the enzymes regulated by PI3K activity by regulating PtdIns-3,4,5-P_3 levels. Its activated form down-regulates Bad, caspase-9, GSK-3beta and forkhead transcription factors, suppressing apoptosis and promoting cell survival [31, 124]. In addition, PKB increased levels of vascular endothelial growth factor (VEGF) under hypoxic conditions [125]. PKB is activated in about 80% of GBM [126]. Activated PKB together with mutant Ras was found to be tumorigenic in a murine glioma model [68]. In another model using astrocytes expressing E6/E7, hTERT (human telomerase reverse transcriptase) and Ras, upgrading of a low-grade to a high-grade tumor was achieved by addition of activated PKB [127]. The effect of PKB in the regulation of cell cycle, apoptosis and angiogenesis of GBM cell lines strongly suggests a role in the development of GBM. The two PKB inhibitors, KP-372-1 and KP-37-2, induced apoptosis in GBM cells [128].

mTOR inhibition

The TOR family of proteins has multiple functions: regulation of mRNA transcription and translation in response to essential nutrients, membrane trafficking, protein degradation, organization of the actin cytoskeleton, and PKC signaling [129, 130]. There is a rapamycin-sensitive mTOR-complex (mTORC1) and a rapamycin-insensitive complex (mTORC2). mTOR activates S6K1 and EBP1. mTOR can also be up-regulated indirectly through activation of PI3K and PKB. Different mTOR inhibitors have been developed and are being assessed in clinical trials: the rapamycin prototype and three rapamycin derivatives RAD001 (everolimus), CCI-779 (temsirolimus), and AP23573. All mTOR inhibitors form a complex with the intracellular immunophilin FKBP12 and inhibit mTOR. The wide spectrum of rapamycin covers infectious, immunosuppressive, endothelial and neurodegenerative disease.

Rapamycin has been shown to have a growth inhibitory effect in several human and murine cancer cell lines both in vitro and in a xenograft model [131–134]. Rapamycin induces a decrease in cyclin D1 expression and an increase in p27 levels, leading to a cell cycle block in late G1/S-phase [135]. Rapamycin has also been shown to induce cell death in a limited number of tumor models, although the molecular mechanism leading to apoptosis is not clear. One of the mechanisms may be that the downstream target of mTOR—S6K1—inactivates the pro-apoptotic molecule BAD [136, 137]. Recently, it has been shown that inhibition of mTOR/S6K1 triggers a negative feedback loop resulting in the activation of AKT signaling probably through a IGF-1R-dependent mechanism. Inhibiting mTOR also has an effect on the angiogenesis by blocking the translation of HIF-α [138]. Anti-angiogenic effects on endothelial cells have further been shown in a rapamycin-sensitive murine tumor model [139]. An interesting paper from Liu et al. showed that NVP-BEZ235, a novel dual PI3K/mammalian target of rapamycin (mTOR) inhibitor, was able to inhibit PI3K and mTOR signaling,and induce cell cycle arrest, down-regulation of VEGF and autophagy in gliomas [140]. Different studies combining mTOR inhibitors together with other anti-cancer compounds have been published with contradictory findings [106, 141, 142]; however, in vitro results may not predict clinical efficacy given the wide spectrum of rapamycin effects. Different clinical trials are now being performed for the rapamycin derivates CCI-779 (http://clinicaltrials.gov/ct2/results?term=CCI-779), RAD001 (http://clinicaltrials.gov/ct2/results?term=RAD001) and AP23573 (http://clinicaltrials.gov/ct2/results?term=AP23573) alone or in combination with other drugs. The most promising results have been described for renal cell carcinoma [143], endometrial cancer [144], and mantle cell lymphoma [145], but also giant cell astrocytoma in tuberous sclerosis patients. Phase II studies have been performed in recurrent GBM patients, with limited anti-tumor activity [146, 147]. Given the negative feedback loop between mTOR and PKB via IGF-1R, an mTOR inhibitor combined with an IGF-1R antibody/inhibitor may be a promising strategy to increase therapeutic efficacy [148].

