

## GENETIC AND SIGNALING PATHWAY ALTERATIONS IN GLIOBLASTOMA: RELEVANCE TO NOVEL TARGETED THERAPIES

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### TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Therapeutic implications of genetic and signaling pathway alterations in GBM
  - 3.1. Overview of aberrant pathways in GBMs
  - 3.2. Epidermal growth factor receptor (EGFR)
  - 3.3. Platelet derived growth factor (PDGF)
  - 3.4. p 53
  - 3.5. Ras
  - 3.6. Phosphatidylinositol 3-kinase (PI3 kinase)
  - 3.7. Drug combinations
4. Perspectives
5. References

### 1. ABSTRACT

Glioblastomas multiforme (GBM) is the most common malignant primary brain tumor in adults. GBM patients have a dismal prognosis, with a median survival of less than 1 year. During the past decade, significant advances have been made in our understanding of the molecular pathogenesis of these tumors. Specific genetic defects have been identified that appear to be important for the development, as well as maintenance of the malignant characteristics that are associated with GBM. Some of these genetic aberrations appear to have prognostic significance. However, even more exciting in this era of molecularly targeted therapy are the clues these gene alterations provide for identifying signaling mechanisms responsible for carcinogenesis, and for identifying potential therapeutic targets. Cancer drug therapy is currently undergoing a major transition with an attempt to move from the use of cytotoxic drugs towards the use of tumor mechanism-based drugs. Advances such as the decoding of the human genome, combinatorial chemistry, and gene expression profiling have led to an increase in the rate at which new drugs are being developed. In this review, we will describe the most common genetic and signaling pathway alterations that have relevance to new drug development for the treatment of GBM.

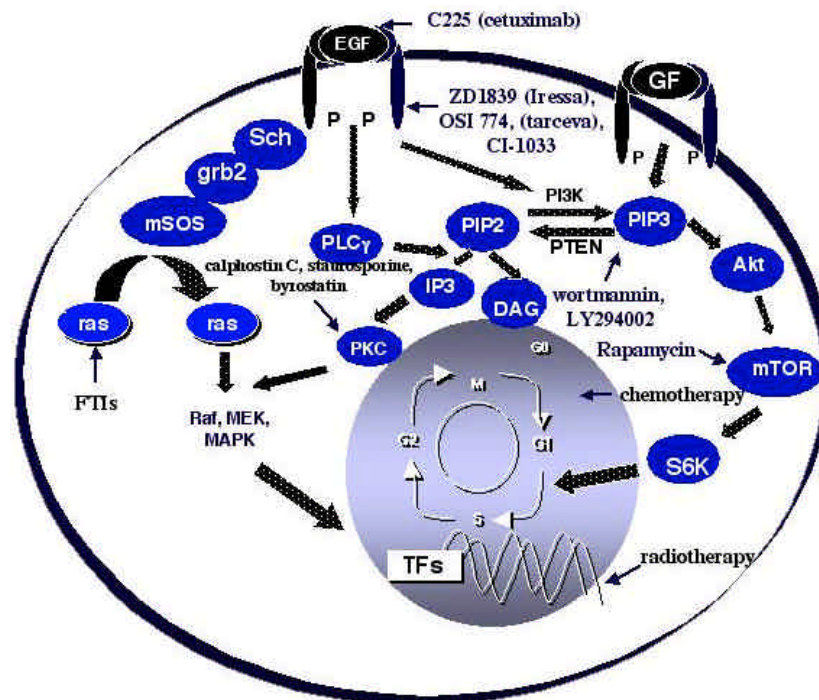
### 2. INTRODUCTION

Approximately 10,000 new cases of GBM are diagnosed in the United States each year. The conventional standard of care for patients with this cancer involves

surgical tumor resection followed by radiation with or without chemotherapy. Most of the chemotherapeutics applied to the treatment of these tumors have been traditional cytotoxic drugs that target cellular DNA or the cytoskeleton. Unfortunately, the use of such agents has not resulted in any significant improvements in GBM response rates, and the prognosis for GBM patients has remained dismal.

