Review Article

Immunoexcitatory mechanisms in glioma proliferation, invasion and occasional metastasis

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Abstract

There is increasing evidence of an interaction between inflammatory cytokines and glutamate receptors among a number of neurological diseases including traumatic brain injuries, neurodegenerative diseases and central nervous system (CNS) infections. A number of recent studies have now suggested a strong relation between inflammatory mechanisms and excitatory cascades and these may play a role in glioma invasiveness and proliferation.

Chronic inflammation appears to be a major initiating mechanism in most human cancers, involving cell-signaling pathways, which are responsible for cell cycling, cancer cell migration, invasion, tumor aggressiveness, and angiogenesis. It is less well appreciated that glutamate receptors also play a significant role in both proliferation and especially glioma invasion. There is some evidence that sustained elevations in glutamate may play a role in initiating certain cancers and new studies demonstrate an interaction between inflammation and glutamate receptors that may enhance tumor invasion and metastasis by affecting a number of cell-signaling mechanisms. These mechanisms are discussed in this paper as well as novel treatment options for reducing immune-glutamate promotion of cancer growth and invasion.

Key Words: Glioblastoma, immunoexcitotoxicity, inflammatory cytokines, metastasis, tumor invasion

INTRODUCTION

Glioblastoma (GBM) is the most commonly diagnosed primary cancer occurring in the central nervous system (CNS) and is characterized by high invasiveness, frequent central necrosis, and a short survival rate (6-18 months) in the vast majority of patients, even with aggressive treatment.[62,80]

Inflammation plays a critical role in carcinogenesis, cancer cell proliferation, angiogenesis, invasion, and metastasis of human cancers, including gliomas.[34] Tumor-associated inflammation is typically characterized by infiltration of innate immune cells, such as microglia/macrophages and the production of cytokines and chemokines. In addition, tissue remodeling and angiogenesis are present in virtually all tumor types.[26,28]

In addition to damage to the deoxyribonucleic acid (DNA) and membrane structures caused by increased levels of reactive oxygen and nitrogen species generated during chronic inflammation and excitotoxicity, one sees activation of a number of cell-signaling pathways that are increasingly recognized as being critical to the growth, progression, and invasion of cancers such as Nuclear Factor kappa B (NF-κB), mammalian

target of rapamycin/Ak-thymoma (mTOR/Akt), and signal transducer and activator of transcription-3 (STAT3).[1,24,29,44]

Of particular interest is the involvement of certain immune cell types in cancer biology, such as macrophage/microglia, mast cells, and T-lymphocytes, all of which have been shown to assume specific immune phenotypes that regulate tumor immunity.[60,108] In addition, recent studies have demonstrated a primary role for cancer stem cells in tumor behavior that not only includes control of tumor proliferation but also can initiate a microenvironment associated with immunosuppression.[32,57,108] Neural stem cells are attracted to sites of inflammation, reactive astrocytosis and angiogenesis primarily by the action of tumor necrosis factor-alpha (TNF-α) on NF-κB activation.[123,124] NF-κB controls stem cell proliferation by up-regulation of cyclin D1.[42] Widera et al. demonstrated a 10-fold increase in NF-κB activity and a 4-fold increase in cyclinD1 positive stem cells with neural stem cell exposure to TNF-α.[124] TNF-α is a growth factor for Hodgkin's lymphoma, cutaneous T-cell lymphoma and gliomas.[3,91] Excitotoxicity also up-regulates NF-κB, which may be a mechanism by which it stimulates GBM proliferation.[77]

Various chemokines and chemokine receptors are also known to play a role in tumor biology. Of particular interest are chemokine receptors CX3CR1 and CXCR4 and their ligands. CXCR4 is the most extensively studied and is associated with aggressive forms of gliomas and poor patient survival.[10,33] The chemokine CX3CL1 regulates microglia and controls the crosstalk between microglia and neurons. In addition, these chemokines play a significant role in tumor resistance to apoptosis, stimulation of angiogenesis and recruitment of microglia and macrophages into the tumor.[13,130] The chemokine macrophage chemoattractant protein-1 (MCP-1) is markedly up-regulated in astrocytoma cells following exposure to TNF-α.[103] MCP-1, which is released during inflammation, is a potent chemoattractant for neural stem cells.[123]

Macrophages and microglia are the most abundant cell types in gliomas, but what is often ignored is that they are both sources of glutamate and other excitatory amino acids.[49,118] Recent studies have found that glutamate and virtually all of the glutamate receptor types are involved in tumor growth, progression, invasion, and metastasis in a wide array of tumor types, including primary brain tumors.[47,94,126] Several studies have also found that inhibiting glutamate receptors suppresses the growth and spread of a number of types of cancer.[101,113]

INFLAMMATION, IMMUNE CELL INFILTRATION, AND GLIOMAS

Chronic inflammation (also observed in non-CNS cancers), appears to play a significant role in all phases of brain tumor development, progression and invasion.[23,24,98,105,109] Yet, in the case of higher grade gliomas, such as GMBs, the microenvironment is mostly immunosuppressive, yet still inflammatory.[51,125] For example, despite the fact that GMBs are known to be immunogenic tumors neither the innate nor the adaptive immune system significantly suppresses these tumors.[37,50]

In a recent study Tafani et al. demonstrated an elevation of inflammation within GBM in 10 tumors removed surgically, selectively sampling deep tumor, peritumoral tissues, and normal surrounding brain.[116] In all samples they found a marked elevation in NF-κB and the pro-inflammatory markers receptor for advanced glycation end-products (RAGE), cyclooxygenase 2 (COX2), nitric oxide synthase-2 (NOS2), P2X7R, and pentraxin 3 (PTX3). For most of these factors, levels were highest in the tumoral tissue with much lower levels in the surrounding brain. Similar elevations in pro-inflammatory proteins have been described in breast and prostate cancers.[97,115] The inducible enzymes COX2 and NOS2 play a role in angiogenesis and signal a worse prognosis when elevated.[48] Both COX2 and NOS2 are also elevated with glutamate excitotoxicity.[2]

Interestingly, tumor reactions to TNF-α vary according to concentration. Low dose TNF-α enhances survivability of glioma cells and in higher doses increases killing of neurons.[9] There is also a selective stimulation of glioma stem cell proliferation as well as tumor necrosis in the center of the tumor and within normal surrounding brain along its border.[58,102] The differential effect on neurons and glia has been attributed to differences in TNF receptors, TNFR1 (p55) and TNFR2 (p75). While neurons express both types of receptors, TNFR1 is activated at lower levels of TNF-α. TNFR1 is neurodestructive, which when overstimulated can lead to necrosis of neurons as seen along the leading edge of the tumor and in the tumor core. TNFR2, primarily located on astrocytes and glioma cells, is neuroprotective.[9] Under conditions of high glutamate levels, as seen with GBMs, stimulation of glial TNFR2 would favor glioma cell survival, thus promoting tumor growth. TNF-α also stimulates stem cell proliferation.[123,124]

ROLE OF TUMOR TISSUE HYPOXIA IN INFLAMMATION-INDUCED INVASION

Hypoxia was also shown to enhance the inflammation and increase invasion and migration of the GBM stem cells.[115] Because GBMs are such fast growing tumors they rapidly outgrow their blood supply, leading to hypoxia/ischemia within the tumor and frequent necrosis. It is now known that angiogenesis is an early process and is driven by hypoxia.[25] Hypoxia inducible factor-1 (HIF-1) is present in high levels in malignant gliomas and necessary for GBM stem cell maintenance.[96] Under

hypoxic conditions, as seen in rapidly growing tumors, HIF-1α is stabilized and translocates to the nucleus where it binds to HIF-1ß.[83] This dimer activates genes involved in angiogenesis, glucose transport, resistance to apoptosis, inflammation and invasion.

