

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# Glioblastoma: Current Chemotherapeutic Status and Need for New Targets and Approaches

Aditi Jain<sup>1</sup>, James CK Lai<sup>1,2</sup>, Golam MI Chowdhury<sup>3,4</sup>,  
Kevin Behar<sup>3,4</sup> and Alok Bhushan<sup>1,2</sup>

<sup>1</sup>Department of Biomedical and Pharmaceutical Sciences  
College of Pharmacy and ISU Biomedical Institute, Idaho State University

<sup>2</sup>Mountain States Tumor and Medical Research Institute, Boise, ID

<sup>3</sup>Magnetic Resonance Research Center

<sup>4</sup>Department of Psychiatry, Yale University School of Medicine, New Haven, CT  
USA

## 1. Introduction

Glioblastoma is the most aggressive, invasive and malignant type of glioma : a tumor which arises from the glial cells in the brain (Wrensch et al., 2002). Glioblastoma represents 54% of all the gliomas and 18% of all brain tumors (CBTRUS, 2011). It is the frequently occurring brain tumor in humans. Patients with glioblastoma usually survive 12-15 months or less after diagnosis. (Krex et al., 2007; Chandana et al., 2008).

Because prognosis for patients with glioblastoma is abysmal and current therapies for the disease are ineffective, this review critically evaluates the current chemotherapeutic status of glioblastoma and highlights the need for new therapeutic targets and approaches. The ultimate goal is to improve the outcome of glioblastoma patients.

## 2. Glioblastoma biology

The incidence of glioblastoma peaks with increasing age and affects adults of ages 50 to 70 (Wrensch et al., 2002). Multiple genetic mutations, upregulation or amplification of genes contribute to glioblastoma carcinogenesis. Depending on the aberrant signaling pathways involved, glioblastoma can develop either as a primary or a secondary tumor (Kleihues & Ohgaki , 1999; Ohgaki & Kleihues, 2007). Primary glioblastoma (representing >60% of all glioblastoma cases) frequently occurs in adults/older patients as a single step transformation with no clinical background. Genetic changes in primary glioblastoma include Epidermal Growth Factor Receptor overexpression (50-60%), mutations in PTEN (30%) and amplification of mdm2 gene. Secondary glioblastoma arises from a slowly progressing low grade astrocytoma or anaplastic astrocytoma to a malignant glioblastoma and affects the young population. Secondary glioblastoma patients survive longer than those with primary glioblastoma multiforme: genetic hallmark of the former group includes Tp53 inactivation (>60%) and overexpression of PDGF ligand and/or receptor. Primary and secondary glioblastomas share similar morphologies, rendering them indistinguishable (Kleihues & Ohgaki , 1999; Ohgaki & Kleihues, 2007).

### 3. Current therapies

#### 3.1 Carmustine

Carmustine (BCNU) was the first tested and approved drug for treating glioblastoma: it showed modest improvement in patient survival in 1960s (Levin, 1999). Belonging to the class of nitrosoureas, it alkylates the O6-guanine position in the DNA and cross-links the DNA, thereby inhibiting cancer growth (Reithmeier et al., 2010). Carmustine is a lipophilic drug and crosses the blood-brain barrier (Louis et al, 2007). After being approved by FDA in 1977, it has been the mainstay of adjuvant therapy for glioblastoma (Salvati et al., 2009). When glioblastoma patients were treated with carmustine after radiation therapy, their survival improved (Brandes et al., 2004). Many still ongoing preclinical and clinical studies have also tested carmustine in combination with other chemotherapeutic drugs (Silvani et al., 2009; Reardon et al., 2004). In 2009, a study comparing temozolomide and carmustine in glioblastoma indicated that carmustine was more toxic than temozolomide alone though both had comparable efficacy (Vinjamuri et al., 2009). Combination therapies with receptor tyrosine kinase inhibitors like genistein, tryphostin and carmustine were synergistic (Liang & Ulliyatt 1998; Khoshyomn et al., 2002). Despite its use as a mainstay therapy, the survival rates of patients on carmustine alone are quite low. Short half-life, chemo-resistance, high systemic toxicity, and difficult delivery to target site, limits carmustine's effectiveness in treating glioblastoma (Johannessen et al., 2008).

#### 3.2 Gliadel wafers

Gliadel wafers are biodegradable polymers loaded with carmustine, approved for treating recurrent glioblastoma (Panigrahi et al., 2011; Aoki et al., 2007). After glioblastoma is surgically removed, the wafers are implanted in its cavity. As the polymer is degraded, it releases the drug slowly (Panigrahi et al., 2011). In 1995, gliadel wafers showed promises because of their local action and high doses of loaded drug delivered to the target site with few off-target effects (Brem et al., 1995). Gliadel-treated patients showed median survival of 7.2 months compared to 5.4 months in the placebo group. In 2003, a randomized, placebo-controlled, multicenter, multi-national, double-blind phase 3 trial demonstrated a higher survival to risk ratio in glioblastoma patients treated with gliadel wafers, with a median survival rate of 13.9 months compared to 11.6 months in placebo controls (Westphal et al., 2003). An ongoing study adopts a multimodality approach of using gliadel wafers with other chemotherapies in glioma patients who have undergone surgery (McGirt et al., 2009). Gliadel wafer with temozolomide increased the median survival of patients with newly diagnosed glioblastoma to 21 months (McGirt et al., 2009). Although beneficial, the use of gliadel wafers for intracranial treatment of glioblastoma is complicated by edema, seizures, post-operative infections, and hydrocephalus (Gallego et al., 2007; Weber & Goebel, 2005): the complications versus benefits merits re-evaluation.

#### 3.3 Cisplatin

In 1965, Barnett Rosenberg *et al* discovered inhibition of *E. coli* cell division by a platinum compound (i.e., cisplatin) formed in electrolysis of platinum electrodes (Rosenberg et al, 1965). Cisplatin was soon employed as an anticancer agent (Rosenberg et al, 1969; Williams, J.M.A Whitehouse, 1979). Cisplatin is a platinum complex with two chloride atoms and two amine groups positioned in a *cis* configuration. Once inside the body, the two chloride atoms are displaced by water molecules, the resulting hydrated complex crosslinks with

DNA strands, triggering programmed cell death (C.J. Williams, J.M.A Whitehouse, 1979). In 1980's cisplatin's efficacy on brain tumor was evaluated (Stewart et al, 1982). Although combination therapy of cisplatin with carmustine and radiation therapy was successful, patients on cisplatin therapy (with or without carmustine) developed ocular and orbital toxicities (Miller et al., 1985). Numerous other clinical trials evaluating the effectiveness of cisplatin chemotherapy in glioblastoma have demonstrated few benefits. Combination of cisplatin with temozolomide, etoposide, thalidomide, or tyrosine kinase inhibitors as a treatment option for glioblastoma have also been studied but have not significantly increased patient survival (Silvani et al., 2004; Lassen et al., 1999; Murphy et al., 2007; Nagane et al., 2007). Additionally, a phase three trial of cisplatin and carmustine administered concurrently with radiation therapy did not have significant improved outcome as compared to carmustine alone and radiation therapy (Buckner et al., 2006).

### 3.4 Temodar

A drug of interest since 1990, temozolomide constitutes the first line chemotherapy for treating glioblastoma following surgery and radiation (Villano et al., 2009). It is an oral alkylating agent that adds a methyl group to the O6 position of guanine residue of the DNA: the resulting methylated adduct induces apoptosis (Villano et al., 2009; Malcolm et al., 2002; Roos et al., 2007). Being lipid soluble, temozolomide shows good bioavailability and readily crosses the blood-brain barrier. In 2005, a breakthrough study demonstrated a regimen of radiation therapy followed by adjuvant and concomitant temozolomide treatment prolonged survival of glioblastoma patients compared to patients treated with radiation alone (Stupp et al., 2005). Since then it has become a standard therapy for glioblastoma with or without modifications. Combination chemotherapies of temozolomide and other drugs are in various phases of clinical trials but none showed benefits compared to temozolomide treatment alone (Ren et al., 2009). One of the major drawbacks of temozolomide therapy is recurrence of glioblastoma. The lesion produced in the DNA by temozolomide could be corrected by the repair enzyme O6 methyl guanine DNA methyl transferase, encoded by the MGMT gene (Sarkaria et al., 2008). Response rates to temozolomide therapy depend on the transferase activity and cancers with high levels of MGMT activity gradually acquire resistance to temozolomide (Nagane et al., 2007). The promoter methylation status of MGMT governs a drug's efficacy towards the tumor. Patients with an increased % of MGMT methylation respond more favorably to temozolomide treatment (Hegi et al., 2008). Drugs mimicking this enzyme are being tested in combination with temozolomide to prevent MGMT actions. One such drug is O6 benzylguanine, which acts as a pseudo-substrate to MGMT enzyme (Kaina et al., 2010; Dolan & Pegg, 1997). However, due to its dose-related hematologic toxicity, its use in combination with alkylating agents is still under investigation (Dolan & Pegg, 1997; Quinn et al., 2009). A recent study showed that patients with unmethylated MGMT tumors benefitted from the combination of interferon  $\beta$  and temozolomide, as compared to patients who received temozolomide alone, highlighting the role of interferon  $\beta$  and also suggesting that methylation status of MGMT is not a sole parameter controlling treatment outcome (Motomura et al., 2011). Tumor resistance to temozolomide as with other alkylating agents is a common phenomenon seen in patients with glioblastoma. Apart from MGMT, many studies have shown that resistance to temozolomide is multifactorial (Sarkaria et al., 2008). Strategies to overcome this resistance

have been analyzed in order to improve temozolomide's activity against glioma and other cancers (Sarkaria et al., 2008; Tentori & Graziani, 2002). With greater understanding of diverse mechanisms responsible for imparting resistance to cancer cells and the role played by growth factor receptors in glioblastoma tumorigenesis, new therapies in combination with temozolomide are being evaluated. Such combination therapies include use of protein tyrosine/serine-threonine kinase inhibitors and temozolomide (Chaponis et al., 2011; Guillard et al., 2009; Peereboom et al., 2010). Though some studies demonstrated beneficial effects, others have reported greater toxicity. Thus, as a promising drug for treating glioblastoma, temozolomide only modestly enhances patient survival. There is a need to find other small molecules with efficacy and safety profiles superior to those of current therapies to be targeted to glioblastoma therapy.

## 4. Potential drug targets and new therapies

### 4.1 Epidermal growth factor receptor (EGFR) and cellular signaling pathways

EGFR plays a critical role in cancer progression and invasion. Present in all germ layers, EGFR is activated by several ligands including EGF. Binding of EGF results in homodimerization and/or heterodimerization with other members of the EGFR family (HER2, HER3 and HER4), leading to tyrosine phosphorylation of its cytoplasmic domain. This activation initiates a series of signaling pathways resulting in cell division. In cancer cells, mutations or amplification of EGFR leads to uncontrolled cell proliferation (Yarden, 2001). Activation of EGFR leads to activation of downstream elements such as phosphatidylinositol-3 kinase (PI3K), which converts phosphatidylinositol diphosphate (PIP<sub>2</sub>) to phosphatidylinositol triphosphate (PIP<sub>3</sub>). Protein kinase B or AKT binds the PIP<sub>3</sub> with the pleckstrin homology (PH) domain, resulting in phosphorylation of AKT at threonine 308 and serine 473 sites. Phosphorylated AKT leads to cell growth, motility and proliferation by activating several downstream signaling pathways (Chakravarti et al., 2004).

PTEN/MMAC/TEP-1, (Phosphatase and tensin homologue deleted on chromosome ten/mutated in multiple advanced cancer/Transforming growth factor  $\beta$  regulated and epithelial cell enriched phosphatase) a tumor suppressor gene located on chromosome 10 is mutated in gliomas. PTEN dephosphorylates PIP<sub>3</sub> to PIP<sub>2</sub> in the PI3 kinase pathway and acts as a negative regulator of this pathway (Cheng & Nicosia, 2001). Continuous activation of PI3K/Akt pathway is associated with malignant transformation of cells. Activation of PI3K pathway and mutations in PTEN occur frequently in GBM tumors when compared to non-GBM tumors (Chakravarti et al., 2004). Mammalian target of rapamycin (mTOR) is a serine/threonine kinase known to function downstream of PI3K/Akt pathway. Akt activates mTOR through inhibition of TSC1/2 complex and activation of Ras homologue-enriched in brain (Rheb). mTOR regulates translation initiation through two pathways - eukaryotic translation initiation factor (eIF4E) binding proteins (4EBP1) and ribosomal p70 S6 kinase (p70S6K) (Hay & Sonenberg, 2004). Inhibition of signaling pathways generated by activation of EGFR are important targets to develop new drugs for the treatment of glioblastoma because EGFR is amplified and overexpressed in such tumors (Lo, 2010; Penar et al., 1997).

### 4.2 PLC $\gamma$ 1

Phospholipase C gamma one (PLC $\gamma$ 1) is an enzyme important in cell signaling. In its phosphorylated active form, it cleaves the membrane phospholipid, phosphatidylinositol 4,



5 biphosphate (PIP<sub>2</sub>) to diacylglycerol (DAG) and inositol triphosphate (IP<sub>3</sub>). IP<sub>3</sub> increases intracellular calcium stores. DAG together with calcium activates protein kinase C which further participates in signal transduction pathways (Kim et al., 2000). Growth factor receptor activation is an important mediator of PLC $\gamma$ 1 phosphorylation and functioning. Overexpression of epidermal growth factor receptors (EGFR) in glioblastoma multiforme (GBM) promotes PLC $\gamma$ 1 activation (Chen et al., 1994; Yang et al., 1994; Wahl et al., 1992). Many functions attributed to PLC $\gamma$ 1 activation include proliferation, differentiation, cell motility and invasion of tumor cells (Jones et al., 2005; Wells & Grandis, 2003). Furthermore, PLC $\gamma$ 1 is associated with breast cancer metastasis and is highly expressed in breast cancer tissues (Arteaga et al., 1991). Activation of PLC $\gamma$ 1 by tyrosine kinase receptors mediates actin cytoskeleton rearrangement, through release of actin modifying proteins gelsolin, profilin, myosin type I (Chen et al., 1996). In the resting state, these proteins are bound to PIP<sub>2</sub> and participate in cell motility only when they are released during hydrolysis of PIP<sub>2</sub> by PLC $\gamma$ 1. Thus, this mechanism defines the role played by the enzyme in cell motility and invasion (Chen et al., 1996). Molecular inhibition of PLC $\gamma$ 1 is associated with decreased invasion in gliomas, prostate, breast and bladder carcinomas (Turner et al., 1997; Katterle et al., 2004; Khoshyomn et al., 1999). A chemical inhibitor of PLC $\gamma$ 1, U73122 inhibits invasion of glioblastoma cells into co-cultured fetal rat brain aggregates (Khoshyomn et al., 1999). During invasion, tumor cells undergo a series of steps to proliferate and thrive locally (Tysnes & Mahesparan, 2001). Thus, these functional considerations of the role of PLC $\gamma$ 1 in glioblastoma proliferation and invasion strongly suggest PLC $\gamma$ 1 could be a drug target.