Over the last decade, however, there has been an increasing understanding about the molecular biology of GBMs, especially with respect to the genetic alterations that are responsible for many of the aggressive phenotypic characteristics of this cancer. A significant amount of data now exists that point to a limited number of gene and signaling pathway alterations as being responsible for the development of GBM, and these involve the epidermal growth factor receptor (EGFR), platelet derived growth factor (PDGF), p53, Ras, phosphatase and tensin analog (PTEN), and phosphatidylinositol 3-kinase (PI-3 kinase) (figure 1).

Results from recent investigations suggest that this new enlightenment will undoubtedly accelerate the development of targeted therapies to exploit enhanced tumor cell properties and/or unique tumor markers. Currently, there is substantial interest in the development of modulators of key molecules in signaling pathways that are dysregulated in cancer. This review will briefly discuss the existing knowledge about the contribution of genetic



**Figure 1.** Signaling pathways important to GBM development and potential therapeutic targets. EGF= Epidermal growth factor ; GF: Growth factors; PIP2= Phosphatidylinositol phosphate-2; PIP3= Phosphatidylinositol phosphate-3; S6K=p70 S6 kinase; mTOR= mammalian target of rapamycin; TF= Transcription factors; PKC=Protein kinase C; SOS= son of sevenless (Sos); Grb2 = growth factor receptor-bound protein-2.

alterations in GBM towards carcinogenesis, and their potential for yielding information about therapeutic targets, along with a brief discussion of a few drugs that are furthest along in their clinical evaluation.

### 3. GENETIC ALTERATIONS AND THERAPEUTIC IMPLICATIONS IN GLIOMAS

#### 3.1. Overview of genetic alterations in Glioblastoma multiforme

Multiple genetic abnormalities have been detected in human gliomas. Reported chromosomal alterations have included loss of heterozygosity (LOH) for 1p, 9p, 10, 13q, 17p, 19q and 22q, and gain of chromosome 7 (1-8). Many of the LOH events are directed at tumor suppressor genes known to encode proteins that play important roles in cell cycle regulation. These include p53 on 17p, and PTEN on 10q (figure 1). In addition to the losses of genetic material, amplifications of genes such as EGFR and MDM2 are common.

To investigate the frequency at which tumor suppressor genes are inactivated in malignant glioma, and thereby highlight the importance of such inactivations, Ishii *et al.* tested 34 human glioma cell lines for the presence of p53, P16<sup>INK4</sup>, p14<sup>ARF</sup>, and PTEN alterations. The results showed genetic inactivation of at least one of the genes in 91% of the cases examined. Sixty percent had at least 2 alterations of these genes, and 26% had alterations of all 4 genes (9). In another study, snap frozen glioma samples were studied for the levels of p53, pRb, PTEN, p14<sup>ARF</sup>, and

P16<sup>INK4</sup> tumor suppressor protein (10). Significant decreases in pRb were observed in 34% of the cases, P16<sup>INK4</sup> in 63%, p14<sup>ARF</sup> in 63%, PTEN in 18%, and a lack of wild type p53 was observed in 38%. Expression of EGFR, cyclin E, cdk4, and hTERT (catalytic subunit of human telomerase) was also examined, and for each, an association was observed between elevated expression and tumor aggressiveness, as well as with reduced survival. Overall, 89% of the malignant gliomas had deficiencies of one or more tumor suppressor genes effecting pRB function. The expression profiles of some of these proteins were found to be useful in distinguishing between GBM and non-GBM tumors, thereby suggesting a potential correlation with prognosis.