Drug combinations

Use of drug combinations is of particular interest, given the limited responses obtained in clinical trials using single drug approaches. In cases of an additive or synergistic effect, drug doses of each respective compound can be reduced, potentially paralleled by a reduction in side effects. We studied the impact of different drug combinations on survival and proliferation of GBM cell lines. Targeting EGFR with AEE788 and PDGFR with Gleevec and/or mTOR with RAD001, we found that single and combined applications did not significantly enhance background apoptosis rates [106]. However, the combination of AEE788 with the microtubuli inhibitor patupilone induced apoptosis in some 50% of cell lines, which was accompanied by simultaneous inactivation of both p-ERK and p-PKB. Asking whether down-regulation of p-ERK and p-PKB is critical for GBM cell survival, we directly blocked the PI3K/PKB and Ras/Raf/MEK/ERK pathways with both the PI3K inhibitor wortmannin and the MEK inhibitor UO126. This combination frequently killed GBM cells, supporting a model of an additive effect by targeting

these two signaling pathways that results in a reduced threshold for the induction of apoptosis. We did not find any correlation between the sensitivity or resistance of GBM cells to apoptosis and their genetic status [106]. Simultaneous treatment with rapamycin and the EGFR inhibitor (EKI-785) resulted in synergistic anti-proliferative and pro-apoptotic effects [149]. At a molecular level, rapamycin alone decreased S6 phosphorylation, while EGFR inhibitor reduced phosphorylation of STAT3 (signal transducer and activator of transcription). Rapamycin alone increased phosphorylation of PKB and promoted the binding of the translational inhibitor eukaryotic initiation factor 4 binding protein (4EBP1) to the eukaryotic translational initiation factor 4E (eIF4E), which are blocked by EGFR inhibition [150]. Simultaneous blocking of multiple enzyme activities may reduce the effect of compensatory signaling which is one of the therapeutic limitations of using single agent therapy. However, suppressing crosstalk may not be accompanied by an enhanced therapeutic effect. Furthermore, combining the anti-malaria drug artesunate (ART) with the EGFR inhibitor OSI-774 resulted in an in vitro cytostatic effect which was most pronounced in a background of constitutively active EGFR [151]. Results of a Phase II study of Imatinib mesylate (Gleevec) plus hydroxyurea showed that this combination was well tolerated and associated with a modest clinical response in a subgroup of patients with recurrent GBM [91]. PI3K inhibitor (as LY294002 and wormannin) sensitizes GBM cells to apoptosis. This mechanism act both activating extrinsic apoptosis through TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) and CD95 and the intrinsic mitochondrial apoptotic pathway [152].

Conclusions

The promise of Gleevec—the concept that each cancer may have its unique molecular signature that can be therapeutically exploited—has not yet been met. In GBM, there are a large number of different molecular targets, and the net effect on signaling by individual mutational patterns may also be unique for each tumor. Nonetheless, empirical drug combinations showed improved therapeutic effects over single agent approaches. A fundamental question is whether GBM have an Achilles heel or whether each tumor requires its individual drug combination. Components of the MAPK pathway, such as Raf [153] and MEK [106], displayed empirical evidence of efficacy when combined with drugs that target PKB/Akt [61] and mTOR [150]. Sublethal damage will select more malignant clones, considering the high degree of genetic instability of malignant human tumors [154]. Hence, therapeutic interference must aim at induction of tumor cell apoptosis. An interesting approach is to use drugs that exploit a general mechanism respecting individual patterns of alterations, e.g., epigenetic signatures of gene expression. For example, histone deacetylase inhibitory drugs induce re-expression of silenced genes in an unpredictable individual way, mimicking the stochastic nature of the disease [155]. Experimentally, HDAC inhibitory drugs displayed a remarkable pro-apoptotic effect upon combination with agents that block glucose utilization, such as 2-deoxy-glucose [156]. Combining new classes of cancer drugs with anti-metabolic strategies may lead to innovative new concepts to attack this disease, leading to enduring clinical responses by successful control of tumor cell proliferation, survival and invasion.

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