#### 4.4 Invasion

The characteristic feature of glioblastoma, which makes it a lethal disease, is its propensity to invade surrounding normal brain tissues (Giese et al., 2003). Unlike other cancers, glioblastoma does not metastasize to distinct organs but infiltrates peritumoral regions (Giese et al., 2003; Nakada et al., 2007). Glioblastoma patients show poor survival because of the inability of current therapies to control the aggressively invasive glioblastoma, which is spurred on by autocrine and paracrine factors released from the tumor and its microenvironment, respectively (Hoelzinger et al., 2007). Overexpression and activity of the epidermal growth factor receptors in almost all glioblastomas is responsible for signaling pathways leading to invasion (Guillamo et al., 2009). These signals help the cancer cells to detach from the bulk of the tumor and adhere to the extracellular matrix components of other cells. The latter process follows only when the tumor invades through the matrix to the surrounding brain parenchyma. Thus, during invasion, a family of proteases known as the matrix metalloproteases and urokinase plasminogen activator receptor system play an active role (VanMeter et al., 2001; Mohanam et al., 2001). Being soluble zinc-dependent enzymes and secreted as inactive zymogens (pro-MMPs) (Visee & Nagase, 2003), matrix metalloproteases (MMPs) cleave the basement membrane and extracellular matrix components like collagen, fibronectin, laminin, and vitronectin. They help in tissue remodeling, angiogenesis, and metastasis (VanMeter et al., 2001). Out of the 24 members in the MMP family, only two forms are overexpressed in glioblastoma and their expression correlates with the degree of malignancy and poor survival rate (Deryugina et al., 1997; Choe et al., 2002). These two forms are MMP-2 (72 kDa type IV collagenase or gelatinase A) and MMP-9 (92 kDa type IV collagenase or gelatinase B) and differ from other members in their substrate preference. Some metalloproteases are not secreted but are expressed on the

surface of tumor cells. MT1-MMP (membrane bound matrix metalloprotease-1) is one such protease which is upregulated in glioblastomas. A major role of MT1-MMP is in the cleavage of pro-MMPs to active MMPs at the cell surface. Together with MMP-2, and MMP-9, MT1-MMP imparts an invasive phenotype to glioblastoma multiforme (Fillmore et al., 2001; Nakada et al., 2001). Urokinase plasminogen activator and receptor (uPA/uPAR) system is important in cancer cell migration and invasion (D'Alessio & Blasi, 2009; Duffy, 2004). Once this system is activated, it converts the inactive plasminogen to the active plasmin. Plasmin degrades the extracellular matrix components (D'Alessio & Blasi, 2009). Increased expression of uPAR on the surface of glioblastoma correlates with poor prognosis. In contrast with normal brain tissue, high grade gliomas (i.e., glioblastoma) exhibit increased activity of the uPA/uPAR system (Mohanam et al., 1998; MacDonald et al., 1998). This system can also activate cell proliferation pathways by interacting with other proteins like vitronectin and integrins (Sidenius & Blasi, 2003). Many studies and clinical trials are now focusing on anti-invasive chemotherapies as a treatment option for glioblastoma. Apart from synthetic derivatives, natural compounds in soy, curcumin can also inhibit glioblastoma invasion *in vitro* (Puli et al., 2006; Senft et al., 2010). Their activity *in vivo* warrants further research. Since a vast signaling network is aberrant in glioblastoma, therapies directed toward a single target cannot be expected to lead to positive outcomes. For example, one clinical trial showed marimastat to be ineffective in increasing survival of patients post-radiosurgery (Levin et al., 2006). Consequently, combination therapies targeting invasion and other pathways in glioblastoma are still ongoing.

#### 4.5 Angiogenesis

Angiogenesis, the process of formation of new blood vessels from existing blood capillaries, is one major contributor to glioblastoma multiforme carcinogenesis and helps the tumor cells to flourish (Tate & Aghi, 2009). To maintain the demand of food, nutrients, and oxygen, tumor cells recruit new blood vessels from those already present (Folkman, 1971; Tate & Aghi, 2009). Likewise, malignant gliomas are highly vascularised and have an angiogenic phenotype (Jain et al., 2007). Angiogenesis takes place with the over-expression of angiogenic factors or when the angiogenic imbalance strikes. One growth factor involved is vascular endothelial growth factor (VEGF), which promotes formation of endothelium in normal cells (Kargiotis et al., 2006). In glioblastoma, VEGF-a, a pro-angiogenic factor from the VEGF family, plays a crucial role in angiogenesis. VEGF-a is secreted in large amounts by glioblastoma cells and can elicit responses like extracellular matrix degradation, endothelial cell proliferation, and expression of other angiogenic factors such as urokinase type plasminogen activator, plasminogen activator inhibitor-1 and matrix metalloproteases (Plate et al., 1992). Secreted VEGF stimulates angiogenesis by binding to specific tyrosine kinase VEGF receptors on endothelial cells of blood vessels surrounding the tumor and initiates proliferation of endothelial cells, thereby ensuring the metabolic demands of the growing tumor are adequately met. There are other pro-angiogenic factors like angiopoietins, IL-8, hepatocyte growth factors, endothelins that are expressed by glioblastoma cells when the “angiogenic switch” is turned on: all these factors have functions similar to that of VEGF in promoting angiogenesis (Argyriou et al., 2009). Some new treatment strategies for GBM include targeting VEGF/VEGF receptors (VEGFR) by monoclonal antibody or VEGFR traps, respectively (Beal et al., 2011). Approved by FDA in 2009 for treating recurrent glioblastoma, bevacizumab (Avastin) is a humanized monoclonal

antibody against VEGF-a (Beal et al., 2011). Bevacizumab decreases tumor blood vessel density and remodels tumor vasculature in a neuroblastoma xenograft model (Dickson et al., 2007). The National Cancer Comprehensive Network recommends bevacizumab with or without other chemotherapy in case of glioblastoma recurrence (National Comprehensive Cancer Network clinical practice guidelines in oncology-central nervous system cancers. v.1.2010). Combination chemotherapy of bevacizumab with irinotecan shows favorable results in phase 2 trials of recurrent malignant glioma (Vredenburgh et al., 2007). Clinical trials are on-going for using bevacizumab in new cases of glioblastoma (Lai et al., 2010). Inclusion of anti-angiogenic therapies to cancer treatment is favorable, because they facilitate increased penetration of conventional chemotherapies and show better response rates (Jain, 2001). Other than bevacizumab, anti-angiogenic agents in various phases of clinical trials include cediranib, cilengitide, and aflibercept (Batchelor et al., 2010; Reardon et al., 2008; Wachsberger et al., 2007). Though favorable responses and anti-tumor effects occur with combination of various anti-angiogenic agents in different cancer models, efficacy of bevacizumab monotherapy in increasing glioblastoma patient survival has not transpired. Emergence of an invasive phenotype while angiogenesis is being targeted in glioblastoma constitutes a major limitation of anti-angiogenic monotherapies (Lamszus et al., 2003; Verhoeff et al., 2009; Keunen et al., 2011). Thus, improved therapies need to be developed to target glioblastoma.

#### 4.6 Metabolism

**Metabolic and other functional roles of astrocytes under physiological conditions:** In mammalian nervous system, neurons and astrocytes are intimately and functionally interrelated: astrocytes play key roles in neurotransmitter and substrate cycling in conjunction with neurons (Chowdhury et al., 2007; Hertz et al., 2007). Moreover, astrocytes protect neurons against various pathophysiological assaults (e.g., oxidative stress, ammonia toxicity) (Dukhande et al., 2006; Wong et al., 2010). However, to what extent these physiological roles are still assumed by astrocytes once they are transformed into astrocytomas has not been elucidated. Nevertheless, recent new evidence suggests that cancer cells, and to a lesser known extent astrocytomas too, exhibit metabolic adaptation and other phenotypic alterations that allow them to survive, proliferate, and invade into their surrounding space occupied by normal cells/tissues (Bhardwaj et al., 2010; Lino & Merlo, 2009; Ordys et al., 2010; Semenza, 2011; Stegh et al., 2008).

**Metabolic adaptation and/or reprogramming in cancer cells in general and astrocytoma/glioblastoma in particular:** As early as the 1920's, Otto Warburg and his associates were the first to note that cancer cells appear to depend on glycolysis for energy production and survival even though oxygen is not in short supply. (Warburg et al., 1927). Warburg's extensive investigation into the metabolic characteristics of multiple types of cancer cells prompted him to hypothesize that cancer cells rely on glycolysis for energy supply because their mitochondrial oxidative metabolism is dysfunctional (Warburg, 1956). His hypothesis has been neglected for some 80 years until the recent resurgence of interests in "the role metabolic reprogramming in cancer progression" (Semenza, 2011). The recent "renaissance of the Warburg Hypothesis" (Warburg et al., 1927, Warburg, 1956) has stimulated a new era in elucidating the aggressive nature of many malignant tumors (including glioblastoma) and their purported dependence on glycolysis for energy and survival (Bhardwaj et al., 2010; Lino & Merlo, 2009; Ordys et al., 2010; Semenza, 2011; Stegh



et al., 2008). While numerous studies have documented mutations in mitochondrial DNA (mtDNA) in a variety of cancers (Czarnecka & Bartnik, 2009) can thus tentatively provide a mechanistic connection to dysfunctional mitochondrial oxidative metabolism as predicted by the Warburg hypothesis, other recent evidence suggests this aspect of the Warburg hypothesis requires critical re-appraisal (Bayley & Devilee, 2010; Dang et al., 2011; Frezza et al., 2011; Ordys et al., 2010; Srivastava & Moraes, 2009).

The aspect of the Warburg hypothesis emphasizing dysfunctional mitochondrial oxidative metabolism in cancer cells deserves a critical re-appraisal because recent studies on cancer cell metabolism have uncovered new mechanistic roles for mTOR and p53. These mechanistic roles are new additions to the already established role of mTOR in tumor development and progression and the fact that in over 50% of the cancer types, p53, a tumor-suppressor, is mutated and their p53 mutation is associated with either decreased apoptosis and/or enhanced proliferation potential in those cancers. Earlier, we have already discussed the importance of these two phenotypic characteristics of glioblastoma.

mTOR complex 1 (mTORC1) is aberrantly activated in many human cancers thereby positioning it to modulate on metabolic changes common to cancer cells (Yecies & Manning, 2011). Furthermore, recent characterization of the metabolism of cancer cells reveals that, mTORC1 activation is adequate to promote an increase in glucose uptake, glycolysis, and lipid synthesis in addition to the pentose phosphate shunt pathway (Ramanathan & Schreiber, 2009; Yecies & Manning, 2011). Because these are all metabolic phenotypes of cancers, mTORC1 has emerged as a central relay for various oncogenic signaling pathways and their convergence in regulating metabolism in cancer cells (Yecies & Manning, 2011). The finding that mTOR functions as a positive regulator of hypoxia-inducible factor 1 (HIF-1) activation by hypoxia (Hudson et al., 2002) highlights the importance of mTOR in regulating signals that lead mammalian cells, especially cancer cells, to adapt to oxygen- and nutrient-poor environments. Furthermore, mTOR is known to exert a direct control of mitochondria (Ramanathan & Schreiber, 2009). Thus, these new mechanistic roles of mTOR strengthen the notion we have discussed earlier that mTOR exhibits good potential as a target for new anti-cancer drug discovery. There has been increasing evidence demonstrating that p53 can regulate multiple metabolic pathways. p53 contributes to the regulation of glycolysis, oxidative phosphorylation, glutamine catabolism, synthesis of nucleotides, fatty acid oxidation, insulin sensitivity, mitochondrial integrity, antioxidant response, autophagy, and mTOR signaling (Maddocks & Vousden, 2011). Inactivation of p53 in cancer cells promotes the Warburg effect as p53 suppresses glycolysis and promotes oxidative phosphorylation. However, there are some known but complex cross-talks between the signaling pathways regulated by mTOR and p53 (Maddocks & Vousden, 2011): nevertheless, whether the interactions between mTOR and p53 signaling favor glioblastoma cell growth and proliferation remains to be fully elucidated.

Some recent cancer cell metabolism studies have further challenged the reliance of cancer cells on glycolysis for energy production and lipid synthesis: in fact, such studies have argued that glycolysis alone is inadequate to maintain the metabolic needs of growing and actively dividing cancer cells (Dang et al., 2011; Maddocks & Vousden, 2011; Shanware et al., 2011). Thus, many researchers have re-discovered the importance of glutamine in meeting the inadequacy of glycolysis to fuel growth and proliferation of many cancer types including gliomas (Dang et al., 2011; Maddocks & Vousden, 2011; Shanware et al., 2011). In this context, as alluded to above, neuroscientists have long recognized the importance of glutamine in astrocytic metabolism and astrocytes-neurons metabolic and neurotransmitter

cycling (Chowdhury et al., 2007; McKenna, 2007). Consequently, these recent interests in the role of glutamine in cancer cell metabolism call into question the need to better appreciate the metabolic and neurotransmitter cycling roles of glutamine in glioblastomas. Because these roles in glioblastomas are poorly understood, these knowledge gaps prompted us to consider the appropriate techniques and approaches to allow the acquisition of this knowledge. Indeed, the recent rapid advances in magnetic resonance imaging (MRI) and nuclear magnetic resonance (NMR) spectroscopy provide exciting new possibilities in this area of research and development.

**Technological advances that can be exploited to diagnose and/or treat glioblastoma:** MRI and other imaging techniques in diagnosing glioblastoma: Recent advances in magnetic resonance imaging (MRI) and related imaging techniques have opened new possibilities in the differential and more accurate clinical assessment of brain tumors including glioblastomas (Cha, 2009; Lemort et al., 2007; Wang & Lam, 2008). Historically, uses of MRI in the diagnosis of brain tumors were initially focused on neuromorphological demonstration, confirmation, and localization of brain tumors. However, the rapid advances of MRI, functional MRI, and magnetic resonance spectroscopy (MRS) spectroscopy in the last decade have allowed the diagnostic imaging of neurotumors combining the use of physiology-based imaging methods that complement the more traditional morphology-based imaging protocols. For example, “High-resolution spectroscopic imaging may contribute to pre-therapeutic grading and characterization of gliomas, as can diffusion techniques. The latter also hold promise in predicting survival in malignant supratentorial astrocytoma and could help to define areas for biopsy. Both methods can differentiate recurrent tumour from radiation injury. Perfusion-weighted magnetic resonance techniques offer potential markers of tumour angiogenesis and capillary permeability, and correlate well with vascular endothelial growth factor expression in grade II and grade III tumours. Functional magnetic resonance imaging can assess whether surgical treatment is feasible and select patients for intraoperative cortical stimulation” (Lemont et al., 2007).

Recently, use of the nanoparticles in the diagnosis and detection of cancers, including glioblastomas, has gained much impetus because of putative enhancement involving their applications in MRI (Bhushan et al., 2010; Cole et al., 2011). The advances in development of newer cancer imaging and therapies based on metallic nanoparticles may help in early detection of cancer and thus contribute to decreasing deaths due to cancer. Various cancer imaging and therapies based on use of metallic nanoparticles are at different stages of preclinical and clinical development. Nanoparticles composed of iron oxide nanoparticles, zinc oxide nanoparticles, gold nanoparticles, silver nanoparticles, and cerium oxide nanoparticles have tremendous potentials to be developed as novel diagnostic and therapeutic agents in cancer (Bhushan et al., 2010; Jain 2010). Furthermore, enhanced cancer biomarker and genetic mutation detection techniques would help in identifying individuals at high risk for developing cancer. In this context, multi-functional metallic nanoparticles show exciting therapeutic potentials and these are currently under development for cancer therapy to be clinically applied in the near future. Metallic nanoparticles can be engineered to enhance the efficacy of current diagnostic and imaging techniques in cancer (Bhushan et al., 2010). Clearly, with the explosive advances in the design and applications of new metallic nanoparticles, this application area in cancer diagnosis and assessment shows exciting new potentials.