#### 3.2. Epidermal growth factor receptor (EGFR)

EGFR is a transmembrane tyrosine kinase. About 40% of GBMs and 15% of anaplastic astrocytomas feature amplifications of the chromosome 7 region that contains the gene encoding EGFR. The concurrent expression of EGFR and its ligands EGF and transforming growth factor-alpha (TGF-alpha) on glioma cell surfaces is suggestive of an autocrine or paracrine stimulatory loop (11-13) that is responsible for elevated EGFR signaling in tumors. In one recent analysis, EGFR amplification was found to have a prognostic significance in a subset of malignant astrocytoma patients (14).

Activation of EGFR is accompanied by activation of downstream kinases such as Map kinase, and this ultimately results in the stimulation of transcriptional

processes that accelerate cell division, mediate resistance to apoptosis, and promote tumor invasion as well as angiogenesis. It is interesting to note that EGF has been shown to inhibit the *in vitro* growth of two tumor cell lines with EGFR amplification: MDA-468(15) and A-431(16). For each of these the growth inhibition has been linked with the induction of p21/Waf1 expression(17,18), suggesting that in certain contexts, elevated EGFR signaling may promote p53-mediated transcription.

Amplification of the EGFR gene appears to be a late event in the development of malignant astrocytoma (19,20), and is commonly followed by gene rearrangements that result in deletions of certain portions of the encoded protein. The most common EGFR rearrangement seen in GBMs involves an in-frame deletion of exons 2-7, resulting in truncation of the extracellular domain of the receptor (EGFR $\Delta$ 6-273, delta EGFR or EGFRvIII). This deleted EGFR occurs in a large fraction of de-novo GBMs, but is not often observed in progressive (or secondary) tumors that generally lack p53 function. EGFR  $\Delta$ 6-273 has constitutive kinase activity, and is associated with enhanced tumorigenicity that is partly attributable to decreased apoptosis via Bcl-XL upregulation(19-21). In addition to EGFR  $\Delta$ 6-273, several other abnormal forms of EGFR have been identified, most of which have an abnormally augmented receptor activity.

The use of EGFR antagonists holds much promise for improved treatment of GBMs (22). Most of the anti-EGFR therapies are cytostatic in effect and, consequently, their toxicity is expected to be less than that associated with conventional chemotherapy. However, in combination with other therapeutic agents, as detailed below, their application in the treatment of GBM may prove to be more effective than as monotherapy.

EGFR inhibition has been accomplished by three approaches. One of these involves the use of monoclonal antibodies to target the extra cellular domain of EGFR, and thereby block ligand binding that stimulates receptor activity (23). C225, a chimeric monoclonal antibody targeting the ligand-binding epitope (24), as well as an antibody targeting a unique epitope created by the EGFRvIII mutation (25), are currently being investigated. A second approach to inhibiting EGFR relies on the use of antisense oligonucleotides that block EGFR synthesis (26). The third, and perhaps the most active area of EGFR-based therapeutic research, involves small molecule tyrosine kinase inhibitors (quinazolines or pyrazolo/pyrrolo/pyrido pyrimidines) that target the intracellular tyrosine kinase domain of EGFR and competitively inhibit ATP binding (27). ZD1839, OSI-774, CI-1033 and PKI-116 are agents among this class of compounds that are being investigated in the treatment of a variety of cancers, including GBM. The current clinical trials are supported by a substantial body of work involving the pre-clinical testing of small molecule EGFR inhibitors. For instance, in a xenograft model the growth rate of EGFR expressing glioma cells was found to be stimulated by the expression of ligand (TGF- $\alpha$ ), but this effect could be inhibited by a type 4-(3-chloroanilino)-6,7-dimethoxy-quinazoline EGFR-

specific tyrosine kinase inhibitor (28). EGFR tyrosine kinase inhibitors have also been shown to reduce the capacity of human glioma cells to infiltrate rat brain aggregates (29). Inhibitors with preferential activity against either the  $\Delta$ 6-273 or normal EGF receptor have been identified (30,31), and this finding will likely influence drug development and further design of clinical trials for GBM patients in which EGFR is a therapeutic target.