Hypoxia is also known to increase expression of CXCR4, which stimulates tumor cell migration and is associated with highly aggressive tumors and a poor prognosis.[10,115] Tafani *et al.* examined the role of hypoxia on pro-inflammatory gene expression in GBMs.[115] Using surgical samples from deep within the tumor, the peritumoral area and surrounding normal brain, they concluded that GBMs express pro-inflammatory genes and proteins that are almost exclusively localized to the tumor tissue and much less so in the peritumoral and host tissue. Under hypoxic conditions GBM stem cells overexpressed pro-inflammatory genes, such as those regulating NF-κB, COX2, iNOS, and PTX3 and this increases migration and invasion of the tumor stem cells. Hypoxia-induced acceleration of invasion and migration of tumors stem cells also appears to play an important role in breast cancer.[115]

In essence, hypoxia within the tumor stimulated HIF-1α and NF-κB activation, which in turn increased generation of pro-inflammatory cytokines and chemokines, leading to accelerated tumor cell invasion. The source of the pro-inflammatory factors was probably macrophages/microglia, since these are the predominant invading cell type. RAGE was clearly over expressed in the GBM fraction at mRNA and protein levels, which is also controlled by hypoxia. The inducible enzymes, COX-2 and NOS2 were also overexpressed in peritumoral and tumor tissue and in the case of the GBM stem cells, expression of these factors was increased by hypoxia.

The picture that is emerging from recent research is that malignant gliomas release high levels of chemokine attractants with subsequent accumulation of large populations of macrophages/microglia immune cells within the tumor and smaller accumulations of lymphocytes and mast cells. Under normal conditions, this would result in the release of high levels of inflammatory cytokines and lead to tumor killing. While many studies have shown either *in vitro* or when using fresh tumor specimens, evidence of increased inflammation, a growing literature suggest that within the tumor there is impaired immune competence for tumor rejection, along with a reduction in the release of critical immune factors from microglia/macrophages and elevation of growth promoting cytokines, such as TGF-1ß, IL-10, IL-23, TNF-α, and IL-6.[51] The main source of this suppressive microenvironment appears to be from factors released from GBM stem cells and the attraction of suppressor T$_{regs}$ to the tumor, which block cytotoxic T-lymphocyte function.[82,86,107,125]

Hypoxia also plays a significant role in malignant glioma initiation, aggressiveness, and invasion, partly by

enhancing immunosuppression and inflammation. In essence, cancer stem cells have converted the cellular immune response to one that is favorable to tumor growth, progression, and invasion.

THE ROLE OF GLUTAMATE AND GLUTAMATE RECEPTORS IN GLIOMA GROWTH AND INVASION AND THE LINK TO INFLAMMATORY CYTOKINES

A growing number of studies show that glutamate and its receptors play a major role in cancer development, progression, growth, invasion and metastasis, including gliomas.[15,17,21,53,78,93,114] Ye and Sontheimer demonstrated using both human GBM cells lines and fresh surgical specimens of GBM a 100-fold lower uptake of glutamate from the extracellular space in comparison to normal astrocytes.[126] They also noted a 3-fold increase in glutamate release over a 12-hour period from glioma cells by active transport. Combined, this greatly increases extraneuronal glutamate concentrations both within the tumor and at the tumor margin.

Subsequent studies have shown that glioma cells, both from GBM cell lines and fresh surgical specimens, lack the principle glutamate transport proteins, EAAT1 (GLAST) and EAAT2 (GLT-1), the latter being most important for control of extracellular glutamate levels in the adult brain.[127] While suppression of glutamate uptake alone is sufficient to trigger excitotoxicity, of particular importance is the reverse transport of glutamate, which can occur by two mechanisms-reverse transport by EAATs and increased activity of the system X$_c$ antiporter. The latter mechanism exchanges extracellular cystine for intracellular glutamate, leading to elevations in extracellular glutamate when the EAAT transporters are simultaneously dysfunctional. That is, normally, the expelled glutamate is rapidly taken up by GLT-1, but in the case of gliomas, GLT-1 is nonfunctional.

Recent studies have shown that glioma cells, particularly GBM cells, demonstrate a dramatic enhancement of system X$_c$ cystine/glutamate exchange activity, which markedly elevates extracellular glutamate levels.[22,79] Furthermore, the release of excess glutamate acts in an autocrine and paracrine manner to stimulate glutamate receptors on nearby neurons in the surrounding brain, leading to excitotoxic cell death and promotion of glioma cell migration and invasion.[79] It has been proposed that induced excitotoxicity is at the leading edge of the expanding glioma, allowing for rapid growth of GBMs in an enclosed cranium[117] [Figure 1]. In addition, excitotoxicity triggered by glutamate release from glioma cells is also responsible for necrosis within gliomas, a hallmark for GBM type gliomas.[85]

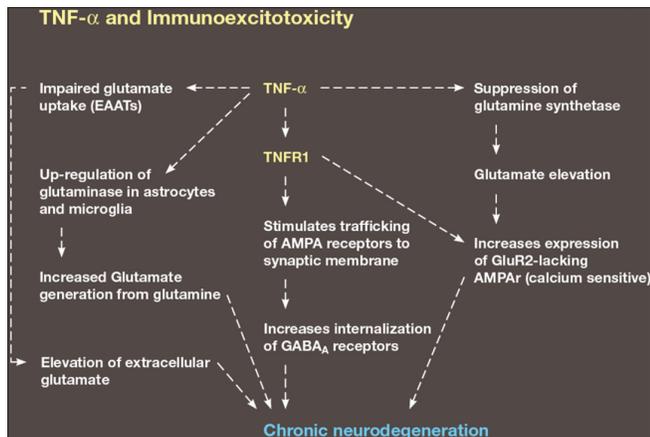Lyons *et al.*, using a variety of GBM cell lines and acute GBM surgical specimens, found that sulfasalazine,

Figure 1: Diagram demonstrating the interactions of TNF-a with membrane receptors leading to an up-regulation of GluR2-lacking AMPA receptor trafficking to synaptic membranes and internalization of GABA receptors. TNF-a also triggers an up-regulation of glutaminase, which stimulates glioma invasion and provides energy molecules for cellular growth and proliferation. In addition, it also suppresses GLT-1 regulated glutamate uptake, leading to excitotoxic levels of extracellular glutamate. The glioma stem cells are mostly resistant to immunoexcitotoxicity

an inhibitor of the system Xc cysteine/glutamate antiporter, reduced glioma cell migration by 40-50% for cells tested *in vitro*.[79] Using a glioma mouse model they injected D54-MG GBM (GBM cell line) cells into the cerebrum of severe combined immunodeficiency (SCID) mice and compared tumor growth and invasion when system Xc was inhibited versus controls with an intact system Xc. They used sulfasalazine and S-(4)-CPG to suppress system Xc.[41,92]

Control animals demonstrated large, highly invasive tumors with distant satellite tumors, whereas both the sulfasalazine and S-(4)-CPG treated animals had much smaller tumors, no evidence of tumor invasion at the boundary and few if any satellite tumors. Others have also shown that inhibition of system Xc slows tumor growth and significantly extends the life of the animal.[22] Lyons *et al*. demonstrated a dramatic up-regulation of system Xc and GluR2-lacking, Ca^{2+}-permeable alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in glioma cells lines and surgical GBM specimens. Elevations in extracellular glutamate and associated calcium oscillations were blocked when system Xc was inhibited.[79]

Sulfasalazine, a drug used to treat ulcerative colitis, penetrates the brain easily and has a good safety profile.[110] The doses used in the above study were comparable to that used in human treatment of bowel diseases. Cystine is rapidly generated by oxidation of cysteine.[68] Lyons *et al*. demonstrated that cystine loading increased glioma cell Ca^{2+} oscillations by stimulating glutamate release. Cystine is also used by glial cells to generate glutathione, which protects cancer cells from apoptosis.