**Technological advances that can be exploited to treat glioblastoma:** As already alluded to above, the recent “renaissance of the Warburg Hypothesis” has stimulated much research into cancer cell metabolism (Bhardwaj et al., 2010; Lino & Merlo, 2009; Ordys et al., 2010; Semenza, 2011; Stegh et al., 2008). Indeed, the presumed dependence of cancer cells on glycolysis for cancer cell growth and proliferation has prompted much new investigation into exploring the glycolytic pathway as a new target for anti-cancer drug discovery (Bhardwaj et al., 2010; Chatterji et al., 2007; Chatterji et al., 2009; Lai et al., 2008; Lino & Merlo, 2009). We have demonstrated that two glycolytic enzyme inhibitors, 3-bromopyruvate and iodoacetate, showed efficacy in lowering the survival of pancreatic cancer (Bhardwaj et al., 2010) and glioblastoma U87 (Chatterji et al., 2007; Chatterji et al., 2009; Lai et al., 2008) cells. Thus, our studies strongly suggest that glycolytic enzyme inhibitors exhibit proof-of-concept potential in discovering new anti-cancer drugs (Bhardwaj et al., 2010; Chatterji et al., 2007; Chatterji et al., 2009; Lai et al., 2008). Consistent with our findings (Bhardwaj et al., 2010; Chatterji et al., 2007; Chatterji et al., 2009; Lai et al., 2008) are the results of human clinical trials employing 2-deoxyglucose (2-DG) in combination therapy with radiation for treatment of glioma (Prasanna et al., 2009). An inhibitor of glucose transport and glycolysis, 2-DG reportedly enhances the effects of radiation in inducing glioma cell death (Prasanna et al., 2009). Thus, the glycolytic pathway in glioblastoma constitutes an excellent target for further anti-cancer drug discovery studies. Nevertheless, because the prognosis of patients diagnosed with glioblastoma is abysmal, there is an urgent need to more fully elucidate the metabolic phenotype of glioblastomas along with exploring glycolysis as a target for discovering new anti-cancer drugs.

**Magnetic resonance spectroscopy to elucidate metabolic adaptations/alterations in glioblastoma:** High lactate accumulation, despite adequate oxygen availability, is a metabolic pattern commonly associated with malignant transformation of the uncontrolled dividing cell. This metabolic phenotype, termed aerobic glycolysis and historically known as the Warburg effect, is characterized by high glycolytic rates and reduced mitochondrial oxidation (Bhardwaj et al., 2010; Lino & Merlo, 2009; Ordys et al., 2010; Semenza, 2011; Stegh et al., 2008), features that favor cell survival in the hypoxic microenvironments found in tumors. This phenotype also favors the routing of key metabolic intermediates away from oxidative metabolism and toward anabolic processes required by rapidly dividing cells (McFate et al., 2008). However, the mechanistic relationship between altered glucose metabolism and malignancy remains poorly understood due to prior lack of appropriate techniques to study such phenomena. MRS has become a major tool in the non-invasive characterization of brain tumor metabolism *in vivo* and *in vitro*. A highly versatile technique, MRS allows measurements of the concentrations of many neurochemicals, including the kinetics of single enzyme-catalyzed reactions such as LDH (Xu et al., 2007) or creatine kinase (Smith et al., 1997), as well as the rates of entire metabolic pathways, such as the TCA cycle and the glutamate/glutamine neurotransmitter cycle (Sibson et al., 2001; de Graaf et al., 2003). MRS is commonly employed with several stable (non-radioactive) isotopes of biological importance such as  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$ , and  $^{31}\text{P}$ , allowing investigation of many aspects of cellular metabolism. Of these nuclei, only  $^1\text{H}$  and  $^{31}\text{P}$  exist at ~100% natural abundance, thus requiring no specific enrichment prior to their measurement. Because  $^{13}\text{C}$  exists at low natural abundance (~1.1%), selective enrichment of  $^{13}\text{C}$  in appropriate substrates allow its use as a metabolic tracer. The high chemical specificity of  $^{13}\text{C}$  MRS, which can distinguish  $^{13}\text{C}$  isotope incorporation into not only different molecules, but also specific carbon positions

within the same compound, allows the fate of the  $^{13}\text{C}$  label into and through multiple metabolic pathways to be followed (Zwingmann and Leibfritz, 2003). Until recently the low sensitivity of  $^{13}\text{C}$  detection (and correspondingly large detection volume) has precluded its use for *in vivo* imaging of tumors, although with the advent of Dynamic Nuclear Polarization (DNP) and hyperpolarized biomarkers of tumor metabolism (see below), direct  $^{13}\text{C}$  detection could become a major tool in tumor staging and response to targeted therapies.

As discussed above, brain tumors such as gliomas produce increased amounts of lactate, which can be measured by  $^1\text{H}$  MRS (Kaibara et al., 1998; Herholz et al., 1992; Terpstra et al., 1998; McKnight, 2004). Glucose production of lactate through glycolysis, whether produced anaerobically (hypoxia) or aerobically, can be determined from the change in lactate concentration verses time in a series of sequentially acquired  $^1\text{H}$  or  $^{13}\text{C}$  NMR spectra. Lactate can be measured either without isotopic labeling (e.g., by differencing of  $^1\text{H}$  spectra or by use of selective lactate-editing techniques), or with  $^{13}\text{C}$  isotopic enrichment of the precursor glucose pool, and both approaches have their specific advantages depending on the desired information. With  $^{13}\text{C}$  isotopic enrichment additional MRS techniques can be employed, such as direct  $^{13}\text{C}$  detection with  $^1\text{H}$ -decoupling to enhance sensitivity and resolution, or direct  $^1\text{H}$  detection with  $^{13}\text{C}$ -editing to differentiate  $^{13}\text{C}$ -labeled from unlabeled proton resonances, so-called heteronuclear  $^1\text{H}$ - $^{13}\text{C}$  MRS. Because the heteronuclear  $^{13}\text{C}$ -edited  $^1\text{H}$  differencing technique (Fitzpatrick et al., 1990) permits both labeled and unlabeled species to be measured from a single set of acquired spectra, the fractional enrichment of lactate-C3 reflects the sum of the pathways contributing carbon atoms (both  $^{13}\text{C}$  and  $^{12}\text{C}$ ) to the C3 position of lactate.

The use of heteronuclear  $^1\text{H}$ - $^{13}\text{C}$  MRS to characterize the C6 glioma metabolic phenotype (high glycolysis and low oxidation) was elegantly demonstrated for the rat brain *in vivo* by Terpstra et al., (1998). These authors found that the glioma metabolized glucose to lactate increased lactate turnover and reduced oxidative metabolism of glucose by the reduced incorporation of  $^{13}\text{C}$  label in glutamate, which is a measure of TCA cycle flux.  $^1\text{H}$  and  $^1\text{H}/^{13}\text{C}$  heteronuclear MRS methods have also been used to study glioma biopsies *ex vitro* (Barton et al., 1999; Martínez-Bisbal et al., 2004) and glial-derived tumor cell lines *in vitro* (Portais et al., 1993; Serkova et al., 1996; Lehtimäki et al., 2003). In ongoing studies in our laboratory, we incubated cultured human glioblastoma U87 cells with  $[1,6\text{-}^{13}\text{C}_2]\text{glucose}$  as a tracer and measured the metabolite profiles in extracts of these cells using  $^1\text{H}$ - $^{13}\text{C}$  MRS (Fig. 1). The extract spectra revealed high levels of lactate, with lower levels of glutamate and alanine, as well as other substances not yet identified. Inspection of  $^{13}\text{C}$  labeled metabolites revealed substantial turnover of lactate-C3 (percentage enrichment,  $\sim 31\%$ ) compared to a lower enrichment in glutamate-C4 ( $\sim 14\%$ ), consistent with a more glycolytic (and less oxidative) metabolic phenotype. The high glycolytic and low oxidative rates displayed by gliomas suggest these pathways as potential therapeutic targets, as emphasized in several reports (Mathupala et al., 2010).

More recently, the introduction of hyperpolarized MRS, which increases the detection sensitivity of an NMR-active nucleus up to 10,000 times, has generated intense excitement in the possibility of imaging low concentration metabolites. Special techniques are employed to achieve hyperpolarization, although the lifetime is brief, decaying according to the spin-lattice relaxation time. Thus,  $^{13}\text{C}$  in carbonyl groups, which exhibit long  $T_1$ 's (many tens of seconds) can be suitably employed as metabolic probes. Particularly promising as a



biomarker of tumor metabolism has been the development of hyperpolarized  $^{13}\text{C}$ -labeled substrates such as  $[1-^{13}\text{C}]$ pyruvate (Kurhanewicz et al., 2011). Tumors express high amounts of LDH, which catalyzes a rapid exchange between pyruvate and lactate. The rapid appearance of hyperpolarized  $[1-^{13}\text{C}]$ lactate can thus serve as an indirect measure of LDH activity and tumorigenicity. Additional metabolic products are seen, such as  $[1-^{13}\text{C}]$ alanine, resulting from alanine transaminase, and  $\text{H}^{13}\text{CO}_3$ , arising through decarboxylation by pyruvate dehydrogenase and subsequent hydration by carbonic anhydrase. Reduced  $\text{H}^{13}\text{CO}_3$  reflecting reduced TCA cycle flux can also be used as a biomarker of tumor mitochondrial metabolism (Terpstra et al., 1998). The method was recently applied to the study of human glioblastoma xenografts in rats (Park et al., 2010) and glioblastoma cells and murine xenografts *in vitro* and *in vivo* to follow the effects of an inhibitor (and anticancer agent) of phosphatidylinositol-3-kinase on tumor growth (Ward et al., 2010). For studies of brain tumors, hyperpolarized molecules such as  $[1-^{13}\text{C}]$ pyruvate and other substrates may prove particularly useful because the blood-brain-barrier, which normally restricts the passage of substrates, is disturbed during tumor growth, allowing for faster uptake and more time for metabolism prior to the decay of polarization.

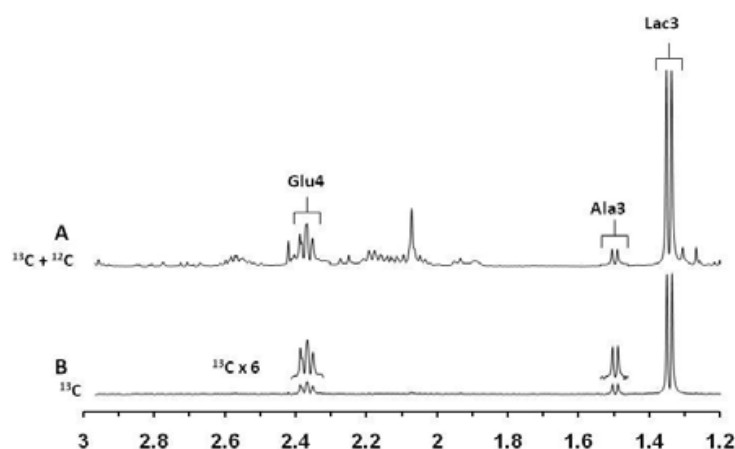


Fig. 1. Representative *in vitro*  $^1\text{H}$ - $^{13}\text{C}$ -NMR spectra of extracts of U87 glioblastoma cell pellet after 30 min incubation of  $[1,6-^{13}\text{C}_2]$ glucose (10 mM): (A) Total metabolite intensity representing the sum of  $^{12}\text{C}$  and  $^{13}\text{C}$ ; (B)  $^{13}\text{C}$ -labeled metabolites arising during  $[1,6-^{13}\text{C}_2]$ glucose infusion (10 mM: 30 min incubation). Highlight (x6) areas of Glu and Ala  $^{13}\text{C}$ -labeled metabolites. Abbreviations: Glu4, glutamate-C4; Ala-C3, alanine-C3 and Lac3, lactate-C3. Spectra were scaled independently to enhance visual clarity owing to the lower amino acid levels observed in U87 glioblastoma cell pellet.

#### 4.7 MicroRNA approach

Gene expression can be regulated by microRNAs. MicroRNAs are 19-25 nucleotides long and are capable of inhibiting translation and mRNA degradation thereby blocking protein production. microRNAs are named by numbers and the number reflects the sequence when they are identified. The next microRNA discovered will be the next number. Also mature microRNAs are named as miR whereas the primary transcript is named as mir. They influence multiple processes in several diseases including cancer (Mendell, 2005). In cancer, their effects are found in invasion, migration and metastasis (Nana-Sinkam & Croce, 2011). They play roles as oncogenes and tumor suppressor genes in several cancer types. Each

miRNA can affect several genes and each gene can be regulated by several microRNAs. The relationship between the target RNA and microRNA in regulating many pathological states is emerging (Perron et al., 2007). Thus, it is timely to investigate the impact of microRNAs in glioblastoma invasion and migration and apply this knowledge in mapping cancer therapeutic strategies. An emerging role of microRNA in resistance may be attributed to its effect on MDR. MicroRNAs miR-27a, miR-451 and miR-138 are known to impact response to chemotherapeutic drugs in several cancers including esophageal, breast, and ovarian cancers and leukemia (Hing et al., 2010; Li et al., 2010; Zhao et al., 2010; Kovalchuk et al., 2008). P-glycoprotein is present in the blood-brain barrier and may be regulated by miR-27a and miR451 (Zhu et al., 2008). In glioblastoma, 10 different miRNA were identified and shown to be predictor of prognosis (Srinivasan et al., 2011). In addition, miR-10b (Gabriely et al., 2011) has been implicated in progression of gliomas. MiR-101 that regulates PcG protein EZH2, a histone methyltransferase, may play a role in glioblastoma progression (Smits et al, 2010). Other microRNA's implicated in glioblastoma progression are listed in Table 1.

#### 4.8 Isoflavones

Micronutrients may be employed as chemopreventive agents: they may be employed to suppress or reverse carcinogenesis, thereby preventing the development of cancer. "Micronutrients include any dietary substance, essential or non-essential that are present in small amounts and brings about a physiological effect" (Greenwald et al., 2002). Example of micronutrients include, but are not limited to, vitamins, minerals, soy phytoestrogens, and other phytochemicals (Russo et al., 2005). Isoflavones are phytoestrogen compounds highly enriched in soy and exhibit anticancer properties. Epidemiological studies have demonstrated that Asian population consuming diets rich in isoflavones show lower incidences of hormone-related cancers (Lee et al., 1991). Genistein (4', 5, 7-trihydroxyisoflavone) and biochanin A (4'-methoxy, 5, 7-dihydroxy isoflavone) are natural isoflavonoid phytoestrogens and are found in soy and subterranean clover, respectively (Persky & Van Horn, 1995). Genistein is a well studied chemopreventive agent (Taylor et al., 2009; Sarkar & Li, 2002). Genistein exerts its anti-cancer properties via several mechanisms: inhibition of tyrosine phosphorylation, weak estrogenic and anti-estrogenic properties, as an anti-oxidant, inhibition of topoisomerase II, inhibition of angiogenesis, and induction of cell differentiation in breast cancer cells (Barnes & Peterson, 1995; Fotsis et al., 1993; Messina et al., 1994). Genistein also competes with ATP for binding to the tyrosine kinase domain, thereby inhibiting tyrosine kinase-mediated signaling processes (Chen et al., 2003). Our work in an *in vitro* co-culture model showed genistein inhibits glioblastoma invasion by inhibiting EGFR tyrosine kinase activity (Penar et al., 1997; Penar et al., 1998). Biochanin A has inhibitory potential on lung tumor development in mice induced by benzo(a)pyrene (Lee et al., 1991). Both genistein and biochanin A inhibit serum- and EGF-stimulated growth of human prostate cancer cells (Peterson & Barnes, 1993; Hempstcok et al., 1998). Biochanin A also inhibits the incidence and growth of xenograft tumors in athymic mice subsequent to injection of prostate cancer cells (LNCap) into the mice (Rice et al., 2002). We have demonstrated that both genistein and biochanin A inhibit invasion of glioblastoma cells by lowering the expression and activity of matrix-degrading enzymes (Puli et al., 2006). Soy isoflavones appear to be safe and effective in pre-clinical studies but clinical trials supporting their efficacy are still required (Virk-Baker et al., 2010).

