

Oxidative Stress and Glutamate Release in Glioma

Robert Ungard and Gurmit Singh
McMaster University
Canada

1. Introduction

Glioma are a family of glial cell tumours of the central nervous system (CNS) well-characterized as aggressive cancers with dismally limited treatment options. The relatively recent discoveries of mechanisms surrounding glioma cell antioxidant protection and neuronal and glial cell destruction have opened the gates to a new therapeutic avenue whose implications to the field of cancer biology extend far beyond the treatment of glioma alone. Stemming from the discovery of significant glutamate release and glutathione production by glioma cells, the mechanisms through which glioma mediate oxidative stress and influence their extracellular microenvironment are now being unravelled.

This chapter will discuss the upregulation of the cystine/ glutamate antiporter, system x_c^- , in glioma and the far ranging consequences that stem from this compensatory action. Specifically, a characteristic shift of cancer cell metabolism away from the tricarboxylic acid (TCA) cycle and towards increased rates of glycolysis, a process termed the Warburg effect, produces a high amount of reactive oxygen species (ROS) that would prove cytotoxic without adequate cellular antioxidant defences. In response to this metabolic abnormality, glioma have demonstrated increased synthesis of the primary cellular antioxidant glutathione and an increased circulation of the cystine/cysteine redox cycle. These antioxidant increases are driven by an upregulation of system x_c^- , which supplies the cell with the rate limiting substrate for glutathione synthesis, cysteine, and acts as one half of the transport machinery for the cystine/cysteine cycle. The increased tolerance to oxidative stress that is conferred by these mechanisms allows glioma survival and growth advantages and mediate chemo- and radiation-resistance to treatment. The corollary effect of cystine import *via* system x_c^- is the export of the neurotransmitter and ubiquitous cell-signalling molecule, glutamate. This release has destructive consequences for the peritumoral brain. Glutamate induces neuronal and glial excitotoxic cell death, and acts in an autocrine and paracrine signalling manner to stimulate glioma cell growth and migration. Treatments based on these mechanisms are currently under development and some have progressed as far as clinical trials. Glutamate receptor antagonists and system x_c^- inhibitors are as of yet the primary avenues of investigation. The potential treatment benefits of targeting these pathways are great, and the discovery of system x_c^- prevalence in other cancers beyond glioma suggests that study of this pathway may produce wide-ranging cancer treatment options.

2. Glioma metabolism and oxidative stress

Cancer cells exist under self-induced conditions of abnormally elevated oxidative stress resulting from a characteristic shift in glucose metabolism away from the TCA cycle and towards a high rate of aerobic glycolysis (Kroemer & Pouyssegur, 2008). This metabolic shift results in less efficient ATP production from glucose by the cell, but serves to confer unique benefits upon the cancer cell allowing survival in conditions of high proliferation, high oxidative stress, and varying access to blood vasculature. One adaptive characteristic of cancer cells is the upregulation of antioxidant defence mechanisms, necessary for protection from the high level of ROS generated by escalated glycolysis.

2.1 Cancer cell metabolism

In normal cells, the metabolism of glucose generates ATP through glycolysis followed by a high rate of pyruvate metabolism through the TCA cycle. The final electron acceptor in the TCA cycle is oxygen, without which, the cycle ceases to function, and pyruvate is converted to lactate *via* anaerobic glycolysis (Kim & Dang, 2006). Anaerobic glycolysis is prevalent in hypoxic environments when the TCA cycle has no access to oxygen, however, glycolysis is also predominant in cancer cells even during aerobic conditions (Kim & Dang, 2006). This phenomenon of cancer cell metabolism was first described in the 1920s by Nobel laureate Otto Warburg and is to this day termed the Warburg effect, or aerobic glycolysis (Warburg et al., 1927). Most cancer cells, limited with regards to energy production by their shift away from the efficiency of the TCA cycle, rely upon an increased rate of glucose uptake for glycolytic ATP production. This allows the cell a number of advantages including the use of glycolytic intermediates for anabolic reactions, without which, rapidly proliferating cells in conditions of fluctuating oxygen availability could not survive (See review by Kroemer & Pouyssegur, 2008). All aerobic respiration generates ROS which induces oxidative damage within the cell (Balendiran et al., 2004). The enhanced metabolic activity of cancer cells raises ROS production to a level that demands adaptation by the cell to survive and proliferate despite the resulting high level of oxidative stress (Halliwell, 2007). The Warburg effect has been identified as a key factor in the increased oxidative stress that cancer cells face, and has also been directly implicated in the activation of oncogenes and the loss of tumour suppressor genes (Le et al., 2010).