In addition to its stimulation of cell proliferation, current literature strongly suggests that EGFR activation mediates radiation resistance. Upon exposure to radiation, EGFR undergoes phosphorylation and activation as if stimulated by ligand binding (32-35), and this results in activation of downstream kinases that inhibit apoptotic response, such as Akt. Further support for the importance of EGFR to radiation resistance is indicated by studies in which the transfection of human glioma cell lines with dominant negative EGFR mutants causes radiosensitization (36-39). The expression of such inactive receptors completely abrogates radiation induced EGFR-phosphorylation, and concomitant growth assays after radiation demonstrate the radio-sensitizing effect of the inactive EGFR. The C225 monoclonal antibody against EGFR has been shown to radio-sensitize human and murine carcinoma cell lines both *in vitro* and *in vivo* after single and repeated radiation exposures (33, 40-43). The radiation sensitizing effect of the small molecule EGFR inhibitor, CI-1033 has been demonstrated in breast cancer cells where single doses of radiation in conjunction with this chemical results in a 23-fold decrease in clonogenic survival compared to radiation alone (44). When multiple fractions of radiation are used, concomitant exposure to CI-1033 leads to an even greater synergy and decreased clonogenic survival by 65-fold compared to radiation alone. In one recent analysis, the response of GBMs to radiation was found to correlate inversely with EGFR expression (45). Patients with GBMs that over-expressed EGFR had a response rate of only 9%, while those with no EGFR immunoreactivity had a response rate of 33%. Although the patients involved in this study were enrolled in different clinical trials with different therapies, the associated data are intriguing in that they suggests the existence of a relationship between a specific molecular defect and response to a particular therapy. Since gliomas commonly have abnormal EGFR activity, and are routinely treated with radiation, inhibition of EGFR activation by any of the methods discussed above may offer an attractive avenue of improving radiation response.

### 3.3. Platelet derived growth factor (PDGF)

PDGFs are homo- or hetero-dimeric proteins formed from 2 subunits, PDGF-A and PDGF-B. The specificity of binding for each of the 3 possible ligand dimers depends upon which of the 2 corresponding receptors is involved: the alpha-alpha receptor binds preferentially to PDGF-A, the beta-beta receptor binds preferentially to PDGF-B, and the affinity of the alpha-beta isoform is intermediate. Four genes encode the receptors and ligands, and their co-expression is common in many cell types and cancers, including gliomas.

Malignant astrocytomas, and to a lesser extent low-grade astrocytomas, express more PDGF-A and PDGF-B than non-neoplastic glial cells. PDGFA and PDGFB ligand genes are expressed in almost all glioma cell lines and in fresh surgical isolates of human malignant astrocytomas (46, 47). Established cell lines typically express a combination of PDGF and PDGF receptors that would conceivably form an autocrine loop. The establishment of these loops may be an early event in the pathogenesis of malignant astrocytoma (12, 48). In addition to their expression in tumor cells, high levels of the PDGF-beta receptor and PDGF-B chain are also found in hyperplastic capillaries that infiltrate gliomas (48).

There are multiple lines of experimental evidence pointing towards the importance of the PDGF pathway in gliomas. The growth of glioma cell lines is significantly enhanced by the introduction of PDGF to cell culture media (49). This response is consistent with the observation of enhanced growth of T98G human glioma cells following their genetic modification with an activated PDGF-B. These cells, which normally do not form tumors in nude mice, acquire tumorigenicity in association with activated PDGF-B gene transfer, suggesting that increased PDGF receptor activity enhances the malignant potential of the tumor cells (50). In a mouse model, the introduction and stable expression of PDGF-beta chain (using a murine retrovirus) has been found to lead to the development of monoclonal and oligoclonal brain tumors (51).