MICRO RNA	FUNCTIONAL EFFECTS	REFERENCES
miR-7	EGFR, Akt pathway	Kefas et al., 2008
miR-10b	Resistance, Invasion	Ujifuku et al., 2010; Sasayama et al., 2009
miR-21	Apoptosis, EGFR, Tumor suppressor, MMP, Resistance	Shi et al., 2010; Ren et al., 2010; Zhou et al., 2010; Li et al., 2009; Conti et al., 2009; Chen et al., 2008; Papagiannakopoulos et al., 2008; Gabriely et al., 2008; corsten et al., 2007; Chan et al., 2005
miR-34a	Oncogenes	Li et al., 2009; Li et al., 2009
miR-93	Angiogenesis, tumor growth	Fang et al., 2011
miR-124a	Migration, Invasion	Fowler et al., 2011; Silber et al., 2008
miR125a	Invasion	Cortez et al., 2010
miR-128	Oncogenes, Stem cell renewal factor	Godlewski et al., 2008
miR-137	Differentiation	Silber et al., 2008
miR-146b-5p	EGFR, Migration, Invasion	Fowler et al., 2011; Xia et al., 2009
miR-153	Apoptosis	Xu et al., 2010; Xu et al., 2011
miR-181	Resistance	Slaby et al., 2010
miR-181a	Radiosensitivity	Chen et al., 2010
miR-181b	Proliferation	Conti et al., 2009
miR-195	Resistance	Ujifuku et al., 2010
miR-196	Prognosis	Guan et al., 2010
miR-221/222	P27(kip) survivin, radiosensitivity, PUMA	Wang et al., 2011; Zhang CZ et al., 2010; Zhang C et al., 2009; Lorimer, 2009; Zhang et al., 2009; Conti et al., 2009; Lukiw et al., 2009; Gillies & Lorimer, 2007
miR-326	Pyruvate kinase M2	Kefas et al., 2010
miR-328	ABCG2 expression, resistance	Li et al., 2010
miR-451	Tumor suppressor, proliferation, migration, resistance, metabolic stress	Nan et al,2010; Godlewski et al., 2010; Godlewski et al., 2010; Gal et al., 2008
miR-455-3p	Resistance	Ujifuku et al., 2010

Table 1. MicroRNA implicated in Glioblastoma Progression and Treatment

#### 4.9 Implications of targeting the blood-brain barrier in developing novel approaches

Targeting drugs that cross the blood-brain barrier (BBB) to reach the extracellular/interstitial space (ECS) in brain pose formidable challenges because the capillary endothelial cells are lined with intercellular tight junctions that restrict transfer of molecules from blood to the ECS (Lino & Merlo, 2009; Patel et al., 2009; Redzic, 2011). Moreover, the capillary endothelium on the brain side is completely surrounded or wrapped by astroglial end feet (Patel et al., 2009; Redzic, 2011). Several strategies have been proposed to deal with the restrictions of the BBB. Such strategies include: chemically modified delivery systems; biologically assisted delivery systems; disruption of the BBB; use of molecular Trojan horses such as peptidomimetic monoclonal antibodies; and particulate drug carrier systems (Juillerat-Jeanneret, 2008; Patel et al., 2009). Nevertheless, among these strategies, which are particularly suitable for delivering drugs to target glioblastomas remain to be definitively ascertained. Glioblastomas are cancer cells that exhibit diverse genotypic and phenotypic characteristics that allow them to adapt to their microenvironment so as to facilitate their proliferation and invasion into the surrounding normal brain tissue (see Lino & Merlo, 2009 and references therein). Consequently, further research is needed to combine a realistic assessment of their genotypic and phenotypic characteristics and how they adapt to their intracerebral niche along with selecting the appropriate strategy to target the desired efficacious chemotherapeutic agent to such gliomas. For example, on the one hand, around low-grade gliomas, the BBB is intact and usually restrictive to drug penetration; on the other hand, around high-grade, more malignant gliomas, the BBB becomes leaky as a result of the tumors actively secreting proteases and other factors that can actively degrade the tight junctions between the endothelial cells at their vicinity (Lino & Merlo, 2009). However, as the tumor grows aggressively, an increasing pressure in the ECS is being built up, ultimately leading to capillary and venous collapse. Consequently, any strategizing in optimizing the delivery of chemotherapeutic agents to the gliomas will have to consider the physiological and/or pathophysiological status of the capillaries that deliver oxygen and nutrients to the gliomas.

### 5. Conclusions

Several new approaches have been developed to treat glioblastomas during the last two decades. However, these approaches have not resulted in lowering the mortality and morbidity of patients suffering from this disease. The reason for this therapeutic inadequacy is that we are dealing with a tumor with highly malignant character and that the presence of the blood-brain barrier precludes easy access for drugs to target to the tumor. We have discussed the progress in understanding the aggressive phenotypic characteristics of glioblastomas and identified multiple drug targets (including key cell signaling and invasive processes) for treatment of this devastating disease. We have also emphasized the importance and the need to fully elucidate metabolic adaptive characteristics of glioblastoma employing the versatile new techniques involving nuclear magnetic resonance spectroscopy and imaging. Additionally, we have highlighted the use of new technologies whereby the restrictions of the blood-brain barrier to drug targeting can be circumvented. We included a brief review of some new roles of micro-RNAs in glioblastoma progression and treatment and showed their potential in mapping new strategies in treating glioblastomas. Ultimately, a successful strategy in treating glioblastomas leading to



improved patient outcome and survival necessarily involves a combination of drug targets based on a deeper appreciation of the metabolic adaptations of glioblastomas.

## 6. References

- Akiyama, T., Ishida, J., Nakagawa, S., Ogawara, H., Watanabe, S., Itoh, N., Shibuya, M., & Fukami, Y. (1987). Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J Biol Chem*, Vol. 262, No.12, pp. 5592-5595.
- Andronesi OC, Blekas KD, Mintzopoulos D, Astrakas L, Black PM, Tzika AA. (2008). Molecular classification of brain tumor biopsies using solid-state magic angle spinning proton magnetic resonance spectroscopy and robust classifiers. *Int J Oncol*, Vol. 33, No.5, pp.1017-1025.
- Aoki ,T., Hashimoto, N., Matsutani, M. (2007). Management of glioblastoma. *Expert Opin Pharmacother*, Vol. 8, No. 18, pp. 3133-46.
- Argyriou, AA., Giannopoulou, E., Kalofonos, HP. (2009). Angiogenesis and anti-angiogenic molecularly targeted therapies in malignant gliomas. *Oncology*, Vol. 77, No.1, pp. 1-11.
- Arteaga, CL., Johnson, MD., Todderud, G., Coffey, RJ., Carpenter, G., & Page, DL. (1991). Elevated content of the tyrosine kinase substrate phospholipase C-gamma 1 in primary human breast carcinomas. *Proc Natl Acad Sci U S A*, Vol. 88, No. 23, pp. 10435-10439.
- Asadi-Moghaddam, K., Chiocca, EA., & Lawler, SE. (2010). Potential role of miRNAs and their inhibitors in glioma treatment. *Expert Rev Anticancer Ther*, Vol.10, No.11, pp. 1753-1762.
- Balmaceda C, Critchell D, Mao X, Cheung K, Pannullo S, DeLaPaz RL, Shungu DC. (2006). Multisection  $^1\text{H}$  magnetic resonance spectroscopic imaging assessment of glioma response to chemotherapy. *J Neurooncol*, Vol. 76, No.2, pp. 185-191.
- Barnes, S., & Peterson, TG. (1995). Biochemical targets of the isoflavone genistein in tumor cell lines. *Proc Soc Exp Biol Med*, Vol. 208, No. 1, pp. 103-8.
- Barton SJ, Howe FA, Tomlins AM, Cudlip SA, Nicholson JK, Bell BA, Griffiths JR.(1999) . Comparison of *in vivo*  $^1\text{H}$  MRS of human brain tumours with  $^1\text{H}$  HR-MAS spectroscopy of intact biopsy samples *in vitro*. *MAGMA*, Vol. 8, No.2, pp.121-128.
- Batchelor, TT., Duda, DG., di Tomaso, E., Ancukiewicz, M., Plotkin, SR., Gerstner, E., Eichler, AF., Drappatz, J., Hochberg, FH., Benner, T., Louis, DN., Cohen, KS., Chea, H., Exarhopoulos, A., Loeffler, JS., Moses, MA., Ivy, P., Sorensen, AG., Wen, PY., & Jain, RK. (2010). Phase II study of cediranib, an oral pan-vascular endothelial growth factor receptor tyrosine kinase inhibitor, in patients with recurrent glioblastoma. *J Clin Oncol*, Vol.28, No.17, pp. 2817-23.
- Bayley, JP. & Devilee, P. (2010). Warburg tumors and the mechanisms of mitochondrial tumor suppressor genes. Barking up the right tree? *Current Opinion in Genetics & Development* , Vol. 20 , pp. 324-329.
- Beal, K., Abrey, LE., & Gutin, PH. (2011). Antiangiogenic agents in the treatment of recurrent or newly diagnosed glioblastoma: analysis of single-agent and combined modality approaches. *Radiat Oncol*, Vol. 6, pp. 2.

- Bhardwaj, V., Rizvi, N., Lai, MB., Lai, JCK., & Bhushan, A. (2010). Glycolytic enzyme inhibitors affect pancreatic cancer survival by modulating its signaling and energetics. *Anticancer Res*, Vol. 30, No.3, pp. 743-749.
- Bhushan, A., Patil, PP., Leung, SW., & Lai, JCK. (2011). Metallic Nanoparticles in Cancer Imaging and Therapy (in press)
- Brandes, AA., Tosoni, A., Amistà, P., Nicolardi, L., Grosso, D., Berti, F., & Ermani, M. (2004). How effective is BCNU in recurrent glioblastoma in the modern era? A phase II trial. *Neurology*, Vol. 63, No. 7, pp. 1281-1284.
- Brem, H., Piantadosi, S., Burger, PC., Walker, M., Selker, R., Vick, NA., Black, K., Sisti, M., Brem, S., & Mohr, G. (1995). Placebo-controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrent Gliomas. *Lancet*, Vol. 345, No. 8956, pp. 1008-1012.
- Buckner, JC., Ballman, KV., Michalak, JC., Burton, GV., Cascino, TL., Schomberg, PJ., Hawkins, RB., Scheithauer, BW., Sandler, HM., Marks, RS., & O'Fallon, JR. North Central Cancer Treatment Group 93-72-52; Southwest Oncology Group 9503 Trials. Phase III trial of carmustine and cisplatin compared with carmustine alone and standard radiation therapy or accelerated radiation therapy in patients with glioblastoma multiforme: North Central Cancer Treatment Group 93-72-52 and Southwest Oncology Group 9503 Trials. *J Clin Oncol*, Vol. 24, No. 24, pp. 3871-9.
- CBTRUS (2011). CBTRUS Statistical report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2004-2007. Central Brain Tumor Registry of the United States, Hinsdale, IL. [www.cbtrus.org](http://www.cbtrus.org)
- Cha, S. (2009). Neuroimaging in neuro-oncology. *Neurotherapeutics*, Vol. 6, pp. 465-477.
- Chakravarti, A., Zhai, G., Suzuki, Y., & Sarkesh, S. (2004). The prognostic significance of phosphatidylinositol 3-kinase pathway activation in human gliomas. *Journal of Clinical Oncology*, Vol. 22, No.10, pp. 1926-1933.
- Chan, JA., Krichevsky, AM., Kosik, KS. (2005). MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Res*, Vol. 65, No.14, pp. 6029-6033.
- Chandana, SR., Movva, S., Arora, M., & Singh, T. (2008). Primary brain tumors in adults. *Am Fam Physician*, Vol. 77, No. 10, pp. 1423-30.
- Chaponis, D., Barnes, JW., Dellagatta, JL., Kesari, S., Fast, E., Sauvageot, C., Panagahy, D., Greene, ER., Ramakrishna, N., Wen, PY., Kung, AL., Stiles, C., & Kieran, MW. (2011). Lomafarnib (SCH66336) improves the activity of temozolomide and radiation for orthotopic malignant gliomas. *J Neurooncol*. [Epub ahead of print].
- Chatterji, T., Dukhande, VV., Bhushan, A., & Lai, JCK. (2009). Glucose Withdrawal Results in Resistance to Cytotoxic Effect of Glycolytic Enzyme Inhibitors in U87 Glioblastoma Cells. *Proceedings of 100<sup>th</sup> Annual Meeting, American Association for Cancer Research*, Denver, CO, April 2009, Vol. 50, p. 828.
- Chatterji, T., Rizvi, N., Isaac, AO., Lai, MB., Dukhande, VV., Bhushan, A., & Lai, JCK. (2007). Metabolic Inhibitors Differentially Alter Astrocytoma and Neuroblastoma Cell Survival and Metabolism. *Annual Meeting, Society for Neuroscience*, San Diego, CA November 2007, (in Abstracts Volume).
- Chen, G., Zhu, W., Shi, D., Lv, L., Zhang, C., Liu, P., & Hu, W. (2010). MicroRNA-181a sensitizes human malignant glioma U87MG cells to radiation by targeting Bcl-2. *Oncol Rep.*, Vol. 23, No. 4, pp. 997-1003.