2.2 Glutathione synthesis response

Glutathione (GSH) is a tripeptide thiol synthesized intracellularly from the amino acids glutamate, cysteine and glycine. In the cell it performs a number of functions, one of which is as the predominant cellular antioxidant in the body (Meister, 1995). GSH fulfils this role by acting as a substrate for several antioxidant enzymes as well as by acting directly upon free radicals in its reduced form, GSH, or in its oxidized form, glutathione disulfide (Meister, 1995). The rate-limiting step in GSH biosynthesis is the availability of cysteine, which in glioma cannot be synthesized intracellularly (Ishii et al., 1992). In glioma, increased oxidation of intracellular GSH and elevated oxidative stress induce the upregulation of cystine transport into the cell, allowing the dual processes of increased GSH biosynthesis, and increased cycling of the cystine/cysteine redox cycle, both of which counter the effects of ROS mediated damage (Banjac et al., 2008; Chung et al., 2005).

3. The System x_c^- antiporter and glioma

In several cancers including glioma, cysteine must be obtained through the import of cystine from the extracellular environment. Cystine is imported into the cell *via* the system x_c^- cystine/glutamate antiporter; a transporter that is a feature of many cancer cell lines and endogenous to many tissues in the body. Cystine is the oxidized form of the amino acid, comprised of two cysteine molecules joined by a covalent double bond, and more prevalent in the oxidizing extracellular space. In the reducing environment of the cell, imported cystine is rapidly reduced to cysteine which is then incorporated as a substrate in GSH biosynthesis or serves to propel the cystine/cysteine redox cycle that plays a large role in maintaining extracellular redox balance (Ishii et al., 1992). The exported glutamate can have a number of deleterious effects upon the surrounding host tissue, many of which favour cancer cell survival and progression (Ishii et al., 1992).

3.1 System x_c^-

System x_c^- is the name given to the Na^+ independent electroneutral exchanger of cystine and glutamate first described in human fibroblasts by Bannai & Kitamura (1980), and later named by Makowske & Christensen (1982). It is classified within the family of heteromeric amino acid transporters, all of which are comprised of a single heavy polypeptide subunit (SLC3 family) and a single light subunit (SLC7 family) coupled *via* a disulfide bridge (Chillarón et al., 2001). These transporters are essential for the import of amino acids to the cell that cannot be intracellularly synthesized. In system x_c^- the heavy subunit is 4F2hc (SLC3A2), a type II membrane glycoprotein common to many amino acid transporters (Verrey et al., 2004). It plays a regulatory role, functioning to traffic and adhere the transporter complex to the cell membrane. It features one transmembrane domain, and has a molecular weight of ~85 kDa (Lim & Donaldson, 2010). 4F2hc is not essential to the transport action of system x_c^- , and can be supplanted with another heavy chain polypeptide with similar transport and adherence capabilities, (ex. rBAT) without losing antiporter function (Wang et al., 2003). The light subunit of system x_c^- is xCT (SLC7A11), which is entirely responsible for the amino acid exchange function of the transporter and unique to system x_c^- . It features 12 transmembrane domains, and has a molecular weight of ~55 kDa (Lim & Donaldson, 2010). Cystine and glutamate are exchanged with a 1:1 stoichiometry that does not require an ionic gradient, rather it is thought that glutamate, which must be eliminated from the cytosol to prevent toxicity, provides the concentration gradient necessary for transporter function (Bannai & Ishii, 1988).