In vivo studies using spectroscopic magnetic resonance imaging (MRI) have confirmed that the peritumoral fluid surrounding high grade oligodendrogliomas contains increased levels of glutamate.[99] This localized elevation in glutamate has also been shown using microdialysis studies from necrotic tissues of GBMs.[100] It should be appreciated that glutamate accumulation also causes rapid activation of adjacent microglia leading to immunoexcitotoxicity[11,12] [Figure 1]. Glutamate, as well as chemokines, stimulate migration of microglia to the tumor, explaining the dense population of microglia seen in high-grade astrocytomas.[76] Microglia are major sources of both glutamate and pro-inflammatory cytokines.

Glutamate and its receptors play a major role in cell migration during brain development.[63] This has been extensively studied in the case of cerebellar granule cell migration during neurodevelopment and has been shown to be dependent on GluR2-lacking, Ca^{+2}-permeable AMPA glutamate receptors, which also play a major role in human malignant glioma and GBM migration and invasion.[31,53,79]

LINK BETWEEN INFLAMMATORY TNF-A AND EXCITATORY GLUTAMATE RECEPTOR ENHANCED SENSITIVITY

In the normal brain, TNF-α is release in low concentrations, whereas most other cytokines are undetectable. A number of studies have shown that TNF-α plays a major role in constitutive plasticity, neural circuit function and LTP by regulating AMPA receptor (AMPAR) trafficking to the neural cell membrane by a process of exocytosis from nearby endoplasmic reticulum.[8,112] AMPA receptor insertion points include the cell body and dendrite spines, with the latter having the highest concentration. Most of the inserted AMPARs are extrasynaptic but approximately 25% will move laterally to be inserted in the postsynaptic density, significantly increasing glutamate excitation.

A real time study of individual living neurons demonstrated rapid (milliseconds) insertion of AMPARs following stimulation by TNF-α.[128] Neuronal sensitivity to glutamate receptor activation was regulated both by constitutional TNF-α release and higher levels of release under pathological conditions, such as with inflammation.[18,43] TNFR1 has been shown to be necessary for AMPAR trafficking, demonstrating a strong link between inflammation and excitatory glutamate receptor activation.[112] Activation of AMPAR trafficking is mostly limited to TNF-α and less so IL-1ß. IL-6 and IL-10 had no effect on AMPAR insertion into cell membranes. Phosphotidylinositol-3 kinase (PI3K) is an essential cell-signaling molecule for TNFR1 activation of AMPAR trafficking. GBMs have been shown to have high concentrations of PI3K.[74]

IL-6 is frequently produced by glioma cells and may play a role in tumor growth, peritumoral edema and angiogenesis. Chang *et al.* found that patients with IL-6 negative GBM tumors had a median survival of 16 months, while those with IL-6 positive GBMs had a median survival of only 7 months.[16] Three patients had survivals longer than 1 year and these showed no IL-6 expression. IL-6, IL-10, and IL-27 can all activate STAT3 (IL-6-STAT3 axis) and thereby can suppress macrophage immunocompetence.[66] In addition, elevations in glutamate were found to stimulate the release of IL-6 in rat hippocampus.[19] As a result, one would expect higher levels of IL-6 in patients with the highest tumoral glutamate levels.

CA²⁺-PERMEABLE AMPA RECEPTORS, INFLAMMATION AND GLIOMA INVASION

Normally, in the adult brain, AMPA receptors are calcium impermeable, primarily because they contain the GluR2 subunit. Of the ionotropic receptors, N-methy-D-aspartate (NMDA), AMPA and kainate receptors, it is the NMDA receptors that normally move extracellular calcium inside the cell, but gliomas do not contain functional NMDA receptors, leaving Ca^{2+}-permeable (GluR2 subunit-lacking) AMPA receptors as the principle ionotropic receptor in gliomas.[53,79] Ca^{2+}-permeable AMPA receptors are increasingly found in a number of neuropathological conditions including amyotrophic lateral sclerosis (ALS), strokes, chronic pain syndromes, spinal cord injury, and brain trauma chronic traumatic encephalopathy (CTE).[14,38,75,119]

Under physiological conditions, transient insertion of Ca^{2+}-permeable AMPA receptors occurs in various brain areas associated with memory and learning processes.[95] The persistent presence and even transient presence of these Ca^{2+}-permeable receptors on neuronal membranes increases the risk of excitotoxicity.[67] One of the most consistent driving forces for pathological insertion of Ca^{2+}-permeable AMPA receptors is the presence of high levels of pro-inflammatory cytokines, in particular TNF-α[67,75,112] [Figure 2]. These observations further emphasize the importance of the interaction between immune factors and glutamate receptors (immunoexcitotoxicity).

Studies have consistently shown rapid, robust insertion of GluR1-containing AMPARs on dendritic membranes following exposure to TNF-α.[8] More recently, it was shown that initially these inserted AMPARs are composed of the GluR2-lacking subunit, which makes them highly calcium permeable. Even a transient presence of Ca^{2+}-permeable AMPA receptors to synaptic membranes increases excitotoxic sensitivity[112,128] [Figure 3].

Compelling evidence suggest Ca^{2+}-permeable AMPA receptors also play a critical role in glioma invasion.[53,79] In the Lyons *et al.* study, all glioma cell lines and surgical specimens of human gliomas demonstrated that GluR2-lacking AMPARs were universally present, with higher concentrations in GBM cells.[79] Blocking these receptors, either with GYKI, a broad-spectrum AMPAR blocker or Joro-spider toxin, a specific GluR2-lacking-AMPAR blocker, reduced glioma cell migration 60%.[31] NASPM, a synthetic joro spider toxin analog, which also blocks Ca^{+2}-permeable GluR2-lacking AMPARs, has been shown to reduce TNF-α enhancement of excitotoxicity, thus demonstrating a possible link between enhanced AMPAR sensitivity and increase glioma cell migration.[67]

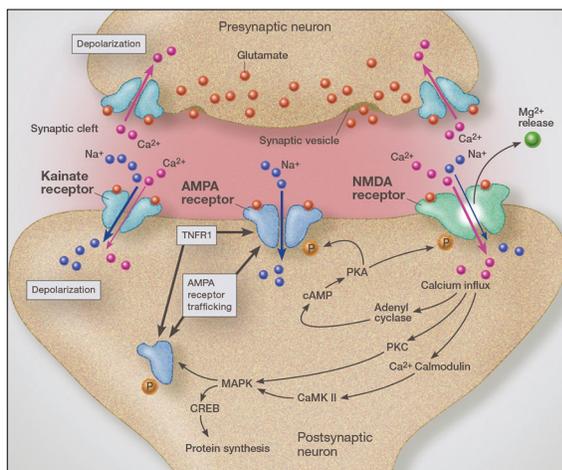Another important process in glioma invasion is glutamate-induced migration along blood vessels and

Figure 2: Illustration demonstrating the trafficking of AMPA receptors stimulated by TNF-α activation of the neurodestructive TNFR1 receptor. This can lead to immunoexcitotoxic destruction of the brain surrounding the tumor, thus allowing for expansion. It can also result in central tumor necrosis commonly seen with in glioblastomas
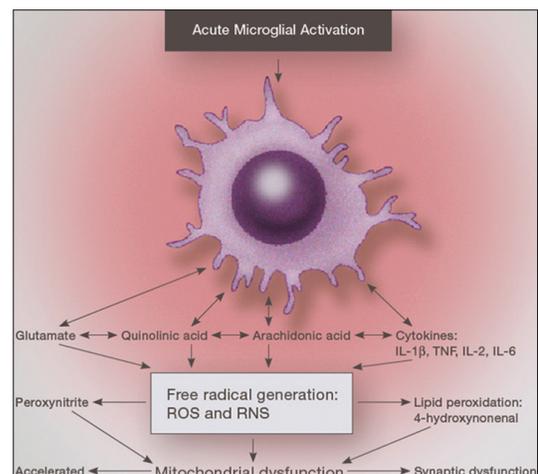

Figure 3: Illustration of immune and excitotoxic factors released by an activated microglia and the effects on mitochondrial function caused by the generation of free radicals, lipid peroxidation products, fatty acid molecules (arachidonic acid), pro-inflammatory cytokines and their interaction resulting in immunoexcitotoxicity

neural pathways. Piao *et al.* found that AMPA receptors containing high levels of the GluR1, such as in the case of GBMs, promoted perivascular glioma invasion by promoting ß-integrin-dependent adhesions to the extracellular matrix.[93] The GluR1 subunit may be linked to the integrin receptor by way of an erythrocyte membrane protein (EMP). Piao *et al.* found that expression of AMPA receptors directly correlated with the level of glioma cell invasion and distant metastasis via perivascular and subpial invasion both *in vitro* and *in vivo*. With TNF-α rapidly increasing AMPA receptors containing GluR1 and GluR2-lacking (Ca^{+2} permeable) AMPA receptors, one would expect increased invasion potential. The TNF-α amplification of AMPA receptor trafficking is dose dependent, meaning that the greater the tumor-associated inflammation, the greater the excitotoxic stimulation of tumor invasion and proliferation.