- Chen, P., Murphy-Ullrich, JE., & Wells, A. (1996). A role for gelsolin in actuating epidermal growth factor receptor mediated cell motility. *J Cell Biol*, Vol. 134, No. 3, pp. 689-98.
- Chen, P., Xie, H., Sekar, MC., Gupta, K., & Wells, A. (1994). Epidermal growth factor receptor-mediated cell motility: phospholipase C activity is required, but mitogen-activated protein kinase activity is not sufficient for induced cell movement. *J Cell Biol*, Vol. 127, pp. 847-857.
- Chen, WF., Huang, MH., Tzang, CH., Yang, M., & Wong, MS. (2003). Inhibitory actions of genistein in human breast cancer (MCF-7) cells. *Biochim Biophys Acta*, Vol. 1638, No.2, pp. 187-96.
- Chen, Y., Liu, W., Chao, T., Zhang, Y., Yan, X., Gong, Y., Qiang, B., Yuan, J., Sun, M., & Peng, X. (2008). MicroRNA-21 down-regulates the expression of tumor suppressor PDCD4 in human glioblastoma cell T98G. *Cancer Lett*, Vol.272, No.2, pp. 197-205.
- Cheng, JQ., & Nicosia, SV. (2001). AKT signal transduction pathway in oncogenesis. Schwab D, editor. *Encyclopedia reference of cancer*, Berlin (Germany): Springer, p. 35-37.
- Choe, G., Park, JK., Jouben-Steele, L., Kremen, TJ., Liau, LM., Vinters, HV., Cloughesy, TF., & Mischel, PS. (2002). Active matrix metalloproteinase 9 expression is associated with primary glioblastoma subtype. *Clin Cancer Res*, Vol. 8, No.9, pp. 2894-901.
- Chowdhury, GM., Gupta, M., Gibson, KM., Patel, AB., & Behar, KL. (2007a). Altered cerebral glucose and acetate metabolism in succinic semialdehyde dehydrogenase-deficient mice: evidence for glial dysfunction and reduced glutamate/glutamine cycling. *J Neurochem*, Vol. 103, pp. 2077-2091.
- Chowdhury, GM., Patel, AB., Mason, GF., Rothman, DL., & Behar, KL. (2007b). Glutamatergic and GABAergic neurotransmitter cycling and energy metabolism in rat cerebral cortex during postnatal development. *J Cereb Blood Flow Metab*, Vol.27, No. 12, pp. 1895-907.
- Chowdhury, GMI., Banasr, M., de Graaf, RA., Rothman, DL., Behar, KL., & Sanacora, G. (2008). Chronic riluzole treatment increases glucose metabolism in rat prefrontal cortex and hippocampus. *J Cereb Blood Flow Metab*, Vol. 28, pp. 1892-1897.
- Chowdhury, GMI., Lai, JCK., & Leung, SW.(2008). Nanotoxicity Studies of the CNS: Potential Application of Magnetic Resonance Spectroscopy Methods. *12th World Multi-Conference on Systemics, Cybernetics and Informatics/14th International Conference on Information Systems Analysis and Synthesis*, VOL II, PROCEEDINGS Pages: 1-5.
- Cole AJ, Yang VC, David AE. (2011). Cancer theranostics: the rise of targeted magnetic nanoparticles. *Trends Biotechnol*. 2011 Apr 11. [Epub ahead of print]
- Conti, A., Aguenouz, M., La Torre, D., Tomasello, C., Cardali, S., Angileri, FF., Maio, F., Cama, A., Germanò, A., Vita, G., Tomasello, F. (2009). miR-21 and 221 upregulation and miR-181b downregulation in human grade II-IV astrocytic tumors. *J Neurooncol*, Vol. 93, No.3, pp. 325-32.
- Corsten, MF., Miranda, R., Kasmieh, R., Krichevsky, AM., Weissleder, R., & Shah, K. (2007). MicroRNA-21 knockdown disrupts glioma growth in vivo and displays synergistic cytotoxicity with neural precursor cell delivered S-TRAIL in human gliomas. *Cancer Res*, Vol. 67, No.19, pp. 8994-9000.
- Cortez, MA., Nicoloso, MS., Shimizu, M., Rossi, S., Gopisetty, G., Molina, JR., Carlotti, C Jr., Tirapelli, D., Neder, L., Brassesco, MS., Scrideli, CA., Tone, LG., Georgescu, MM., Zhang, W., Puduvalli, V., & Calin, GA. (2010). miR-29b and miR-125a regulate

- podoplanin and suppress invasion in glioblastoma. *Genes Chromosomes Cancer*, Vol. 49, No.11, pp. 981-990.
- Czarnecka, A., & Bartnik, E. (2009). Mitochondrial DNA mutations in tumors. In: *Cellular Respiration and Carcinogenesis*, Apte, SP., & Sarangarajan, R. (eds.), pp. 119-130, Humana Press, New York, NY.
- D'Alessio, S., & Blasi, F. (2009). The urokinase receptor as an entertainer of signal transduction. *Front Biosci*, Vol. 14, pp. 4575-87.
- Dang, CV., Hamaker, M., Sun, P., Le, A. & Gao, P. (2011). Therapeutic targeting of cancer cell metabolism. *Journal of Molecular Medicine*, Vol. 89, pp. 205-212.
- Darkes, Malcolm JM., Plosker, Greg L., & Jarvis, B. (2002).Temozolomide:A Review of its Use in the Treatment of Malignant Gliomas,Malignant Melanoma and Other Advanced Cancers. *Am J Cancer*, Vol. 1, No. 1, pp. 55-80.
- De Graaf, RA., Mason, GF., Patel, AB.,Behar, KL., & Rothman, DL. (2003). *In vivo*  $^1\text{H}$ - $^{13}\text{C}$ -NMR spectroscopy of cerebral metabolism. *NMR Biomed*, Vol. 16, pp. 339-357
- Deryugina, EL., Bourdon, MA., Luo, GX., Reisfeld, RA., & Strongin, A. ( 1997). Matrix metalloproteinase-2 activation modulates glioma cell migration. *J Cell Sci*, Vol. 110, No. 19, pp. 2473-82.
- Dickson, PV., Hamner, JB., Sims, TL., Fraga, CH., Ng, CY., Rajasekeran, S., Hagedorn, NL., McCarville, MB., Stewart, CF., Davidoff, AM. (2007). Bevacizumab-induced transient remodeling of the vasculature in neuroblastoma xenografts results in improved delivery and efficacy of systemically administered chemotherapy. *Clin Cancer Res*, Vol.13, No.13, pp. 3942-3950.
- Dolan, ME.,& Pegg, AE. (1997). O6-benzylguanine and its role in chemotherapy. *Clin Cancer Res*, Vol. 3, No. 6, pp. 837-47.
- Duffy, MJ. (2004). The Urokinase Plasminogen Activator System: Role in Malignancy. *Current Pharmaceutical Design*, Vol. 10, pp. 39-49.
- Dukhande, VV., Malthankar-Phatak, GH., Hugus, JJ., Daniels, CK., & Lai, JCK. (2006). Manganese Induced Neurotoxicity is Differentially Enhanced by Glutathione Depletion in Astrocytoma and Neuroblastoma Cells. *Neurochem. Res*, Vol. 31, No.11, pp. 1349-1357.
- Fang, L., Deng, Z., Shatseva, T., Yan,g J., Peng, C., Du, WW., Yee, AJ., An,g LC., He, C., Shan, SW., & Yang BB. (2011). MicroRNA miR-93 promotes tumor growth and angiogenesis by targeting integrin- $\beta$ 8. *Oncogene*, Vol. 30, No. 7, pp. 806-821.
- Fillmore, HL., VanMeter, TE., & Broaddus, WC. (2001). Membrane-type matrix metalloproteinases (MT-MMPs): expression and function during glioma invasion. *J Neurooncol*, Vol. 53, No. 2, pp. 187-202.
- Fotsis, T., Pepper, M., Adlercreutz, H., Fleischmann, G., Hase, T., Montesano, R., & Schweigerer, L. (1993). Genistein, a dietary-derived inhibitor of in vitro angiogenesis. *Proc Natl Acad Sci U S A*, Vol. 90, No.7, pp. 2690-2694.
- Fowler, A., Thomson, D., Giles, K., Maleki, S., Mreich, E., Wheeler, H., Leedman, P., Biggs, M., Cook, R., Little, N., Robinson, B.,& McDonald K. (2011). miR-124a is frequently down-regulated in glioblastoma and is involved in migration and invasion. *Eur J Cancer*, Vol. 47, No. 6, pp. 953-963.



- Fitzpatrick SM, Hetherington HP, Behar KL, Shulman RG .(1990). The flux from glucose to glutamate in the rat brain *in vivo* as determined by  $^1\text{H}$ -observed,  $^{13}\text{C}$ -edited NMR spectroscopy. *J Cereb Blood Flow Metab*, Vol. 10, No. 2, pp.170-179.
- Frezza, C., Pollard, PJ., & Gottlieb, E. (2011). Inborn and acquired metabolic defects in cancer. *Journal of Molecular Medicine*, Vol. 89, pp. 213-220.
- Gabriely, G., Wurdinger, T., Kesari, S., Esau, CC., Burchard, J., Linsley, PS., & Krichevsky, AM. (2008). MicroRNA 21 promotes glioma invasion by targeting matrix metalloproteinase regulators. *Mol Cell Biol*, Vol. 28. No.17, pp. 5369-5380.
- Gabriely, G., Yi, M., Narayan, RS., Niers, JM., Wurdinger, T., Imitola, J., Ligon, KL., Kesari, S., Esau, C., Stephens, RM., Tannous, BA., & Krichevsky, AM. (2011). Human glioma growth is controlled by microRNA-10b. *Cancer Res*. ePub Apr 11.
- Gal, H., Pandi, G., Kanner, AA., Ram, Z., Lithwick-Yanai, G., Amariglio, N., Rechavi, G., & Givol, D. (2008). MIR-451 and Imatinib mesylate inhibit tumor growth of Glioblastoma stem cells. *Biochem Biophys Res Commun*, Vol. 376, No.1, pp. 86-90.
- Gallego, JM., Barcia, JA., Barcia-Mariño, C. (2007). Fatal outcome related to carmustine implants in glioblastoma multiforme. *Acta Neurochir (Wien)*, Vol. 149, No. 3, pp. 261-265.
- Giese, A., Bjerkvig, R., Berens, ME., & Westphal, M. (2003). Cost of migration: invasion of malignant gliomas and implications for treatment. . *J Clin Oncol*, Vol. 21. No. 8, pp. 1624-36.
- Gillies, JK., & Lorimer, IA. (2007). Regulation of p27Kip1 by miRNA 221/222 in glioblastoma. *Cell Cycle*, Vol.6. No.16, pp. 2005-2009.
- Godlewski, J., Bronisz, A., Nowicki, MO., Chiocca, EA., & Lawler, S. (2010). microRNA-451: A conditional switch controlling glioma cell proliferation and migration. *Cell Cycle*. Vol. 9, No. 14, pp. 2742-2748.
- Godlewski, J., Nowicki, MO., Bronisz, A., Nuovo, G., Palatini, J., De Lay, M., Van Brocklyn, J., Ostrowski, MC., Chiocca, EA., Lawler, SE. (2010). MicroRNA-451 regulates LKB1/AMPK signaling and allows adaptation to metabolic stress in glioma cells. *Mol Cell*, Vol. 37, No.5, pp. 620-632.
- Godlewski, J., Nowicki, MO., Bronisz, A., Williams, S., Otsuki, A., Nuovo, G., Raychaudhury, A., Newton, HB., Chiocca, EA., & Lawler, S. (2008). Targeting of the Bmi-1 oncogene/stem cell renewal factor by microRNA-128 inhibits glioma proliferation and self-renewal. *Cancer Res*, Vol. 68, No.22, pp. 9125-9130.
- Greenwald, P. (2002). Cancer prevention clinical trials. *J Clin Oncol*, Vol. 20, No.18, pp. 14S-22S.
- Greenwald, P., Milner, JA., Anderson, DE., & McDonald, SS. (2002). Micronutrients in cancer chemoprevention. *Cancer Metastasis Rev.*, Vol. 21, No. 3-4, pp. 217-30.
- Griffin JL, Lehtimäki KK, Valonen PK, Gröhn OH, Kettunen MI, Ylä-Herttuala S, Pitkänen A, Nicholson JK, Kauppinen RA .(2003). Assignment of  $^1\text{H}$  nuclear magnetic resonance visible polyunsaturated fatty acids in BT4C gliomas undergoing ganciclovir-thymidine kinase gene therapy-induced programmed cell death. *Cancer Res*, Vol. 63, No.12, pp.3195-3201.
- Grossman, SA., Ye, X., Piantadosi, S., Desideri, S., Nabors, LB., Rosenfeld, M., & Fisher, J. (2010). Survival of patients with newly diagnosed glioblastoma treated with

- radiation and temozolomide in research studies in the United States. *Clin Cancer Res*, Vol. 16, No. 8, pp. 2443–2449.
- Guan, Y., Mizoguchi, M., Yoshimoto, K., Hata, N., Shono, T., Suzuki, SO., Araki, Y., Kuga, D., Nakamizo, A., Amano, T., Ma, X., Hayashi, K., & Sasaki, T. (2010). MiRNA-196 is upregulated in glioblastoma but not in anaplastic astrocytoma and has prognostic significance. *Clin. Cancer Res.*, Vol. 16, No. 16, pp. 4289-4297.
- Guillamo, JS., de Boüard, S., Valable, S., Marteau, L., Leuraud, P., Marie, Y., Poupon, MF., Parienti, JJ., Raymond, E., & Peschanski, M.(2009). Molecular Mechanisms Underlying Effects of Epidermal Growth Factor Receptor Inhibition on Invasion, Proliferation, and Angiogenesis in Experimental Glioma. *Clin Cancer Res*, Vol.15, pp. 3697-3704.
- Guillard, S., Clarke, PA., Te Poele, R., Mohri, Z., Bjerke, L., Valenti, M., Raynaud,F., Eccles, SA., & Workman, P.(2009). Molecular pharmacology of phosphatidylinositol 3-kinase inhibition in human glioma. *Cell Cycle*, Vol. 8, No. 3, pp. 443-53.
- Hay, N., & Sonenberg, N.(2004) . Upstream and downstream of mTOR. *Genes Dev*, Vol.18, pp. 1926-1945.
- Hegi, ME., Liu, L., Herman, JG., Stupp, R., Wick, W., Weller, M., Mehta, MP.,& Gilbert ,MR. (2008). Correlation of O6-Methylguanine Methyltransferase (MGMT) Promoter Methylation With Clinical Outcomes in Glioblastoma and Clinical Strategies to Modulate MGMT Activity. *Journal of Clinical Oncology*, Vol. 26, No. 25, pp. 4189-4199.
- Hempstock, J., Kavanagh, JP., & George, NJ. (1998). Growth inhibition of prostate cell lines in vitro by phyto-oestrogens. *Br J Urol*, Vol.82, NO.4, pp. 560-563.
- Hertz L, Peng L, Dienel GA .(2007). Energy metabolism in astrocytes: high rate of oxidative metabolism and spatiotemporal dependence on glycolysis/glycogenolysis. *J Cereb Blood Flow Metab*, Vol. 27, pp. 219-249.
- Hoelzinger, DB., Demuth, T., & Berens, ME. (2007). Autocrine Factors That Sustain Glioma Invasion and Paracrine Biology in the Brain Microenvironment. *J Natl Cancer Inst* , Vol. 99, No. 21, pp. 1583-1593.
- Hong L., Han Y., Zhang H., Li M., Gong T., Sun L., Wu K., Zhao, Q., & Fan, D. (2010). The prognostic and chemotherapeutic value of miR-296 in esophageal squamous cell carcinoma. *Ann Surg.*, Vol. 251, No. 6, pp. 1056-1063.
- Hudson, CC., Liu, M., Chiang, GG., Otterness, DM., Loomis, DC., Kaper, F., Giaccia, AJ., & Abraham, RT. (2002). Regulation of hypoxia-inducible factor 1 $\alpha$  expression and function by the mammalian target of rapamycin. *Mol Cell Biol*, Vol. 22, pp. 7004-7014.
- Hu S, Lustig M, Balakrishnan A, Larson PE, Bok R, Kurhanewicz J, Nelson SJ, Goga A, Pauly JM, and Vigneron DB .(2010). 3D compressed sensing for highly accelerated hyperpolarized  $^{13}\text{C}$  MRSI with *in vivo* applications to transgenic mouse models of cancer. *Magn Reson Med*, Vol. 63, No.2, pp. 312–321.
- Jain KK.(2010). Advances in the field of nanooncology. *BMC Med*, Vol.8, pp.83.
- Jain, RK.(2001). Normalizing tumor vasculature with anti-angiogenic therapy: a new paradigm for combination therapy. *Nat Med*, Vol. 7, No.9, pp. 987-989.
- Jain, RK., di Tomaso, E., Duda, DG., Loeffler, JS., Sorensen, AG.,& Batchelor, TT. (2007). Angiogenesis in brain tumours. *Nat Rev Neurosci*, Vol.8, No.8, pp. 610-622.