Approaching the importance of PDGF from the opposite direction in which the intent is to suppress receptor signaling, the transfection of dominant negative mutants of PDGF ligand has been shown to reverse the malignant phenotype in human glioma cell lines (52). In addition, the introduction of a truncated, inactive PDGF beta-receptor into rat glioma cells was determined to significantly reduce cell growth rate both *in vitro* and *in vivo* (53). Finally, the use of anti-PDGF agents such as anti-PDGF antibodies and suramin result in reduced glioma cell malignancy as manifested by diminished DNA synthesis, inhibition of tumor colony growth, and reversion of the transformed phenotype (54). A similar effect was determined with the administration of another PDGF antagonist, trapidil (55). The effectiveness of small-molecule inhibition of the PDGF receptor is in fact being examined in current clinical trials. Imatinib mesylate (Gleevec), an inhibitor of the 2-phenylaminopyrimidine class that can be taken orally, was developed to target the Abl oncoprotein and certain members of the subgroup III receptor tyrosine kinase family that includes PDGF receptors (56, 57). This drug is currently approved by the Food and Drug Administration (FDA) for treatment of chronic myeloid leukemia (CML). In addition, Imatinib mesylate has demonstrated activity on glioma cell cultures as well as in a nude mouse model of GBM (58), and is currently in phase I and II studies to evaluate its efficacy in GBM patients.

### 3.4. p53

p53 is a transcription factor encoded by a tumor suppressor gene that resides on chromosome 17p; a site of

frequent deletion in GBMs. p53 regulates the expression of a number of genes such as p21<sup>WAF1/CIP1</sup>, BAX, GADD45, and FAS/APO1, and is thereby an important regulator of various biological functions including cell cycling, apoptosis, differentiation, and angiogenesis (59-63). Mutations of p53 are common in cancers, and occur in up to 30% of astrocytomas. In addition, as much as 10% of GBMs and glioma cell lines have amplification of the murine double minute-2 gene (MDM-2) that suppresses p53 activity (64, 65). Thus, functional suppression of p53 plays a role in a substantial proportion of malignant astrocytomas. The importance of p53 function to the biology of many cancers, including glioblastoma, has been demonstrated in numerous studies. For instance, re-introduction of wild type p53 into tumor cells with defective (null or mutant) p53 has been shown to lead to cell cycle arrest and apoptosis (66). Wild type p53 has also been implicated as a suppressor of angiogenesis since its mutation promotes the neo-vascularization of tumors (67-69). Based on the results of investigations such as these, as well as the high incidence of p53 mutations in GBM, a phase I gene therapy study is currently underway to assess the safety of using an adenoviral-p53 construct for introducing exogenous wild-type p53 into the tumors of malignant glioma patients.

An additional consideration regarding loss of p53 function in GBM concerns the role p53 plays in guarding the integrity of the genome by coordinating a complex series of responses to DNA damage (70, 71). Not surprisingly, therefore, the status of the p53 gene correlates with tumor radio-responsiveness (70-73). Cells with wild type p53 are more radio-resistant than glioma cells transfected with mutant (inactive) p53. The basis of p53-associated radio-resistance has been attributed to effects on the G1 checkpoint, which result in a higher fraction of cells entering the radio resistant S phase (70). These results suggest that the status of p53 in a glioma can be used to guide the radiation fractionation schedule, and that manipulation of p53 function could potentially increase radio sensitivity in these tumors.