Using a Matrigel Transwell migration/invasion assay they confirmed a direct correlation between GluR1 and GluR4 expression and the degree of glial migration and invasion.[93] This finding shows that subunit composition of AMPA receptors determines the behavior of glioma cells in terms of invasiveness and that gliomas (especially the higher grade gliomas) have a special, pro-invasive subunit composition (High GluR 1 and low or absent GluR2).

Piao *et al.* also found that cells overexpressing the GluR1 subunit have significantly more focal adhesions (Fas) than control cells.[93] A dynamic process of adhesion and release determines migration along perivascular pathways, and both processes can be controlled by GluR1 expression.[30,93] Other studies have confirmed that glutamate can increase integrin surface expression in neurons.[72]

Invasion of normal brain requires penetration of the extracellular matrix, mainly by proteolytic enzymes. Matrix metalloproteinase (MMPs), a group of proteolytic enzymes, can erode through the extracellular matrix, and are released from the invadopodia. Studies have shown that their expression is increased by elevated glutamate levels.[55]

GLUTAMATE-INDUCED CELL SIGNALING AND GLIOMA INVASION

A number of studies have examined cell signaling linkage to glutamate receptor activation and how these pathways enhance glioma invasion and metastasis.[53-55,93] Several key processes seem to be involved and include PTEN, NF-κB, Akt, and calpain2, all of which are linked to glutamate receptors, either directly or indirectly.[4,53,55,84,85,90]

PTEN, a phosphatase and tensin homolog protein, is produced by the PTEN gene as a tumor suppressor and is frequently mutated in astrocytomas; more often in GBM than low-grade astrocytomas.[3,69] Interestingly, PTEN is more frequently mutated in primary than in secondary GBM and is known to accelerate progression of low-grade astrocytomas to GBM.[61,65]

PTEN suppresses tumor growth and acceleration by inhibiting Akt, another tumor growth promoter.[46] Akt expression is linked to NF-κB activation, which is in turn activated by glutamate.[56,84] The latter observation provides a link between inflammation, glutamate receptor activation (immunoexcitotoxicity), and tumor invasion and aggressiveness.

Akt expression has been shown to confer a more aggressive phenotype to malignant tumor cells by suppression of tumor cell apoptosis.[6] The combination of PTEN mutations along with elevated extracellular glutamate levels leads to Akt pathway activation resulting in tumor growth stimulation.[85]

One of the principle cell signaling pathways linking tumor aggressiveness to glutamate receptor activation is calpain2, which is activated by Ca^{2+} entry into glial cells.[55] One of the early links between calpain and cancer invasion was demonstrated by Shiba *et al.*, who found increased levels of calpain in breast cancer tissue compared with normal breast tissue cells.[106] Subsequently it was found that knocking down calpain2 reduced breast cancer invasion by ~50%, mainly by regulating invadopodia projections necessary for movement through the extracellular matrix.[27]

Ca^{2+}-permeable AMPA receptors allow entry of Ca^{2+} into the glioma cells, which not only increases intracellular Ca^{2+} levels but also initiates Ca^{2+} oscillations that extend from the Ca^{2+}- channel inward. It is the Ca^{2+} oscillations that control migration of glioma cells and invadopodia activity.[79] These oscillations have also been shown to play a critical role in focal adhesion assembly and disassembly necessary for invasion of GBM cells into surrounding tissues.[40]

Jang *et al.*, using a Matrigel Transwell migration/invasion assay of U87MG human GBM cells with knockdown of calpain2 expression, found that invasion was ~90% lower in the calpain2 knockdown cells compared with control glia.[55] They also observed a 39% lower level of MMP-2 in the knockdown cells, a major mechanism of glioma invasion. Reactive oxygen species also play important roles in signaling activation pathways of invadopodia and are abundantly generated during immunoexcitotoxicity.[122]

More than 95% of human primary brain tumors express astrocyte-elevated gene-1 (AEG-1) a factor that may provide GBMs with significant growth stimulation.[35,57] AEG-1 protects glioma cells from apoptosis and acts via the Akt pathway.[68] AEG-1 also activates the promoter of NF-κB and enhances formation of the NF-κB transcription activator p50/p65 complex in the nucleus, which activates numerous pro-inflammatory cytokines, several of which act as tumor growth promoters.[35]

Interestingly, AEG-1, like NF-κB and TNF-α, have a negative effect on the EAAT2 (GLT-1) glutamate transporter, which, when impaired, raises extracellular glutamate levels.[57] Thus, there appears to be a cyclic buildup of glutamate involved in glioma progression and invasion, involving numerous interacting mechanism and this significantly enhances the proliferation, aggressiveness and invasion potential of glial tumors.

FUTURE DIRECTION IN CONTROLLING GLIOMA GROWTH AND INVASIVENESS

Conventional treatment of anaplastic astrocytomas and GBMs has been overall a failure. Death rates and length of survival of GBM patients is essentially the same as it was 40 years ago.[87] This failure is based on the fact that most such treatments are directed against single cancer cell mechanisms that can be quickly overcome by the cancer cells. Recent evidence suggest that of more importance to resistance is the finding that malignant gliomas arise from GBM stem cells that are resistant to virtually all chemotherapy agents and radiation.[7,32,36,39] These tumor stem cells create an immunosuppressive microenvironment within the tumor that prevents successful immune system elimination of the tumor but yet the tumor itself is associated with significant inflammation at the same time. In addition, the pro-inflammatory cytokine TNF-α has been shown to stimulate neural stem cell proliferation via NF-κB signaling, which is also activated by glutamate stimulation. This suggests that glutamate may stimulate GBM stem cell proliferation via NF-κB activation as well.

The role of glutamate excess in tumor growth, progression, invasion, and on rare occasions, metastasis, has been firmly established for a great number of cancers, including primary brain cancers. A number of recent studies have shown that reducing glutamate levels or blocking specific glutamate receptors can dramatically reduce tumor induction, proliferation and in particular, invasion.[79,101,113]

Now that glutamate receptor activation has been linked to both tumor expansion by increased proliferation as well as other mechanisms, such as a stimulation of invasion and progressive necrosis of the normal brain along its leading edge, what should the neurosurgeon do to reduce this immunoexcitotoxic effect? That is, should we change our targets for a more successful treatment protocol?

Olney and other studies have shown that many commonly eaten foods contain free glutamate levels that can produce brain lesions in animals when orally ingested.[88,89,111] Because the blood–brain barrier is designed only to prevent brain injury from acute elevations in glutamate, chronic ingestion of high glutamate-containing foods presents a special problem. A great number of processed foods contain one of more sources of free glutamate and/or aspartate.

During chronic ingestion the elevated levels of plasma glutamate can enter the brain via the circumventricular organs (CVO) and in the case of gliomas, especially GBM, the blood–brain barrier is often compromised, allowing glutamate to freely enter the area of the tumor.