- Johannessen, TC., Bjerkvig, R., & Tysnes, BB. (2008). DNA repair and cancer stem-like cells--potential partners in glioma drug resistance? *Cancer Treat Rev*, Vol. 34, No.6, pp. 558-567.
- Jones, NP., Peak, J., Brader, S., Eccles, SA., & Katan, M. (2005). PLCgamma1 is essential for early events in integrin signalling required for cell motility. *J Cell Sci*, Vol. 118, No. 12, pp. 2695-2706.
- Juillerat-Jeanneret, L. (2008). The targeted delivery of cancer drugs across the blood-brain barrier: chemical modifications of drugs or drug-nanoparticles? *Drug Discov Today*, Vol. 13, pp. 1099-1106.
- Kaina, B., Margison, GP., & Christmann, M. (2010). Targeting O<sup>6</sup>-methylguanine-DNA methyltransferase with specific inhibitors as a strategy in cancer therapy. *Cell Mol Life Sci*, Vol. 67, No. 21, pp. 3663-3681.
- Kargiotis, O., Rao, JS., & Kyritsis, AP. (2006). Mechanisms of angiogenesis in gliomas. *J Neurooncol*, Vol. 78, No. 3, pp. 281-293.
- Katakowski, M., Zheng, X., Jiang, F., Rogers, T., Szalad, A., & Chopp, M. (2010). MiR-146b-5p suppresses EGFR expression and reduces in vitro migration and invasion of glioma. *Cancer Invest*, Vol. 28, No. 10, pp. 1024-1030.
- Katterle, Y., Brandt, BH., Dowdy, SF., Niggemann, B., Zänker, KS., & Dittmar, T. (2004). Antitumour effects of PLC-gamma1-(SH2)2-TAT fusion proteins on EGFR/c-erbB-2-positive breast cancer cells. *Br J Cancer*, Vol. 90, No. 1, pp. 230-235.
- Kefas, B., Comeau, L., Erdle, N., Montgomery, E., Amos, S., & Purow, B. (2010). Pyruvate kinase M2 is a target of the tumor-suppressive microRNA-326 and regulates the survival of glioma cells. *Neuro Oncol*, Vol. 12, No. 11, pp. 1102-1112.
- Kefas, B., Godlewski, J., Comeau, L., Li, Y., Abounader, R., Hawkinson, M., Lee, J., Fine, H., Chiocca, EA., Lawler, S., & Purow, B. (2008). microRNA-7 inhibits the epidermal growth factor receptor and the Akt pathway and is down-regulated in glioblastoma. *Cancer Res*, Vol. 68, No. 10, pp. 3566-3572.
- Keunen, O., Johansson, M., Oudin, A., Sanzey, M., Rahim, SA., Fack, F., Thorsen, F., Taxt, T., Bartos, M., Jirik, R., Miletic, H., Wang, J., Stieber, D., Stuhr, L., Moen, I., Rygh, CB., Bjerkvig, R., & Niclou, SP. (2011). Anti-VEGF treatment reduces blood supply and increases tumor cell invasion in glioblastoma. *Proc Natl Acad Sci U S A*, Vol. 108, No. 9, pp. 3749-3754.
- Khoshyomn, S., Nathan, D., Manske, GC., Osler, TM., & Penar, PL. (2002) Synergistic effect of genistein and BCNU on growth inhibition and cytotoxicity of glioblastoma cells. *J Neurooncol*, Vol. 57, No. 3, pp. 193-200.
- Khoshyomn, S., Penar, PL., Rossi, J., Wells, A., Abramson, DL., & Bhushan, A. (1999). Inhibition of phospholipase C-gamma1 activation blocks glioma cell motility and invasion of fetal rat brain aggregates. *Neurosurgery*, Vol. 44, No. 3, pp. 568-577.
- Kim, MJ., Kim, E., Ryu, SH., & Suh, PG. (2000). The mechanism of phospholipase C-γ1 regulation. *Experimental and Molecular Medicine*, Vol. 32, No. 3, pp. 101-109.
- Kleihues, P. & Ohgaki, H. (1999). Primary and secondary glioblastomas: from concept to clinical diagnosis. *Neuro Oncol*, Vol. 1, pp. 44-51.
- Kovalchuk, O., Filkowski, J., Meservy, J., Ilnytsky, Y., Tryndyak, VP., Chekhun, VF., & Pogribny, IP. (2008). Involvement of microRNA-451 in resistance of the MCF-7

- breast cancer cells to chemotherapeutic drug doxorubicin. *Mol Cancer Ther.* , Vol. 7, No. 7, pp. 2152-2159.
- Krex, D., Klink, B., Hartmann, C., von Deimling, A., Pietsch, T., Simon, M., Sabel, M., Steinbach, J.P., Heese, O., Reifenberger, G., Weller, M. & Schackert, G. (2007). Long-term survival with glioblastoma multiforme. *Brain*, Vol. 13, pp. 2596-2606.
- Kurhanewicz J, Vigneron DB, Brindle K, Chekmenev EY, Comment A, Cunningham CH, Deberardinis RJ, Green GG, Leach MO, Rajan SS, Rizi RR, Ross BD, Warren WS, Malloy CR .(2011). Analysis of cancer metabolism by imaging hyperpolarized nuclei: prospects for translation to clinical research. *Neoplasia*, Vol.13, No.2, pp. 81-97.
- Lai, A., Tran, A., Nghiemphu, PL., Pope, WB., Solis, OE., Selch, M., Filka, E., Yong, WH., Mischel, PS., Liau, LM., Phuphanich, S., Black, K., Peak, S., Green, RM., Spier, CE., Kolevska, T., Polikoff, J., Fehrenbacher, L., Elashoff, R., & Cloughesy, T. (2011). Phase II study of bevacizumab plus temozolomide during and after radiation therapy for patients with newly diagnosed glioblastoma multiforme. *J Clin Oncol*, Vol. 29, No.2, pp. 142-148.
- Lai, JCK., Bhardwaj, V., Chatterji, T., Rizvi, N., Isaac ,AO., Lai, MB., Johnson, T., Leung, SW., Daniels, CC., & Bhushan, A .(2008). Inhibitors of Glycolytic Enzymes: Induction of Cancer Cell Death & Alteration in Cell Signaling. In *Advances in Cancer Research in Idaho Symposium, organized by Idaho Cancer Research Association in association with the 50<sup>th</sup> Annual Meeting of the Idaho Academy of Science*, College of Western Idaho, Nampa, ID March 2008, in Program & Abstracts, p. 12.
- Lamszus, K., Kunkel, P., & Westphal, M. (2003). Invasion as limitation to anti-angiogenic glioma therapy. *Acta Neurochir Suppl*, Vol.88, pp. 169-177.
- Lassen, U., Kristjansen, PE., Wagner, A., Kosteljanetz, M., & Poulsen, HS.( 1999). Treatment of newly diagnosed glioblastoma multiforme with carmustine, cisplatin and etoposide followed by radiotherapy. A phase II study. *J Neurooncol*, Vol. 43, No.2, pp. 161-166.
- Lawler, S., & Chiocca, EA. (2009). Emerging functions of microRNAs in glioblastoma. *J Neurooncol*, Vol.92, No.3, pp. 297-306
- Lebon, V., Petersen, KF., Cline, GW., Shen, J., Mason, GF., Dufour, S., Behar, KL., Shulman, GI., & Rothman, DL. (2002). Astroglial contribution to brain energy metabolism in humans revealed by <sup>13</sup>C nuclear magnetic resonance spectroscopy: elucidation of the dominant pathway for neurotransmitter glutamate repletion and measurement of astrocytic oxidative metabolism. *J Neurosci*, Vol. 22, pp. 1523-1531.
- Lee, HP., Gourley, L., Duffy, SW., Esteve, J., Lee, J., & Day, NE. (1991). Dietary effects on breast-cancer risk in Singapore. *Lancet*, Vol. 337, No.8751 , pp. 1197-200.
- Lee, YS., Seo, JS., Chung, HT., & Jang, JJ.( 1991). Inhibitory effects of biochanin A on mouse lung tumor induced by benzo(a)pyrene. *J Korean Med Sci*, Vol. 6, No. 4, pp. 325-328.
- Lehtimäki KK, Valonen PK, Griffin JL, Väisänen TH, Gröhn OH, Kettunen MI, Vepsäläinen J, Ylä-Herttuala S, Nicholson J, Kauppinen RA .(2003). Metabolite changes in BT4C rat gliomas undergoing ganciclovir-thymidine kinase gene therapy-induced programmed cell death as studied by <sup>1</sup>H NMR spectroscopy *in vivo*, *ex vivo*, and *in vitro*. *J Biol Chem*, Vol. 278, No.46, pp.45915-45923.



- Lemort, M., Canizares-Perez, AC., Van der Stappen, A., & Kampouridis, S. (2007). Progress in magnetic imaging of brain tumors. *Curr Opin Oncol*, pp. 616-622.
- Levin, VA. (1999). Chemotherapy for brain tumors of astrocytic and oligodendroglial lineage: the past decade and where we are heading. *Neuro Oncol*, Vol. 1, No.1, pp. 69-80.
- Levin, VA., Phuphanich, S., Yung, WK., Forsyth, PA., Maestro, RD., Perry, JR., Fuller, GN., & Baillet, M. (2006). Randomized, double-blind, placebo-controlled trial of marimastat in glioblastoma multiforme patients following surgery and irradiation. *J Neurooncol*, Vol. 78, No. 3, pp. 295-302.
- Li, WQ., Li, YM., Tao, BB., Lu, YC., Hu, GH., Liu, HM., He, J., Xu, Y., & Yu, HY. (2010). Downregulation of ABCG2 expression in glioblastoma cancer stem cells with miRNA-328 may decrease their chemoresistance. *Med Sci Monit*, Vol. 16, No. 10, pp. HY27-30.
- Li, Y., Guessous, F., Zhang, Y., Dipierro, C., Kefas, B., Johnson, E., Marcinkiewicz, L., Jiang, J., Yang, Y., Schmittgen, TD., Lopes, B., Schiff, D., Purow, B., & Abounader, R. (2009). MicroRNA-34a inhibits glioblastoma growth by targeting multiple oncogenes. *Cancer Res*, Vol. 69, No.19, pp. 7569-7576.
- Li, Y., Li, W., Yang, Y., Lu, Y., He, C., Hu, G., Liu, H., Chen, J., He, J., Yu, H. (2009). MicroRNA-21 targets LRRFIP1 and contributes to VM-26 resistance in glioblastoma multiforme. *Brain Res*, Vol. 1286, pp. 13-18.
- Li, ZM, Hu, S., Xiao, L., Wang, J., Cai, J., Yu, LL., & Wang, ZH. (2010). Expression of microRNA 27a and its correlation with drug resistance in human ovarian cancer A2780/Taxol cells. *Zhonghua Fu Chan Ke Za Zhi.*, Vol. 45, No. 5, pp. 372-375.
- Liang, BC., & Ulliyatt, E. (1998). Chemosensitization of glioblastoma cells to bis-dichloroethyl-nitrosourea with tyrphostin AG17. *Clin Cancer Res.*, Vol. 4, No. 3, pp. 773-781.
- Lino, M., & Merlo, A. (2009). Translating biology into clinic: the case of glioblastoma. *Current Opinion Cell Biol*, Vol. 21, pp. 311-316.
- Lo, HW. (2010). EGFR-targeted therapy in malignant glioma: novel aspects and mechanisms of drug resistance. *Curr Mol Pharmacol*, Vol. 3, No.1, pp. 37-52.
- Lorimer, IA. (2009). Regulation of p27Kip1 by miRNA 221/222 in glioblastoma. *Cell Cycle*, Vol. 8, No. 17, pp. 2685.
- Lukiw, WJ., Cui, JG., Li, YY., & Culicchia, F. (2009). Up-regulation of micro-RNA-221 (miRNA-221; chr Xp11.3) and caspase-3 accompanies down-regulation of the survivin-1 homolog BIRC1 (NAIP) in glioblastoma multiforme (GBM). *J Neurooncol*, Vol. 91, No.1, pp. 27-32.
- MacDonald, TJ., DeClerck, YA., & Laug, WE. (1998). Urokinase induces receptor mediated brain tumor cell migration and invasion. *Neurooncol*, Vol. 40, No.3, pp.215-226.
- Maddocks, ODK., & Vousden, KH. (2011). Metabolic regulation by p53. *J Mol Med*, Vol. 89, pp. 237-245.
- Malthankar-Phatak, GH., Patel, AB., Xia, Y., Hong, S., Chowdhury, GM., Behar, KL., Orina, IA., & Lai, JC. (2008). Effects of continuous hypoxia on energy metabolism in cultured cerebro-cortical neurons. *Brain Res*, Vol. 1229, pp. 147-154.
- Martínez-Bisbal MC, Martí-Bonmatí L, Piquer J, Revert A, Ferrer P, Llácer JL, Piotto M, Assemat O, Celda B. (2004). <sup>1</sup>H and <sup>13</sup>C HR-MAS spectroscopy of intact biopsy

- samples *ex vivo* and *in vivo*  $^1\text{H}$  MRS study of human high grade gliomas. *NMR Biomed*, Vol.17, No.4, pp.191-205.
- Mathupala SP, Ko YH, Pedersen PL. (2010). The pivotal roles of mitochondria in cancer: Warburg and beyond and encouraging prospects for effective therapies. *Biochim Biophys Acta*. 1797(6-7), pp. 1225-1230.
- McGirt, MJ., Than, KD., Weingart, JD., Chaichana, KL., Attenello, FJ., Olivi, A. (2009). Gliadel (BCNU) wafer plus concomitant temozolomide therapy after primary resection of glioblastoma multiforme. *J Neurosurg*, Vol. 110, pp. 583-588.
- McKnight TR.(2004). Proton magnetic resonance spectroscopic evaluation of brain tumor metabolism. *Semin Oncol*, Vol. 31, No.5, pp.605-617.
- Mendell, JT. (2005). MicroRNAs: critical regulators of development, cellular physiology and malignancy. *Cell Cycle*, Vol.4, No.9, pp. 1179-1184.
- Messina, MJ., Persky, V., Setchell, KD.,& Barnes, S. (1994). Soy intake and cancer risk: a review of the in vitro and in vivo data. *Nutr Cancer*, Vol. 21, No.2, pp. 113-131.
- Miller, DF., Bay, JW., Lederman, RJ., Purvis, JD., Rogers, LR., Tomsak, RL. (1985). Ocular and orbital toxicity following intracarotid injection of BCNU (carmustine) and cisplatin for malignant gliomas. *Ophthalmology*, Vol. 92, No.3, pp. 402-406.
- Mohanam, S., Gladson, CL., Rao, CN., & Rao, JS. (1999). Biological significance of the expression of urokinase-type plasminogen activator receptors (uPARs) in brain tumors. *Front Biosci*, Vol. 15, No. 4, pp. D178-87.
- Motomura, K., Natsume, A., Kishida, Y., Higashi, H., Kondo, Y., Nakasu, Y., Abe, T., Namba, H., Wakai, K.,& Wakabayashi, T. (2011). Benefits of Interferon- $\beta$  and Temozolomide Combination Therapy for Newly Diagnosed Primary Glioblastoma With the Unmethylated MGMT Promoter. A Multicenter Study. *Cancer*, Vol. 117, No.8, pp. 1721-1730.
- Murphy, S., Davey, RA., Gu, XQ., Haywood, MC., McCann, LA., Mather, LE., & Boyle, FM.(2007). Enhancement of cisplatin efficacy by thalidomide in a 9L rat gliosarcoma model. *J Neurooncol*, Vol. 85, No.2, pp. 181-189.
- Nagane, M., Kobayashi, K., Ohnishi, A., Shimizu, S., & Shiokawa, Y. (2007). Prognostic significance of O6-methylguanine-DNA methyltransferase protein expression in patients with recurrent glioblastoma treated with temozolomide. . *Jpn J Clin Oncol*, Vol. 37, No. 12, pp. 897-906.
- Nagane, M., Narita, Y., Mishima, K., Levitzki, A., Burgess, AW., Cavenee, WK.,& Huang, HJ.(2001). Human glioblastoma xenografts overexpressing a tumor-specific mutant epidermal growth factor receptor sensitized to cisplatin by the AG1478 tyrosine kinase inhibitor. *J Neurosurg*, Vol.95, No. 3, pp. 472-479.
- Nakada, M., Kita, D., Futami, K., Yamashita, J., Fujimoto, N., Sato, H., & Okada, Y. (2001). Roles of membrane type 1 matrix metalloproteinase and tissue inhibitor of metalloproteinases 2 in invasion and dissemination of human malignant glioma. *J Neurosurg*, Vol. 94, No. 3, pp. 464-473.
- Nakada, M., Nakada, S., Demuth, T., Tran, NL., Hoelzinger, DB., & Berens, ME. (2007). Molecular targets of glioma invasion. *Cell Mol Life Sci*, Vol. 64, No. 4, pp. 458-478.
- Nan, Y., Han, L., Zhang, A., Wang, G., Jia, Z., Yang, Y., Yue, X., Pu, P., Zhong, Y., Kang, C. (2010). MiRNA-451 plays a role as tumor suppressor in human glioma cells. *Brain Res.*, Vol. 1359, pp.14-21.