### 3.5. Ras

Ras is an important mitogenic enzyme whose activity is elevated in many cancers. This activity is upregulated by signaling from growth factor receptors such as EGFR and PDGFR. When EGFR and/or PDGFR are activated and undergo auto-phosphorylation, growth factor receptor-bound protein-2 (Grb2) and son of sevenless (Sos) proteins are recruited to the activated receptors to form a complex. This complex activates Ras by catalyzing its exchange of GDP for GTP. Activated Ras recruits Raf, a serine/threonine kinase, which in turn phosphorylates and activates Map-kinase kinase (MEK). MEK in turn phosphorylates and activates Map kinase; activated Map kinase translocates to the nucleus to phosphorylate various transcription factors whose activities promote cellular proliferation. In addition to its cell proliferation effects, aberrant Ras activity in tumor cells increases the expression of FasL, which promotes cytotoxicity in infiltrating T lymphocytes, thus suppressing a local immune response that may otherwise retard tumor growth (74, 75). Gliomas

express high levels of Fas ligand (FasL), and this expression has been shown to be responsive to changes in Ras activity (76).

Ras activation through corresponding gene mutation occurs in 30% of all cancers. Interestingly, however, mutations of Ras are rare in GBM. Nonetheless, elevated Ras activity is thought to be common in and perhaps obligate for GBM development. Whereas the basis of the elevated activity is not certain, it seems likely that aberrant growth factor receptor activity is a mitigating factor (77-79). Other GBM gene alterations, such as PTEN inactivation, probably influence Ras activity as well.

Various approaches have been taken to inhibit Ras activity in tumor cells. *In vitro* investigation has shown that blocking Ras activation by expression of a dominant-negative (inactive) Ras mutant decreases proliferation of human glioblastoma cell lines (79). Antisense oligonucleotides have been used to block Ras protein expression (80, 81), and inhibitors of key enzymes involved in Ras modifications have been used, including inhibitors of geranylgeranyl transferases (82-84), Raf kinase (80, 85-87), and farnesyl transferases (88). Most of the small molecule inhibitors have shown anti-cancer activity in pre-clinical studies, and phase I studies are planned or are underway to test some of these for safety in cancer patients. Farnesyl transferase inhibitors (FTIs) are farthest along in their development as anti-cancer agents and will be briefly discussed below.

Because Ras oncoproteins are latent when initially synthesized and require modification by addition of a farnesyl group, inhibition of farnesyl transferases has been shown to have a significant inhibitory effect on glioma cells *in vitro*. Treatment with the FTI, SCH-66336 inhibits the viability, anchorage independent cell growth, and cell cycling of glioma cell lines (89). Similarly, treatment of glioma cell lines with lovastatin, which also inhibits Ras farnesylation, has been shown to inhibit cell proliferation and migration (90). Guha *et al.* demonstrated that blocking Ras activation with FTIs, as well as by expression of the Ha-Ras-Asn17 dominant-negative mutant, decreases *in vitro* proliferation of human astrocytoma cell lines (79). Several FTIs are currently undergoing clinical evaluation in phase I and II trials (88) that include a Pediatric Brain Tumor Consortium (PBTC) phase I study of SCH-66336 in children with refractory or recurrent brain tumors.

Ras activity has also been implicated in tumor radiation resistance (91-93). Tumors with endogenous Ras activation are more resistant to radiation than their counterparts in whom Ras activity has been silenced (94-97). Inhibition of Ras activity leads to radio-sensitization of rodent cells transfected with activated Ras, and of human tumor cells bearing endogenous Ras mutations (96, 97). Lovastatin (96) and other farnesyl transferase inhibitors (94, 98), have been shown to radio-sensitize tumor cells.

### 3.6. Phosphatidylinositol 3-kinase (PI-3 kinase) pathway

Elevated signaling through the PI-3 kinase pathway has been implicated in the pathogenesis of GBMs

as well as in GBM resistance to therapy. PI-3 kinase transduces cellular signals from upstream growth factor receptors by generating second messengers phosphatidylinositol-3, 4-bisphosphates (PIP2) and phosphatidylinositol-3, 4, 5 trisphosphate (PIP3). The atypical kinase Akt and Protein kinase C (PKC) are downstream effectors of PI-3 kinases. Akt activates multiple signaling pathways that regulate processes such as cell cycling, transcription (via activation of mTOR and p70S6 kinase), angiogenesis, DNA repair, and apoptosis (via activation of BAD).