With impaired glutamate transport, orally ingested and intrinsically generated glutamate can add to the already elevated extracellular glutamate levels seen in gliomas and could potentially increase tumor invasion. A number of commercial enteral feeding formulas have high concentrations of free glutamate, cysteine, and aspartate manufactured from processed hydrolyzed casein, hydrolyzed whey protein, crystalline L-amino acids, and soy protein isolate, all known to provide very high concentrations of excitotoxic amino acids.[81]

Neurosurgeons and neurologists should counsel their patients to avoid foods high in glutamate and processed foods containing excitotoxin additives, such as monosodium glutamate, hydrolyzed protein, soy protein concentrates and isolates, whey protein isolates, autolyzed yeast, sodium or calcium caseinate, and other food label names for disguised forms of glutamate.

In terms of clinical treatment, a wide array of natural substances have been shown to regulate cancer cell signaling pathways and reduce cancer growth, aggressiveness and invasion by a great number of well-defined mechanisms.[45,59,129] By inhibiting a large number of essential cellular signaling mechanisms used by cancer cells for survival, growth, and invasion, these natural products may hold more promise than conventional chemotherapy and can also enhance the effectiveness and safety of traditional treatments.

Of particular interest is curcumin, an extract from the spice turmeric, which inhibits a number of cancer signaling pathways including NF-κB, the oncogenes c-myc, c-fos, c-jun, MAPKs, ERK, PI3K, Akt, CDKs, iNOS, mTOR, PKC, and EGFR tyrosine kinase.[73] Studies have demonstrated antiangiogenesis effects and reduced metastasis and invasion in cancer models.[64] In addition, curcumin has been shown to be a chemosensitizer and radiosensitizer for cancer cells and protects surrounding normal cells from damage by these same treatments.[70,71]

Of considerable interest is the ability of curcumin to down-regulate multidrug-resistance (MDR) and inhibit STAT3.[20,52] Curcumin has a very high safety profile even when given in large doses. Curcumin also shows significant activity against GBM cells *in vitro* and *in vivo*.[5] Quercetin suppresses NF-κB, LOX, and PI3K, which are linked to inflammation, tumor proliferation, and AMPAR trafficking.[120]

Clinically, the main difficulty with curcumin, and some of the other flavonoids, is with poor absorption orally when used in a powdered form. Newer techniques in

pharmacology and drug delivery, such as nanosizing, lipid encapsulation and mixing it with certain oils, such as extravirgin olive oil, medium-chain triglycerides (MCT) oil, and coconut oil, have greatly increased the bioavailability of these extracts. Once in the blood, curcumin readily enters the brain.

Other cancer inhibiting extracts, such as quercetin, ellagic acid, hesperidin, silymarin, resveratrol, tea catechins, epigallocatechin gallate, and luteolin hold great promise in providing safer and more effective treatments for gliomas. A discussion of these extracts will be covered in an upcoming article.

Another approach is the suppression of glutaminase, the enzyme that converts glutamine to glutamate within glial cells. Studies targeting this rate-limiting enzyme have shown slowed glioma cell growth *in vitro*.[104,121]

In conclusion, given the multifactorial nature of the GBMs, the rationale behind conventional chemotherapeutic treatment methods aimed at targeting a single pathway should be reconsidered. A much better option for treating these highly aggressive tumors may instead, lie in a multi-prong attack on diverse cooperating cell signaling and metabolic factors, which are essential for cancer cell survival and invasion. In this regard, several natural compounds hold a great and yet much underutilized therapeutic potential.

REFERENCES

1. Abou-Ghazal M, Yang DS, Qiao W, Reina-Ortiz C, Wei J, Kong LY, *et al.* The incidence, correlation with tumor-infiltrating inflammation, and prognosis of phosphorylated STAT3 expression in human gliomas. Clin Cancer Res 2008;14:8228-35.
2. Acarin L, Peluffo H, Gonsalez B, Castellano B. Expression of inducible nitric oxide synthase and cyclooxygenase-2 after excitotoxic damage to the immature rat brain. J Neurosci Res 2002;68:745-54.
3. Aggarwal BB. Nuclear factor-kappaB: The enemy within. Cancer Cell 2004;6:203-8.
4. Ali IU, Schriml LM, Dean M. Mutational spectra of PTEN/MMAC1 gene: A tumor suppressor with lipid phosphatase activity. J Natl Cancer Inst 1999;91:1922-32.
5. Aoki H, Takada Y, Kondo S, Sawaya R, Aggarwal BB, Kondo Y. Evidence that curcumin suppresses the growth of malignant gliomas *in vitro* and *in vivo* through induction of autophagy: Role of Akt and extracellular signal-regulated kinase signaling pathways. Mol Pharmacol 2007;72:29-39.
6. Bacus SS, Altomare DA, Lyass L, Chin DM, Farrell MP, Gurova K, *et al.* Akt2 is frequently upregulated in HER-2/neu-positive breast cancers and may contribute to tumor aggressiveness by enhancing cell survival. Oncogene 2002;21:3532-40.
7. Bao S, Wu Q, Mclendon RE, Hao Y, Shi Q, Hielmeland AB, *et al.* Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 2006;444:756-60.
8. Beattie EC, Stellwagen D, Mortishita W, Bresnahan JC, Ha BK, Von Zastrow M, *et al.* Control of synaptic strength by glial TNFalpha. Science 2002;295:2282-85.
9. Bernadino L, Xapelli S, Silva AP, Jakobsen B, Poulsen FR, Oliverira CR, *et al.* Modular effects of interleukin-1beta and tumor necrosis factor-alpha on AMPA-induced excitotoxicity in mouse organotypic slice cultures. J Neurosci 2005;25:6734-44.
10. Bian XW, Yang SX, Chen JH, Ping YF, Zhou XD, Wang QL, *et al.* Preferential expression of chemokine receptor CXCR4 by highly malignant human gliomas and its association with poor patient survival. Neurosurgery 2007;61:570-8.
11. Biber K, Neumann H, Inuoue K, Boddeke HW. Neurons 'On' and 'Off' signals control microglia. Trends Neurosci 2007;30:569-602.
12. Blaylock R, Maroon J. Immunoexcitotoxicity as a central mechanism in chronic traumatic encephalopathy-a unifying hypothesis. Surg Neurol Int 2011;2:107.
13. Bochme SA, Lio FM, Maciejewski-Lenoir D, Bacon KB, Conlon PJ. The chemokine fractalkine inhibits Fas-mediated cell death of brain microglia. J Immunol 2000;165:397-403.
14. Bogaert E, d'Ydewalle C, Van Den Bosch L. Amyotrophic lateral sclerosis and excitotoxicity: From pathological mechanism to therapeutic target. CNS Neurol Disord Drug Targets 2010;9:297-304.
15. Brocke KS, Stufner C, Luksch H, Geiger KD, Stepulak A, Marzahn J, *et al.* Glutamate receptors in pediatric tumors of the central nervous system. Cancer Biol Ther 2010;9:455-68.
16. Chang CY, Li MC, Liao SL, Huang YL, Shen CC, Pan HC. Prognostic and clinical implications of IL-6 expression in glioblastoma multiforme. J Clin Neurosci 2005;12:930-3.
17. Chang HJ, Yoo BC, Lim SB, Jeong SY, Kim WH, Park JG. Metabotropic glutamate receptor 4 expression in colorectal carcinoma and its prognostic significance. Clin Cancer Res 2005;11:3288-95.
18. Chao CC, Hu S. Tumor necrosis factor-alpha potentiates glutamate neurotoxicity in human fetal brain cell cultures. Dev Neurosci 1994;16:172-9.
19. Chaparro-Huerta V, Rivera-Cervantes MC, Flores-Soto ME, Gomez-Pinedo U, Beas-Zarate C. Proinflammatory cytokines and apoptsis following glutamate-induced excitotoxicity mediated by p38 MAPK in the hippocampus of neonatal rats. J Neuroimmunol 2005;165:53-62.
20. Choi BH, Kim CG, Lim Y, Shin SY, Lee YH. Curcumin down-regulates the multidrug-resistance mdr1b gene by inhibiting the PI3K/Akt/NF Kappa B pathway. Cancer Lett 2008;259:111-8.
21. Choi KY, Chang K, Pickel JM, Badger JD 2nd, Roche KW. Expression of the metabotropic glutamate receptor 5 (mGluR5) induces melanoma in transgenic mice. Proc Natl Acad Sci USA 2011;108:15219-24.
22. Chung WJ, Lyons SA, Nelson GM, Hamza H, Gladson CL, Gillespie GY, *et al.* Inhibition of cystine uptake disrupts the growth of primary brain tumors. J Neurosci 2005;25:7101-10.
23. Cobbs CS, Brenman JE, Aldape KD, Bredt DS, Israel MA. Expression of nitric oxide synthase in human central nervous system tumors. Cancer Res 1995;55:727-30.
24. Cobbs CS, Samanta M, Harkins LE, Gillespie GY, Merrick BA, MacMillan-Crow LA. Evidence for peroxinitrite-mediated modifications to p53 in human gliomas: Possible functional consequences. Arch Biochem Biophys 2001;394:167-72.
25. Collet G, Skrzypek K, Grillion C, Matejuk A, El Hafni-Rahbi B, Lamerant-Favel N, *et al.* Hypoxia control to normalize pathologic angiogenesis: Potential role for endothelial precursor cells and miRNAs regulation. Vascul Pharmacol 2012;56:252-61.
26. Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: Links to genetic instability. Carcinogenesis 2009;30:1073-81.
27. Cortesio CL, Chan KT, Perrin BJ, Burton NO, Zhang S, Zhang ZY, *et al.* Calpain 2 and PTP1B function in a novel pathway with Src to regulate invadopodia dynamics and breast cancer cell invasion. J Cell Biol 2008;180:957-71.
28. Coussens LM, Werb Z Inflammation and cancer. Nature 2002;420:860-7.
29. Coussens LM, Werb Z Inflammatory cells and cancer: Think different! J Exp Med 2001;193:F23-6.
30. de Groot JF, Piao Y, Lu L, Fuller GN, Yung WK. Knockdown of GluR1 expression by RNA interference inhibits glioma proliferation. J Neurooncol 2008;88:121-33.
31. Deng W, Rosenberg PA, Volpe JJ, Jensen FE. Calcium-permeable AMPA/kainite receptors mediate toxicity and preconditioning by oxygen-glucose deprivation in oligodendrocyte precursors. Proc Natl Acad Sci USA 2003;100:6801-6.
32. Dimov I, Tasic-Dimov D, Conic I, Stefanovic V. Glioblastoma multiforme stem cells. Scientific World Journal 2011;11:930-58.
33. Ehtesham M, Mapara KY, Stevenson CB, Thompson RC. CXCR4 mediates the proliferation of glioblastoma progenitor cells. Cancer Lett 2009;274:305-12.
34. Eiro N, Vizoso FJ Inflammation and cancer. World Gastrointest Surg 2012;4:62-72.
35. Emdad L, Sarkar D, Su ZZ, Randolph A, Boukerche H, Valerie K, *et al.* Activation of the nuclear factor kappaB pathway by astrocyte gene-1: Implications for tumor progression and metastasis. Cancer Res