- Nana-Sinkam, SP., & Croce, CM. (2011). MicroRNAs as therapeutic targets in cancer. *Transl Res.*, Vol. 157, No.4, pp. 216-225.
- National Comprehensive Cancer Network clinical practice guidelines in oncology-central nervous system cancers. v.1.2010.  
[http://www.nccn.org/professionals/physician\\_gls/PDF/cns.pdf](http://www.nccn.org/professionals/physician_gls/PDF/cns.pdf)
- Ohgaki, H., & Kleihues, P. (2007). Genetic pathways to primary and secondary glioblastoma. *Am J Pathol*, Vol. 170, No. 5, pp. 1445-1453.
- Ordys, BB., Launay, S., Deighton, RF., McCulloch, J., & Whittle, IR. (2010). The role of mitochondria in glioma pathophysiology. *Mol Neurobiol*, Vol. 42, pp. 64-75.
- Panigrahi, M., Das, PK., & Parikh, PM. (2011). Brain tumor and Gliadel wafer treatment. *Indian J Cancer*, Vol. 48, No. 1, pp. 11-17.
- Papagiannakopoulos, T., Shapiro, A., & Kosik, KS. (2008). MicroRNA-21 targets a network of key tumor-suppressive pathways in glioblastoma cells. *Cancer Res*, Vol.68, No.19, pp. 8164-8172.
- Park I, Larson PE, Zierhut ML, Hu S, Bok R, Ozawa T, Kurhanewicz J, Vigneron DB, Vandenberg SR, James CD, Nelson SJ. (2010). Hyperpolarized  $^{13}\text{C}$  magnetic resonance metabolic imaging: application to brain tumors. *Neuro Oncol*, Vol.12, No.2, pp.133-144.
- Patel AB, de Graaf RA, Mason GF, Rothman DL, Shulman RG, Behar KL (2005) The contribution of GABA to glutamate/glutamine cycling and energy metabolism in the rat cortex *in vivo*. *Proc Natl Acad Sci USA*, Vol. 102, No.15, pp.5588-5593.
- Patel, MM., Goyal, BR., Bhadada, SV., Bhatt, JS., & Amin, AF. (2009). Getting into the brain. Approaches to enhance brain drug delivery. *CNS Drugs*, Vol. 23, pp. 35-58.
- Peereboom, DM., Shepard, DR., Ahluwalia, MS., Brewer, CJ., Agarwal, N., Stevens, GH., Suh, JH., Toms, SA., Vogelbaum, MA., Weil, RJ., Elson, P., & Barnett, GH. (2010). Phase II trial of erlotinib with temozolomide and radiation in patients with newly diagnosed glioblastoma multiforme. *J Neurooncol*, Vol. 98, No. 1, pp. 93-9.
- Penar, PL., Khoshyomn, S., Bhushan, A., & Tritton, TR. (1997). Inhibition of epidermal growth factor receptor-associated tyrosine kinase blocks glioblastoma invasion of the brain. *Neurosurgery*, Vol.40, No.1, pp. 141-151.
- Penar, PL., Khoshyomn, S., Bhushan, A., & Tritton, TR. (1998). Inhibition of glioma invasion of fetal brain aggregates. *In Vivo*, Vol. 12, No.1, pp. 75-84.
- Perron MP., Boissonneault V., Gobeil LA., Ouellet, DL., & Provost, P. (2007). Regulatory RNAs: future perspectives in diagnosis, prognosis, and individualized therapy. *Methods Mol Biol.*, Vol. 361, pp. 311-26.
- Persky, V., & Van Horn, L. (1995). Epidemiology of soy and cancer: perspectives and directions. *J Nutr*, Vol. 125, No. 3, pp. 709S-712S.
- Peterson, G., & Barnes, S. (1993). Genistein and biochanin A inhibit the growth of human prostate cancer cells but not epidermal growth factor receptor tyrosine autophosphorylation. *Prostate*, Vol. 22, No.4, pp. 335-345.
- Petroff, OA., Errante, LD., Rothman, DL., Kim, JH., & Spencer, DD. (2002). Glutamate-glutamine cycling in the epileptic human hippocampus. *Epilepsia*, Vol. 43, pp. 703-10.

- Plate, KH., Breier, G., Weich, HA., & Risau, W. (1992). Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vivo. *Nature*, Vol. 359, pp. 845-848.
- Portais JC, Schuster R, Merle M, Canioni P .(1993) . Metabolic flux determination in C6 glioma cells using carbon-13 distribution upon [1-<sup>13</sup>C]glucose incubation. *Eur J Biochem*, Vol. 217, No.1, pp.457-468.
- Prasanna, VK., Venkataramana, NK., Dwarakanath, BS., & Santhosh, V. (2009). Differential responses of tumors and normal brain to the combined treatment of 2-DG and radiation in glioblastoma. *J Cancer Res Ther*, Vol. 5, No. 1, pp. S44-S47.
- Puli S, Lai JC, Bhushan A: Inhibition of matrix degrading enzymes and invasion in human glioblastoma (U87MG) cells by isoflavones. *J Neurooncol* 79(2):135-42, 2006.
- Quinn, JA. (2009). Phase II trial of temozolomide plus o6-benzylguanine in adults with recurrent, temozolomide-resistant malignant glioma. *J Clin Oncol*, Vol. 27, No. 8, pp. 1262-1267.
- Rae, C., Moussa, Cel-H., Griffin, JL., Bubb, WA., Wallis, T., & Balcar, VJ. (2005). Group I and II metabotropic glutamate receptors alter brain cortical metabolic and glutamate/glutamine cycle activity: a <sup>13</sup>C NMR spectroscopy and metabolomic study. *J Neurochem*, Vol. 02, pp. 405- 416.
- Ramanathan, A., & Schreiber, SL. (2009). Direct control of mitochondrial function by mTOR. *Proc Natl Acad Sci*, Vol. 106, pp. 22229-22232.
- Reardon, DA., Fink, KL., Mikkelsen, T., Cloughesy, TF., O'Neill, A., Plotkin, S., Glantz, M., Ravin, P., Raizer, JJ., Rich, KM., Schiff, D., Shapiro, WR., Burdette-Radoux, S., Dropcho, EJ., Wittemer, SM., Nippgen, J., Picard, M., & Nabors, LB. (2008). Randomized phase II study of cilengitide, an integrin-targeting arginine-glycine-aspartic acid peptide, in recurrent glioblastoma multiforme. *J Clin Oncol*, Vol. 26, No. 34, pp. 5610-5617.
- Reardon, DA., Quinn, JA., Rich, JN., Gururangan, S., Vredenburgh, J., Sampson, JH., Provenzale, JM., Walker, A., Badruddoja, M., Tourt-Uhlig, S., Herndon, JE 2<sup>nd</sup>., Dowell, JM., Affronti, ML., Jackson, S., Allen, D., Ziegler, K., Silverman, S., Bohlin, C., Friedman, AH., Bigner, DD., & Friedman, HS. (2004). Phase 2 trial of BCNU plus irinotecan in adults with malignant glioma. *Neuro Oncol*, Vol. 6, No.2, pp. 134-144.
- Redzic, Z. (2011). Molecular biology of the blood-brain and the blood-cerebrospinal fluid barriers: similarities and differences. *Fluids and Barriers of the CNS*, Vol. 8, No. 3, pp. 1-25.
- Reithmeier, T., Graf, E., Piroth, T., Trippel, M., Pinsker, MO., & Nikkhah, G.(2010). BCNU for recurrent glioblastoma multiforme: efficacy, toxicity and prognostic factors. *BMC Cancer* , Vol. 10, No. 30, pp. 1-8.
- Ren, H., Tan, X., Dong, Y., Giese, A., Chou, TC., Rainov, N., & Yang, B. (2009). Differential Effect of Imatinib and Synergism of Combination Treatment with Chemotherapeutic Agents in Malignant Glioma Cells. *Basic & Clinical Pharmacology & Toxicology*, Vol. 104, pp. 241-252.
- Ren, Y., Zhou, X., Mei, M., Yuan, XB., Han, L., Wang, GX., Jia, ZF., Xu, P., Pu, PY., Kang, CS. (2010) MicroRNA-21 inhibitor sensitizes human glioblastoma cells U251 (PTEN-mutant) and LN229 (PTEN-wild type) to taxol. *BMC Cancer*., Vol. 10, No. 27.



- Rice, L., Samedì, VG., Medrano, TA., Sweeney, CA., Baker, HV., Stenstrom, A., Furman, J., & Shiverick, KT. (2002). Mechanisms of the growth inhibitory effects of the isoflavonoid biochanin A on LNCaP cells and xenografts. *Prostate*, Vol. 52, No.3, pp. 201-212.
- Roos, WP., Batista, LF., Naumann, SC., Wick, W., Weller, M., Menck, CF., & Kaina B. (2007). Apoptosis in malignant glioma cells triggered by the temozolomide-induced DNA lesion O6-methylguanine. *Oncogene*, Vol. 26, pp. 186-197.
- Rosenberg, B., Van Camp, L., & Krigas, T. (1965). Inhibition of cell division in *Escherichia coli* by electrolysis products from a platinum electrode. *Nature*, Vol. 205, No.4972, pp. 698-699.
- Rosenberg, B., Vancamp, L., Trosko, JE., & Mansour, VH. (1969). Platinum Compounds: a New Class of Potent Antitumour Agents. *Nature*, Vol. 222, No. 5191, pp. 385-386.
- Russo, M., Tedesco, I., Iacomino, G., Palumbo, R., Galano, G., & Russo, GL. (2005). Dietary Phytochemicals in Chemoprevention of Cancer. *Current Medicinal Chemistry - Immunology, Endocrine & Metabolic Agents*, Vol, 5, No, 1, pp. 61-72.
- Salvati, M., D'Elia, A., Formichella, AI., & Frati, A. (2009). Insights into pharmacotherapy of malignant glioma in adults. *Expert Opin Pharmacother*, Vol. 10, No. 14, pp. 2279-90.
- Sarkar, FH., Li, Y. (2002). Mechanisms of cancer chemoprevention by soy isoflavone genistein. *Cancer Metastasis Rev*, Vol. 21, No.3-4, pp. 265-80.
- Sarkaria, JN., Kitange, GJ., James, CD., Plummer, R., Calvert, H., Weller, M., Wick, W. (2008). Mechanisms of Chemoresistance to Alkylating Agents in Malignant Glioma. *Clin Cancer Res*, Vol. 14, No.10, pp. 2900-2908.
- Sasayama, T., Nishihara, M., Kondoh, T., Hosoda, K., & Kohmura, E. (2009). MicroRNA-10b is overexpressed in malignant glioma and associated with tumor invasive factors, uPAR and RhoC. *Int J Cancer*, Vol.125, No.6, pp. 1407-1413.
- Semenza, GL. (2011). A return to cancer metabolism. *Journal of Molecular Medicine*, Vol. 89, pp. 203-204.
- Senft, C., Polacin, M., Priester, M., Seifert, V., Kögel, D., & Weissenberger, J. (2010). The nontoxic natural compound Curcumin exerts anti-proliferative, anti-migratory, and anti-invasive properties against malignant gliomas. *BMC Cancer*, Vol. 10, No. 491, pp. 1-8.
- Serkova N, Brand A, Christians U, Leibfritz D. (1996). Evaluation of the effects of immunosuppressants on neuronal and glial cells *in vitro* by multinuclear magnetic resonance spectroscopy. *Biochim Biophys Acta*, Vol. 1314. No. 1-2, pp. 93-104.
- Shi, L., Chen, J., Yang, J., Pan, T., Zhang, S., & Wang, Z. (2010). MiR-21 protected human glioblastoma U87MG cells from chemotherapeutic drug temozolomide induced apoptosis by decreasing Bax/Bcl-2 ratio and caspase-3 activity. *Brain Res.*, Vol. 1352, pp. 255-264.
- Sibson, NR., Mason, GF., Shen, J., Cline, GW., Herskovits, AZ., Wall, JE., Behar, KL., Rothman, DL., & Shulman, RG. (2001). *In vivo* <sup>13</sup>C NMR measurement of neurotransmitter glutamate cycling, anaplerosis and TCA cycle flux in rat brain during [2-<sup>13</sup>C]glucose infusion. *J Neurochem*, Vol. 76, pp. 975-989.
- Sidenius, N., & Blasi, F. (2003). The urokinase plasminogen activator system in cancer: recent advances and implication for prognosis and therapy. *Cancer Metastasis Rev*, Vol. 22, No. 2-3, pp. 205-222.