The deregulation of PI-3 kinase in tumors can occur through at least 3 mechanisms. Since PI-3 kinase is downstream of PDGFR and EGFR, the elevated expression and signaling of these receptor tyrosine kinases can increase PI-3 kinase activity. In addition to elevated growth factor receptor signaling, PI-3 kinase activity is increased through amplification and overexpression of its 110 Kd catalytic subunit in some cancers (99). Finally, the downstream effects of PI-3 kinase are elevated as a result of PTEN inactivation in GBM. PTEN is a dual specific phosphatase that works in opposition to the activity of PI-3 kinase by de-phosphorylating PIP2 and PIP3. *In vitro* studies have demonstrated that PTEN plays a role in cell migration, spreading and invasion (100, 101), since both integrin expression and focal adhesion formation are down regulated by wild-type, but not by inactive PTEN. In human GBM cells, the introduction of PTEN into PTEN-null cells leads to cell cycle arrest. PTEN expression also has been demonstrated to potently suppress human glioblastoma cell growth and tumorigenicity (102). Intracranial xenografts from tumor cells with reconstituted wild-type PTEN have been shown to exhibit decreased angiogenesis and significantly decreased growth and extends the life of host animals, as compared to animals harboring intracranial tumors from cells lacking PTEN (103).

The PTEN gene is located at chromosomal region 10q23.3. Loss of heterozygosity (LOH) of chromosome 10q, where PTEN is located, is one of the most common genetic defects noted in GBM, occurring in 70-90% of these tumors (3, 104-106). In addition to its deletion, PTEN is commonly inactivated by mutations in GBM (1). There is evidence suggesting that PTEN mutation is a marker for poor prognosis in glioma patients (107-109).

PTEN acts as a regulator of protein synthesis by modulating the activity of Akt and its downstream effectors such as mammalian target of rapamycin (mTOR), p70 S6 kinase, and 4EBP1 (110, 111) which functions as a translation repressor. Through its influence of these protein activities, PTEN function is important to the regulation of a number of cellular processes, including angiogenesis through hypoxia-inducible factor 1-associated inhibition of VEGF synthesis (112).

PI-3 kinase and its downstream effectors provide several potential targets for therapy. Among those currently being investigated are inhibitors of PI-3 kinase, protein kinase C (PKC), mTOR, and cyclin dependent kinases.

Inhibitors of protein kinase C such as calphostin, byrstatin and staurosporine are being examined (113), and have demonstrated *in vitro* activity against GBM. Inhibitors of mTOR such as rapamycin and CCI-779 have also demonstrated activity against GBM in preclinical studies, either alone or in combination with other agents (114, 115). These drugs have demonstrated safety in humans through recently conducted phase I trials, and are now being tested in patients with GBMs.

### 3.7. Drug combinations

In the near future, it will be possible to determine entire spectrums of molecular defects in individual tumors, and to tailor therapies based on collections of specific genetic alterations. In the current absence of the ability to determine exact tumor genotypes, as well as the absence of an ability to predict relationships between tumor genotypes and tumor response to therapy, it seems reasonable to base therapeutic strategies on the assumption that multiple pathways are de-regulated in most, if not all, tumors. Therefore, simultaneous, direct targeting of the pathways most often involved in human tumor development may, at this time, represent the optimal approach to cancer therapy. Many inhibitors for different cell signaling pathways are being developed and are undergoing clinical trials, and research is underway to test combinations of inhibitors in various cancer models. Results from a few, current studies suggest that certain combinations of novel agents with traditional chemotherapeutic agents may have synergistic anti-tumor effects (21, 116-123). Some of the studies in which multi-agent synergy has been observed will be discussed below.