2006;66:1509-16.

36. Eramo A, Ricci-Vitiani L, Zeuner A, Pallini R, Lotti F, Sette G, *et al*. Chemotheray resistance of glioblastoma stem cells. Cell Death Differ 2006;13:1238-41.

37. Fathallah-Shaykh HM, Gao W, Cho M, Herrera MA. Priming in the brain, an immunologically privileged organ, elicits anti-tumor immunity. In J Cancer 1998;75:266-76.

38. Ferguson AR, Christensen RN, Gensel JC, Miller BA, Sun F, Beattie EC, *et al*. Cell death after spinal cord injury is exacerbated by rapid TNF-alpha-induced trafficking of GluR2-lacking AMPARs to the plasma membrane. J Neurosci 2008;28:11391-400.

39. Ghods AJ, Irvin D, Liu G, Yuan X, Abdulkadir IR, Tunici B, *et al*. Spheres isolated from 9 L gliosarcoma rat cell line possess chemoresistant and aggressive cancer stem-like cells. Stem Cells 2007;25:1645-53.

40. Giannone G, Ronde P, Gaire M, Haiech J, Takeda K. Calcium oscillations trigger focal adhesion disassembly in human U87 astrocytoma cells. J Biol Chem 2002;277:26364-71.

41. Gout PW, Buckley AR, Simms CR, Bruchovsky N. Sulfasalazine, a potent suppressor of lymphoma growth by inhibition if the x(c)-cystine transporter: A new action for an old drug. Leukemia 2001;15:1633-40.

42. Graham V, Khudyakov J, Ellis P, Pevny L. SOX2 functions to maintain neural progenitor identity. Neuron 2003;39:749-65.

43. Grassi F, Miles AM, Monaco L, Puntuieri A, Santoni A, Eusebi F. TNF-alpha increases the frequency of spontaneous miniature synaptic currents in cultured rat hippocampal neurons. Brain Res 1994;65226-30.

44. Grivennikov SI, Karin M. Dangerous liasions: STAT3 and NF-kappaB collaboration and crosstalk in cancer. Cytokine Growth Factor Rev 2010;21:11-9.

45. Gupta SC, Kim JH, Prasad S, Aggarwal BB. Regulation of survival, proliferation, invasion, angiogenesis, and metastasis of tumor cells through modulation of inflammatory pathways by nutraceuticals. Cancer Metastasis Rev 2010;29:405-34.

46. Haas-Kogan D, Shalev N, Wong M, Mills G, Yount G, Stokoe D. Protein kinase B (PKB/Akt) activity is elevated in glioblastoma cells due to mutation of the tumor suppressor PTEN/MMAC. Curr Biol 1998;8:1195-8.

47. Haas HS, Linecker A, Pfragner R, Sadjak A. Peripheral glutamate signaling in head and neck areas. Head Neck 2010;32:1554-72.

48. Hara A, Okayasu I. Cycloogenase-2 and inducible nitric oxide synthase expression in human astrocytic gliomas: Correlation with angiogenesis and prognostic significance. Acta Neuropathol 2004;108:43-8.

49. Hendriks JJ, Teunissen CE, de Varies HE, Dijkstra CD. Macrophages and neurodegeneration. Brain Res Brain Res Rev 2005;48:185-95.

50. Holladay FP, Choudhuri R, Heitz T, Wood GW. Generation of cytotoxic immune responses during the progression of a rat glioma. J Neurosurg 1994;80:90-6.

51. Hussain SF, Yang D, Suki D, Aldape K, Grimm E, Heimberger AB. The role of human glioma-infiltrating microglia/macrophages in mediating antitumor immune responses. Neuro Oncol 2006;8:261-79.

52. Hutzen B, Friedman L, Sobo M, Likn L, Cen L, De Angelis S, *et al*. Curcumin analogue GO-YO30 inhibits STAT3 activity and cell growth in breast and pancreatic carcinomas. In J Oncol 2009;35:867-72.

53. Ischiuchi S, Yoshida Y, Sugawara K, Aihara M, Ohtani T, Watanabe T, *et al*. Ca^{2+}-permeable AMPA receptors regulate growth of human glioblastoma via Akt activation. J Neurosci 2007;27:7987-8001.

54. Ischiuchi S, Tsuzuki K, Yoshida N, Hagimura N, Okado H, Miwa A, *et al*. Blockage of $Ca(2+)$-permeable AMPA receptors suppresses migration and induces apoptosis in human glioblastoma cells. Nat Med 2002;8:971-8.