- Silber, J., Lim, DA., Petritsch, C., Persson, AI., Maunakea, AK., Yu, M., Vandenberg, SR., Ginzinger, DG., James, CD., Costello, JF., Bergers, G., Weiss, WA., Alvarez-Buylla, A., & Hodgson, JG. (2008). miR-124 and miR-137 inhibit proliferation of glioblastoma multiforme cells and induce differentiation of brain tumor stem cells. *BMC Med.* 2008, Vol.6, pp. 14.
- Silvani, A., Eoli, M., Salmaggi, A., Lamperti, E., Maccagnano, E., Broggi, G., & Boiardi, A. (2004). Phase II trial of cisplatin plus temozolomide, in recurrent and progressive malignant glioma patients. *J Neurooncol*, Vol. 66, No. 1-2, pp. 203-208.
- Silvani, A., Gaviani, P., Lamperti, EA., Eoli, M., Falcone, C., Dimeco, F., Milanesi, IM., Erbetta, A., Boiardi, A., Fariselli, L., & Salmaggi, A. (2009). Cisplatin and BCNU chemotherapy in primary glioblastoma patients. *J Neurooncol*, Vol. 94, No. 1, pp. 57-62.
- Slaby, O., Lakomy, R., Fadrus, P., Hrstka, R., Kren, L., Lzicarova, E., Smrcka, M., Svoboda, M., Dolezalova, H., Novakova, J., Valik, D., Vyzula, R., & Michalek, J. (2010). MicroRNA-181 family predicts response to concomitant chemoradiotherapy with temozolomide in glioblastoma patients. *Neoplasma*, Vol. 57, No. 3, pp. 264-269.
- Smith CD, Landrum W, Carney JM, Landfield PW, Avison MJ .(1997). Brain creatine kinase with aging in F-344 rats: analysis by saturation transfer magnetic resonance spectroscopy. *Neurobiol Aging*, Vol. 18, No.6, pp. 617-622.
- Smits, M., Nilsson, J., Mir, SE., van der Stoop, PM., Hulleman, E., Niers, JM., de Witt Hamer, PC., Marquez, VE., Cloos, J., Krichevsky, AM., Noske, DP., Tannous, BA., & Würdinger, T. (2010). miR-101 is down-regulated in glioblastoma resulting in EZH2-induced proliferation, migration, and angiogenesis. *Oncotarget.*, Vol. 1, No. 8, pp. 710-720.
- Srinivasan, S., Patric, IR., & Somasundaram, KA. (2011). Ten-microRNA Expression Signature Predicts Survival in Glioblastoma. *PLoS One.*, Vol. 6, No. 3, e17438.
- Srivastava, S., & Moraes, CT. (2009). Cellular adaptations to oxidative phosphorylation defects in cancer. In: *Cellular Respiration and Carcinogenesis*, Apte, SP., & Sarangarajan, R. (eds.), pp. 55-72, Humana Press, New York, NY.
- Stegh, AH., Chin, L., Louis, DN., & DePinho, RA. (2008). What drives intense apoptosis resistance and propensity for necrosis in glioblastoma? A role for Bcl2L12 as a multifunctional cell death regulator. *Cell Cycle*, Vol. 7, No.18, pp. 2833-2839.
- Stewart, DJ., Wallace, S., Feun, L., Leavens, M., Young, SE., Handel, S., Mavligit, G., & Benjamin, RS. (1982). A phase I study of intracarotid artery infusion of cis-Diamminedichloroplatinum(II) in patients with recurrent malignant intracerebral tumors. *Cancer Res*, Vol. 42, No. 5, pp. 2059-2062.
- Stupp, R., Mason, WP., van den Bent, MJ., Weller, M., Fisher, B., Taphoorn, MJ., Brandes, AA., Marosi, C., Bogdahn, U., Curschmann, J., Janzer, RC., Ludwin, SK., Gorlia, T., Allgeier, A., Lacombe, D., Cairncross, JG., Eisenhauer, E., & Mirimanoff, RO. (2005). Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N. Engl. J. Med*, Vol. 352, pp. 987-996.
- Tate, MC., & Aghi, MK. (2009). Biology of angiogenesis and invasion in glioma. *Neurotherapeutics*, Vol. 6, No.3, pp. 447-457.

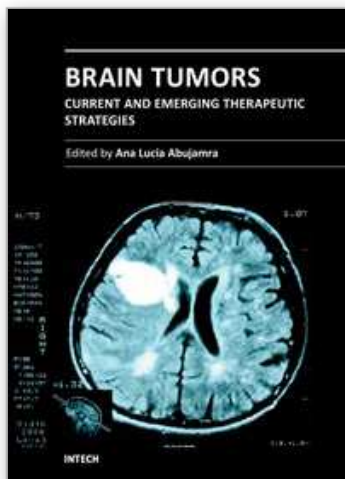
- Taylor, CK., Levy, RM., Elliott, JC., & Burnett, BP. (2009). The effect of genistein aglycone on cancer and cancer risk: a review of in vitro, preclinical, and clinical studies. *Nutr Rev*, Vol. 67, No.7, pp. 398-415.
- Tentori, L., & Graziani, G. (2002). Pharmacological Strategies to Increase the Antitumor Activity of Methylating Agents. *Current Medicinal Chemistry*, Vol. 9, pp. 1285-1301.
- Terpstra M, Gruetter R, High WB, Mescher M, DelaBarre L, Merkle H, Garwood M. (1998). Lactate turnover in rat glioma measured by *in vivo* nuclear magnetic resonance spectroscopy. *Cancer Res*, Vol. 58, No.22, pp.5083-5088.
- Turner, T., Epps-Fung, MV., Kassis, J., & Wells, A. (1997). Molecular inhibition of phospholipase C $\gamma$  signaling abrogates DU-145 prostate tumor cell invasion. *Clin Cancer Res*, Vol. 3, No. (12 Pt 1), pp. 2275-2282.
- Tysnes, BB., & Mahesparan, R. (2001). Biological mechanisms of glioma invasion and potential therapeutic targets. *Journal of Neuro-Oncology*, Vol. 53, pp. 129-147.
- Ujifuku, K., Mitsutake, N., Takakura, S., Matsuse, M., Saenko, V., Suzuki, K., Hayashi, K., Matsuo, T., Kamada, K., Nagata, I., & Yamashita, S. (2010). miR-195, miR-455-3p and miR-10a( \*) are implicated in acquired temozolomide resistance in glioblastoma multiforme cells. *Cancer Lett.*, Vol. 296, No. 2, pp. 241-248.
- VanMeter, TE., Rooprai, HK., Kibble, MM., Fillmore, HL., Broadus, WC., & Pilkington, GJ. (2001). The role of matrix metalloproteinase genes in glioma invasion: co-dependent and interactive proteolysis. *J Neurooncol*, Vol. 53, No.2, pp. 213-235.
- Verhoeff, JJ., van Tellingen, O., Claes, A., Stalpers, LJ., van Linde, ME., Richel, DJ., Leenders, WP., & van Furth, WR. (2009). Concerns about anti-angiogenic treatment in patients with glioblastoma multiforme. *BMC Cancer*, Vol.9, pp. 444.
- Villano, JL., Seery, TE., & Bressler, LR. (2009). Temozolomide in malignant gliomas: current use and future targets. *Cancer Chemother Pharmacol*, Vol. 64, pp. 647-655.
- Vinjamuri, M., Adumala, RR., Altaha, R., Hobbs, GR., & Crowell, EB Jr. (2009). Comparative analysis of temozolomide (TMZ) versus 1,3-bis (2-chloroethyl)-1 nitrosourea (BCNU) in newly diagnosed glioblastoma multiforme (GBM) patients. *J Neurooncol*, Vol. 91, No. 2, pp.221-225.
- Virk-Baker, MK., Nagy, TR., & Barnes, S. (2010). Role of phytoestrogens in cancer therapy. *Planta Med*, Vol. 76, No.11, pp. 1132-1142.
- Visse, R., & Nagase, H. (2003). Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res*, Vol. 92, No. 8, pp. 827-839.
- Vredenburgh, JJ., Desjardins, A., Herndon, JE II., Dowell, JM., Reardon, DA., Quinn, JA., Rich, JN., Sathornsumetee, S., Gururangan, S., Wagner, M., Bigner, DD., Friedman, AH., & Friedman, HS. (2007). Phase II trial of bevacizumab and irinotecan in recurrent malignant glioma. *Clin Cancer Res*, Vol.13, No.4, pp.1253-1259.
- Wachsberger, PR., Burd, R., Cardi, C., Thakur, M., Daskalakis, C., Holash, J., Yancopoulos, GD., & Dicker, AP. (2007). VEGF trap in combination with radiotherapy improves tumor control in u87 glioblastoma. *Int J Radiat Oncol Biol Phys*, Vol. 67, No.5, pp. 1526-1537.
- Wahl, MI., Jones, GA., Nishibe, S., Rhee, SG., & Carpenter, G. (1992). Growth factor stimulation of phospholipase C-gamma 1 activity. Comparative properties of control and activated enzymes. *J Biol Chem*, Vol. 267, No. 15, pp. 10447-10456.

- Wang, X., Han, L., Zhang, A., Wang, G., Jia, Z., Yang, Y., Yue, X., Pu, P., Shen, C., Kang, C. (2011). Adenovirus-mediated shRNAs for co-repression of miR-221 and miR-222 expression and function in glioblastoma cells. *Oncol Rep*, Vol. 25, No. 1, 97-105.
- Wang, YX., & Lam, WWM. (2008). Characterisation of brain disorders and evaluation of therapy by functional and molecular magnetic resonance techniques. *Hong Kong Med J*, Vol. 14, pp. 469-478.
- Warburg, O. (1956). On the origin of cancer cells. *Science*, Vol. 123, pp. 309-314.
- Warburg, O., Wind, F., & Negelein, E. (1927). The metabolism of tumors in the body. *Journal of General Physiology*, Vol. 8, pp. 519-530.
- Ward CS, Venkatesh HS, Chaumeil MM, Brandes AH, Vancrinkinge M, Dafni H, Sukumar S, Nelson SJ, Vigneron DB, Kurhanewicz J, James CD, Haas-Kogan DA, Ronen SM. (2010). Noninvasive detection of target modulation following phosphatidylinositol 3-kinase inhibition using hyperpolarized  $^{13}\text{C}$  magnetic resonance spectroscopy. *Cancer Res*, Vol. 70, No.4, pp.1296-1305.
- Weber, EL., Goebel, EA. (2005). Cerebral edema associated with Gliadel wafers: two case studies. *Neuro-Oncol*, Vol. 7, pp. 84-89.
- Wells, A., & Grandis, JR. (2003). Phospholipase C-gamma1 in tumor progression. *Clin Exp Metastasis*, Vol. 20, No. 4, pp. 285-290.
- Wen, PY., & Kesari, S. (2008). Malignant gliomas in adults. *N Engl J Med*, Vol. 359, No.5, pp. 492-507.
- Westphal ,M., Hilt, DC., Bortey, E., Delavault, P., Olivares, R., Warnke, PC., Whittle, IR., Jääskeläinen, J., & Ram, Z. (2003). A phase 3 trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel wafers) in patients with primary malignant glioma. *Neuro Oncol*, Vol.5, No.2, pp. 79-88.
- Williams, CJ., & Whitehouse, JMA. (1979). Cis-platinum: a new anticancer agent. *British Medical Journal*, Vol. 1, pp. 1689-1691.
- Wong, YYW., Jaiswal, AR., Bhushan, A., Leung, SW., & Lai, JCK. (2010). Further Elucidation of Neuroprotective Properties of Astrocytoma (Astrocytes-like) Cells. *Journal of the Idaho Academy of Science*, Vol. 46, No. 1, pp. 52-57.
- Wrensch, M., Minn, Y., Chew, T., Bondy, M., & Berger, MS. (2002). Epidemiology of primary brain tumors: Current concepts and review of the literature. *Neuro-oncology*, Vol. 4, No. 4, pp. 278- 299.
- Xia, H., Qi, Y., Ng, SS., Chen, X., Li, D., Chen, S., Ge, R., Jiang, S., Li, G., Chen, Y., He, ML., Kung, HF., Lai, L., & Lin, MC. (2009). microRNA-146b inhibits glioma cell migration and invasion by targeting MMPs. *Brain Res*, Vol. 1269, pp. 158-165.
- Xu, J., Liao, X., Lu, N., Liu, W., & Wong, CW. (2011). Chromatin-modifying drugs induce miRNA-153 expression to suppress Irs-2 in glioblastoma cell lines. *Int J Cancer*. (Epub ahead of print).
- Xu, J., Liao, X., Wong, C. (2010). Downregulations of B-cell lymphoma 2 and myeloid cell leukemia sequence 1 by microRNA 153 induce apoptosis in a glioblastoma cell line DBTRG-05MG. *Int J Cancer*, Vol. 126, No.4, pp. 1029-1035.
- Xu S, Yang J, Shen J. (2007). In vivo  $^{13}\text{C}$  saturation transfer effect of the lactate dehydrogenase reaction. *Magn Reson Med*, Vol. 57, No.2, pp. 258-264.
- Yarden, Y. (2001). The EGFR family and its ligands in human cancer. signalling mechanisms and therapeutic opportunities. *Eur J Cancer*, Vol.37, No. 4, pp. S3-8.



- Yecies, JL., & Manning, BD. (2011). mTOR links oncogenic signaling to tumor cell metabolism. *J Mol Med*, Vol. 89, pp. 221-228.
- Zhang, C., Kang, C., You, Y., Pu, P., Yang, W., Zhao, P., Wang, G., Zhang, A., Jia, Z., Han, L., & Jiang, H. (2009). Co-suppression of miR-221/222 cluster suppresses human glioma cell growth by targeting p27kip1 in vitro and in vivo. *Int J Oncol*, Vol. 34, No. 6, pp. 1653-1660.
- Zhang, C., Wang, G., Kang, C., Du, Y., & Pu, P. (2009). Up-regulation of p27 (kip1) by miR-221/222 antisense oligonucleotides enhances the radiosensitivity of U251 glioblastoma. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi*, Vol. 26, No. 6, pp. 634-638.
- Zhang, CZ., Zhang, JX., Zhang, AL., Shi, ZD., Han, L., Jia, ZF., Yang, WD., Wang, GX., Jiang, T., You, YP., Pu, PY., Cheng, JQ., & Kang CS. (2010). MiR-221 and miR-222 target PUMA to induce cell survival in glioblastoma. *Mol Cancer*, Vol. 9, pp. 229.
- Zhao, X., Yang, L., Hu, J., & Ruan, J. (2010). miR-138 might reverse multidrug resistance of leukemia cells. *Leuk Res*, Vol. 34, No. 8, pp. 1078-1082.
- Zhou, X., Ren, Y., Moore, L., Mei, M., You, Y., Xu, P., Wang, B., Wang, G., Jia, Z., Pu, P., Zhang, W., & Kang, C. (2010). Downregulation of miR-21 inhibits EGFR pathway and suppresses the growth of human glioblastoma cells independent of PTEN status. *Lab Invest*. Vol. 90, No. 2, pp. 144-155.
- Zhu, H., Wu, H., Liu, X., Evans, BR., Medina, DJ., Liu, CG., & Yang, JM. (2008). Role of MicroRNA miR-27a and miR-451 in the regulation of MDR1/P-glycoprotein expression in human cancer cells. *Biochem Pharmacol*, Vol. 76, No.5, pp. 582-588.
- Zhu, Y., & Parada, LF. (2002). Molecular biology and genetics of neurologic tumors. *Nature Reviews Cancer*, Vol. 2, No.8, pp. 616-626.
- Zwingmann, C., & Leibfritz, D. (2003). Regulation of glial metabolism studied by <sup>13</sup>C-NMR. *NMR Biomed*, Vol. 16, pp. 370-99.

IntechOpen



## **Brain Tumors - Current and Emerging Therapeutic Strategies**

Edited by Dr. Ana Lucia Abujamra

ISBN 978-953-307-588-4

Hard cover, 422 pages

**Publisher** InTech

**Published online** 23, August, 2011

**Published in print edition** August, 2011

Brain Tumors: Current and Emerging Therapeutic Strategies focuses on tumor models, the molecular mechanisms involved in the pathogenesis of this disease, and on the new diagnostic and treatment strategies utilized to stage and treat this malignancy. A special section on immunotherapy and gene therapy provides the most up-to-date information on the pre-clinical and clinical advances of this therapeutic venue. Each chapter in Brain Tumors: Current and Emerging Therapeutic Strategies is authored by international experts with extensive experience in the areas covered.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Aditi Jain, James CK Lai, Golam MI Chowdhury, Kevin Behar and Alok Bhushan (2011). Glioblastoma: Current Chemotherapeutic Status and Need for New Targets and Approaches, Brain Tumors - Current and Emerging Therapeutic Strategies, Dr. Ana Lucia Abujamra (Ed.), ISBN: 978-953-307-588-4, InTech, Available from: <http://www.intechopen.com/books/brain-tumors-current-and-emerging-therapeutic-strategies/glioblastoma-current-chemotherapeutic-status-and-need-for-new-targets-and-approaches>

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike-3.0 License](https://creativecommons.org/licenses/by-nc-sa/3.0/), which permits use, distribution and reproduction for non-commercial purposes, provided the original is properly cited and derivative works building on this content are distributed under the same license.

IntechOpen

IntechOpen