Chakravarti *et al.* studied 2 glioma cell lines with equivalent upregulation of EGFR, but with differences in apoptotic response when EGFR signaling was inhibited. These cell lines did not differ in Map kinase activity, but the more resistant cell line was found to have increased levels of activated Akt and p70S6 kinase. The resistant cell line was found to have upregulated insulin-like growth factor receptor (IGFR) function, and inhibition of this receptor increased the sensitivity of the cell line to EGFR inhibition (121). Thus, differential expression of multiple growth factor receptors may account for some of the observed heterogeneity of response to a specific therapy. This finding supports the use of combinations of inhibitors to achieve optimal cell cycle inhibition and apoptosis.

In another study, the EGFR inhibitor CI-1033 was found to act synergistically when used in combination with a topoisomerase I inhibitor, SN-38 (a metabolite of Irinotecan/CPT 11), in a glioma cell line (123). The basis of this synergy was determined to be the inhibition of SN-38 efflux by CI-1033 resulting in higher intracellular levels and activity of SN-38. A phase I study based on this discovery is due to begin soon at our institution. Synergy between EGFR inhibitors and cisplatin has also been demonstrated in glioma cells. Inhibition of receptor signaling by the EGFR-specific tyrosine kinase inhibitor, AG1478, sensitized xenografts to the cytotoxic effects of CDDP, and the combined CDDP/AG1478 treatment significantly suppressed growth of subcutaneous xenografts

in nude mice relative to therapy with CDDP or AG1478 alone. The combined effect of these agents was determined to result from increased apoptosis as well as reduced cell proliferation (117).

In pre-clinical *in vitro* and *in vivo* (xenograft) testing using ovarian cancer cell lines, a synergistic pro-apoptotic effect was observed with the combined use of paclitaxel and LY294002, a PI-3 kinase inhibitor (122). Conversely, when active PI-3 kinase was transfected into the ovarian cancer cells, cellular resistance to apoptosis upon exposure to paclitaxel was significantly enhanced. Conceivably, this treatment would be applicable to other tumors where the PI-3 kinase pathway is upregulated, as it is in gliomas.

## 4. PERSPECTIVES

GBMs are heterogeneous tumors with multiple genetic and molecular defects resulting in the aberrant activity of several signaling pathways. The most commonly altered molecular activities are for EGFR, PDGFR, PI-3 kinase, Ras, PTEN and p53. Research performed over the last few years has demonstrated that in pre-clinical models, inhibition of these pathways can reverse the malignant phenotype and modulate treatment resistance in GBMs. One limitation to the ready application of these agents to CNS tumors concerns the general lack of information regarding their ability to cross the blood brain barrier. Some of the agents discussed are lipid soluble, and would be expected to distribute throughout the CNS. An alternative and utilitarian view regarding the importance of lipid solubility stems from the use of cisplatin and vincristine that do not readily cross the blood-brain barrier, but have shown activity against CNS tumors. Perhaps their effectiveness is due, in part, to local blood-brain barrier disruptions in GBM patients, which promote their CNS distribution in spite of low lipid solubility.

Many small molecule inhibitors of signaling effector proteins are currently in phase I clinical trials to test their safety in humans, and a few are in phase II trials to test efficacy. There are also human trials planned to test combinations of these agents for potential synergistic effects. At this time there is no data from which to predict which of the signaling effector proteins are most critical to the growth of GBM, and should therefore serve as the primary focus of novel therapeutic strategies. There is a literature base from which to support a key growth stimulatory effect of each genetic alteration discussed in this review, as well as for the associated signaling pathways that are affected. However, the encouraging results from recent clinical trials involving the targeting of proteins associated with specific gene alterations in breast cancer (HER-2/neu) and CML (BCR-ABL) suggest a conceptually similar approach should be adopted in the treatment of glioblastoma.

Future challenges in this field will be to develop an understanding of the role of these new agents in combination therapy, their proper sequencing or schedule of administration, and the determination of relationships

between tumor expression profile patterns and response to a specific agent or combination of agents.

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