55. Jang HS, Lai S, Greenwood JA. Calpain 2 is required for glioblastoma cell invasion: Regulation of matrix metalloproteinase 2. Neurochem Res 2010;35:1796-804.

56. Kaltschmidt C, Kaltschmidt B, Bacuerle PA. Stimulation of inotropic glutamate receptors activates transcription factor NF-κB in primary neurons. Proc Nat Acad Sci U S A 1995;92:9618-22.

57. Kang DC, Su ZZ, Sarkar D, Emdad L, Volsky DJ, Fisher PB. Cloning and characterization of HIV-1-inducible astrocyte elevated gene-1, AEG-1. Gene 2005;353:8-15.

58. Kato T, Sawamura Y, Tada M, Sakuma S, Sudo M, Abe H. P55 and p75 tumor necrosis factor receptor expression in human glioblastoma cells. Neural Med Chir (Tokyo) 1995;35:567-74.

59. Kaur M, Agarwal R. Silymarin and epithelial cancer chemoprevention: How

60. Kim R, Emi M, Tanabe K. Cancer immunosuppression and autoimmune disease: Beyond immunosuppressive networks for tumor immunity. Immunology 2006;119:254-64.

61. Kleihues P, Ohgaki H. Primary and secondary glioblastomas: From concept to clinical diagnosis. Neuro-Oncolol 1999;1:44-51.

62. Kleihues P, Ohgaki H. Phenotype vs genotype in the evolution of astrocytic brain tumors. Toxicol Pathol 2000;28:164-70.

63. Kumuro H, Rakic P. Intracellular Ca2+ fluctuations modulate the rate of neuronal migration. Neuron 1996;17:275-85.

64. Kunnumakkara AB, Anand P, Aggarwal BB. Curcumin inhibits proliferation, invasion, angiogenesis and metastasis of different cancers through interaction with multiple cell signaling proteins. Cancer Lett 2008;269:199-225.

65. Kwon CH, Zhao D, Chen J, Alcantara S, Li Y, Burns DK, *et al*. Pten haploinsufficency accelerates formation of high-grade astrocytomas. Cancer Res 2008;68:3286-94.

66. Lang R. Tuning of macrophage responses by Stat3-inducing cytokines: Molecular mechanisms and consequences in infection. Immunobiology 2005;210:63-76.

67. Leonoudakis D, Zhao P, Beattie EC. Rapid tumor necrosis factor alpha-induced exocytosis of glutamate receptor 2-lacking AMPA receptors to extrasynaptic plasma membrane potentiates excitotoxicity. J Neurosci 2008;28:2119-30.

68. Liang HP, Brophy TM, Hogg PJ. Redox properties of the tissue factor Cys186-Cys209 disulfide bond. Biochem J 2011;437:455-60.

69. Li J, Yen C, Liaw D, Podsypania K, Bose S, Wamg SI, *et al*. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 1997;275:1943-7.

70. Li M, Zhang Z, Hill DL, Wang H, Zhang R. Curcumin, a dietary component, has anticancer, chemosensitization, and radiosensitization effects through the PI3K/mTOR/ETS2 pathway. Cancer Res 2007;67:1988-96.

71. Limtrakul P. Curcumin as a chemosensitizer. Adv Exp Med Biol 2007;595:269-300.

72. Lin CY, Lynch G, Gall CM. AMPA receptor stimulation increases alpha5beta1 integrin surface expression, adhesive function and signaling. J Neurochem 2005;94:531-46.

73. Lin JK. Molecular targets of curcumin. Adv Exp Med Biol 2007;595:227-43.

74. Lino MM, Merio A. PI3Kinase signaling in glioblastoma. J Neurooncol 2011;103:417-27.

75. Liu B, Liao M, Mielke JG, Ning K, Chen Y, Li L, *et al*. Ischemic insults direct glutamate receptor subunit 2-lacking AMPA receptors to synaptic sites. J Neurosci 2006;26:5309-19.

76. Liu GJ, Nagarajah R, Banati RB, Bennett MR. Glutamate induces directed chemotaxis of microglia. Eur J Neurosci 2009;29:1108-18.

77. Lubin FD, Jhonston LD, Sweatt JD, Anderson AE. Kainate mediates nuclear factor-kappa B activation in hippocampus via phosphatidylinositol-3 kinase and extracellular signal-regulates kinase. Neuroscience 2005;133:969-81.

78. Luksch H, Uckermann O, Stepulak A, Hendruschk S, Marzahn J, Bastain S, *et al*. Silencing of selected glutamate receptor subunits modulates cancer growth. Anticancer Res 2011;313181-92.

79. Lyons SA, Chung WJ, Weaver AK, Ogunrinu T, Sontheiner H. Autocrine glutamate signaling promotes glioma cell invasion. Cancer Res 2007;67:9463-71.

80. Maher EA, Furnari FB, Bachoo RM, Rowitch DH, Louis DN, Cavenee WK, *et al*. Malignant glioma: Genetics and biology of a grave matter. Gene Dev 2001;15:1311-33.

81. Malone A. Enteral formula selection: A product Review of Selected Product Catagories. Pract Gastroenterol 2006;44-74.

82. Misra N, Bayry J, Lacroix-Desmazes S, Kazatchkine MD, Kaveri SV. Cutting edge: Human CD4+CD25+Tcells restrain the maturation and antigen-presenting function of dendritric cells. J Immunol 2004;172:4676-80.

83. Mobasheri A, Richardson S, Mobasheri R, Sakibael M, Hoyland JA. Hypoxia inducible factor-1 and faculititive glucose transporters GLUT1 and GLUT3: Putative molecular components of the oxygen and glucose sensing apparatus in articular chondrocytes. Histol Histopathol 2005;20:1327-38.

84. Morales M, Gonzales-Mejia ME, Bernabe A, Hernandez-Kelly LC, Ortega A. Glutamate activates protein knase B (PKB/Akt) through AMPA receptors in cultured Bergmann glia. Neurochem Res 2006;31:423-9.

85. Noch E, Khalili K. Molecular mechanisms of necrosis in glioblastoma: The role of glutamate excitotoxicity. Cancer Biol Ther 2009;8:1791-7.

close are we to bedside? Toxicol Appl Pharmacol 2007;224:350-9.

86. Oberg HH, Juricke M, Kabelitz D, Wesch D. Regulation of T cell activation by TLR ligands. Eur J Cell Biol 2011;90:582-92.

87. Ohka F, Natsume A, Wakabayashi T. Current ternds in targeted therapies for glioblastoma multiforme. Neurol Res Int 2012;2012:878425.

88. Olney JW, Ho OL. Brain damage in infant mice following oral intake of glutamate, aspartate or cysteine. Nature 1970;227:609-11.

89. Olney JW, Labruyere J, de Gubareff T. Brain damage in mice from voluntary ingestion of glutamate and aspartate. Neurobiol Toxicol 1980;2:125-9.

90. O'Neill LA, Kaltschmidt C. NF-κB: A crucial transcription factor for glial and neuronal cell function. Trends Neurosci 1997;20:252-58.

91. Pahl HL. Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene 1999;18:6853-66.

92. Patel SA, Warren BA, Rhoderick JF, Bridges RJ. Differentiation of substrate and non-substrate inhibitors of transport system xc(-): And obligate exchanger of L-glutamate and L-cystine. Neuropharmacology 2004;46:273-84.

93. Piao Y, Lu L, de Groot J. AMPA receptors promote perivascular glioma invasion via beta1 integrin-dependent adhesion to the extracellular matric. Neuro Oncol 2009;11:260-73.

94. Pissimissis N, Papageorgiou E, Lembessis P, Armakolas A, Koutsilieris M. The glutamatergic system expression in human PC-3 and LNCaP prostate cancer cells. Anticancer Res 2009;29:371-7.

95. Plant K, Pelkey KA, Bortolotto ZA, Morita D, Terashima A, McBain CJ, *et al.* Transient incorporation of native GluR2-lacking AMPA receptors during hippocampal long-term potentiation. Nat Neurosci 2006;9:602-4.

96. Qiang L, Wu T, Zhang HW, Lu N, Hu R, Wang YJ, *et al.* HIF-1α is critical for hypoxia-mediated maintenance of glioblastoma stem cells by activating Notch signaling pathway. Cell Death Differ 2012;19:284-94.

97. Ravenna L, Sale P, Di Vito M, Russo A, Salvatori L, Tafani M, *et al.* Up-regulation of the inflammatory-reparative phenotype in human prostate carcinoma. Prostate 2009;69:1245-55.

98. Reynes G, Vila V, Martin M, Parada A, Fleitas T, Reganon E, *et al.* Circulating markers of angiogenesis, inflammation, and coagulation in patients with glioblastoma. J Neurooncol 2011;102:35-41.

99. Rijpkema M, Schuuring J, van der Meulen Y, van der Graaf M, Bernsen H, Boerman R, *et al.* Characterization of oligodendrogliomas using short echo time 1 H MR spectroscopic imaging. NMR Biomed 2003;16:12-8.

100. Roslin M, Henricksson R, Bergstrom P, Ungerstedt U, Bergenheim AT. Baseline levels of glucose metabolites, glutamate and glycerol in malignant gliom assessed by sterotatic microdialysis. J Neuro-oncol 2003;61:151-60.

101. Rzeski W, Turski L, Ikonomidou C. Glutamate antagonists limit tumor growth. Proc Natl Acad Sci U S A 2001;98:6372-7.

102. Sakuma S, Sawamura Y, Tada M, Aida T, Abe H, Suzuki K, *et al.* Responses of human glioblastoma cells to human natural tumor necrosis factor-alpha: Susceptibility, mechanics of resistance and cytokine production studies. J Neurooncol 1993;15:197-208.

103. Schwanborn J, Lindecke A. Elvers M, Horojschi V, Kerick M, Rafigh M, *et al.* Microarray analysis of tumor necrosis factor alpha induced gene expression in U373 human glioblastoma cells. BMC Geonomics 2003;4:46.

104. Seltzer MJ, Bennett BD, Joshi AD, Gao P, Thomas AG, Ferraris DV, *et al.* Inhibition of glutaminase preferentially slows growth of glioma cells with mutant IDH1. Cancer Res 2010;70:8981-7.

105. Sen E. Targeting inflammation-induced transcription factor activation: An open frontier for glioma therapy. Drug Discov Today 2011;16:1044-51.

106. Shiba E, Kim S, Fujitani M, Kambayashi I, Tsujimoto S, Shimomura K, *et al.* Possible involvement of calpain in the growth of estrogen receptor positive breast cancer cells. Anticancer Res 1996;16:773-7.

107. Shnaper S, Desbaillets I, Brown DA, Murat A, Migliavacca E, Schluep M, *et al.* Elevated levels of MIC-1/GDF15 in the cerebrospinal fluid of patients are associated with glioblastoma and worst outcome. In J Cancer 2009;125:2624-30.

108. Sica A, Larghi P, Mancino A, Rubino L, Porta C, Totaro MG, *et al.* Macrophage polarization in tumor progression. Semin Cancer Biol 2008;18:349-55.

109. Siveen KS, Kuttan G. Role of macrophages in tumor progression. Immunol Lett 2009;123:97-102.

110. Sontheimer H, Bridges RJ. Sulfasalazine for brain cancer fits. Expert Opin Investig Drugs 2012;21:575-8.

111. Stegink LD, Filer LJ Jr, Baker GL. Plasma amino acid concentrations in normal adults ingesting aspartame and monosodium L-glutamate as part of a soup/beverage meal. Metabolism 1987;36:1073-9.

112. Stellwagen D, Beattie EC, Seo JY, Malenka RC. Differential regulation of AMPA receptor and GABA receptor trafficking by tumor necrosis factor-alpha. J Neurosci 2005;25:3219-28.

113. Stepulak A, Sifringer M, Rzeski W, Endesfelder S, Gratopp A, Pohl EE, *et al.* NMDA antagonist inhibits the extracellular signal-regulated kinase pathway and suppresses cancer growth. Proc Natl Acad Sci U S A 2005;102:15605-10.

114. Stepulak A, Luksch H, Gebhardt C, Uckermann O, Marzahn J, Rzeski W, *et al.* Expression of glutamate receptor subunits in human cancers. Histochem Cell Biol 2009;132:435-45.

115. Tafani M, Russo A, Di Vito M, Sale P, Pellegrini L, Schito L, *et al.* Up-regulation of pro-inflammatory genes as adaptation to hypoxia in MCF-7 cells and in human mammary invasive carcinoma microenvironment. Cancer Sci 2010;101:1014-23.

116. Tafani M, Di Vito M, Frati A, Pelligrini L, De Dantis E, Sette G, *et al.* Pro-inflammatory gene expression in solid glioblastoma microenvironment and in hypoxic stem cells from human glioblastoma. J Neuroinflammation 2011;8:32.

117. Takano T, Lin JH, Aruino G, Gao Q, Yang J, Nedergaard M. Glutamate release promotes growth of malignant gliomas. Nat Med 2001;7:1010-5.

118. Takeuchi H Neurotoxicity by microglia: Mechanisms and potential therapeutic strategy. Clin Exp Neuroimmunol 2010;1:12-21.

119. Van Damme P, Bracken D, Callewaert G, Robberecht W, Van Den Bosh L. GluR2 deficiency accelerates motor neuron degeneration in a mouse model of amyotrophic lateral sclerosis. J Neuropathol Exp Neurol 2005;64:605-12.

120. Walker EH, Pacold ME, Perisic O, Stephens L, Hawkins PT, Wymann MP, *et al.* Structural determinants of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and stauroporine. Mol Cell 2000;6:909-19.

121. Wang JB, Erickson JW, Fuji R, Ramachandran S, Gao P, Dinavahi R, *et al.* Targeting mitochondrial glutaminase activity inhibits oncogenic transformation. Cancer Cell 2010;18:207-19.

122. Weaver AM. Regulation of cancer invasion by reactive oxygen species and Tks family scaffold proteins. Sci Signal 2009;2:pe56.

123. Widera D, Holtkamp W, Entschladen F, Niggemann B, Zanker K, Kaltschmidt B, *et al.* MCP-1 induces migration of adult stem cells. Eur Cell Biol 2004;83:381-7.

124. Widera D, Mikenberg I, Elvers M, Kaltschmidt C, Kaltschmidt B. Tumor necrosis factor α triggers proliferation of adult neural stem cells via IKK/KF-kB signaling. BMC Neurosci 2006;7:64.

125. Wu A, Wei J, Kong LK, Wang Y, Priebe W, Oiao W, *et al.* Glioma cancer stem cells induce immunosuppressive macrophages/microglia. Neuro Oncol 2010;12:1113-25.

126. Ye ZC, Sontheimer H. Glioma cells release excitotoxic concentrations of glutamate. Cancer Res 1999;59:4383-91.

127. Ye ZC, Rothstein JD, Sontheimer H. Compromised glutamate transport in human glioma cells: Reduction-mislocalization of sodium-dependent glutamate transporters and enhanced activity of cystine-glutamate exchange. J Neurosci 1999;19:10767-77.

128. Yudowski GA, Puthenveedu MA, Leonoudakis D, Panicker S, Thorn KS, Beattie EC, *et al.* Real-time imaging of discrete exocytic events mediating surface delivery of AMPA receptors. J Neurosci 2007;27:11112-21.

129. Zaveri NT. Green tea and its polyphenol catechins: Medicinal uses in cancer and noncancer applications. Life Sci 2006;78:2073-80.

130. Zujovic V, Benavides J, Vige X, Carter C, Taupin V. Fractalkine modulate TNF-alpha secretion and neurotoxicity induced by microglial activation. Glia 2000;29:305-15.