3.1.1 System x_c^- in glia

System x_c^- is expressed endogenously in a number of tissues in the body. In the human brain, it is a feature of both neurons and glial cells. Specifically, xCT was found to be expressed in neurons of the cerebral cortex, GFAP positive glial cells, vascular endothelial cells and the leptomeninges (Burdo et al., 2006). The prominence of system x_c^- in the brain is thought to be related to the organ's relatively high rate of glucose metabolism and the need for antioxidants to protect highly sensitive neurons from the resulting ROS production (Conrad & Sato, 2011). The expression of xCT fluctuates greatly, and can be readily induced under a number of stimuli including low levels of extracellular cystine (Bannai & Kitamura, 1982), and oxidative stress (Bannai et al., 1991). In astrocytes, the induced upregulation of xCT increased GSH synthesis and release and conferred antioxidant protection on immature

neurons in an *in vitro* co-culture model (Shih et al., 2006). Due to its critical role in maintaining antioxidant and glutamate balance, the misregulation of system x_c^- has the potential for great damage, and the transporter has been implicated in a number of CNS pathologies, including some characteristic features of morbidity in glioma.

3.1.2 System x_c^- in glioma

The ability of xCT to be readily induced upon exposure to oxidative stress or cysteine deficit is thought to be responsible for the presence of system x_c^- as a cell-culture induced artifact in some cell lines. In glioma cell lines this is not the case; system x_c^- has not only been demonstrated in established cell lines (Chung et al., 2005), but also in normal glia (Burdo et al., 2006), and in glioma tumour samples from patients (Lyons et al., 2007).

In glioma, glucose metabolism is significantly escalated, as is characteristic of cancer cell metabolism. The resulting increase in the production of ROS from aerobic glycolysis induces the upregulation of xCT expression (Kim et al., 2001). With 4F2hc present in abundance, this xCT increase is sufficient to initiate an upregulation of system x_c^- characteristic of glioma (Sontheimer, 2008). The consequences of this system x_c^- upregulation are immensely detrimental to the patient as a result of both of the substrate actions of the antiporter. The greater import of cystine by system x_c^- allows the cell to survive and proliferate in conditions of oxidative stress that would be lethal to other cells. This increased resistance has a destructive outcome for the patient, allowing the glioma to survive and progress to a greater extent, and endowing the cell with resistance to cancer treatments, many of which attack cancer cells through increased oxidative stress. The simultaneous export of high levels of glutamate into the microenvironment of glutamate-sensitive brain tissues induces neuron and glial cell death and promotes the growth and migration of the tumour. These uniquely destructive outcomes from the action of system x_c^- ultimately aid the progression of the cancer. (See Fig. 1 concept model).

4. System x_c^- and oxidative stress in glioma

The upregulation of xCT readily occurs in response to oxidative stress. To mediate oxidative damage, the system x_c^- antiporter acts in two cystine-dependent manners to provide antioxidant capabilities to the cell and surrounding microenvironment. By increasing the availability of intracellular cysteine, this rate-limiting substrate is provided for both the synthesis of GSH, and for the completion of one half of the cystine/cysteine redox cycle.

4.1 System x_c^- drives glutathione synthesis

Within the cell, the tripeptide GSH is synthesized from its constituent amino acids, glycine, glutamate and cysteine *via* the enzymes γ -glutamylcysteine synthetase, adding glutamate; and glutathione synthetase, adding glycine in two steps (See Fig. 2). Oxidized GSH can be reduced back to its active form *via* glutathione reductase (Conrad & Sato, 2011). The rate-limiting factor in this pathway is the availability of intracellular cysteine (Ishii et al., 1987). Most mammalian cells have the ability to directly import cysteine with a number of transporters (Lo et al., 2008), however cysteine is not prevalent in the extracellular space to the degree of cystine. Upon export from the cell, cysteine, the reduced and more prominent intracellular form of the amino acid, is rapidly oxidized to cystine, which is vastly more

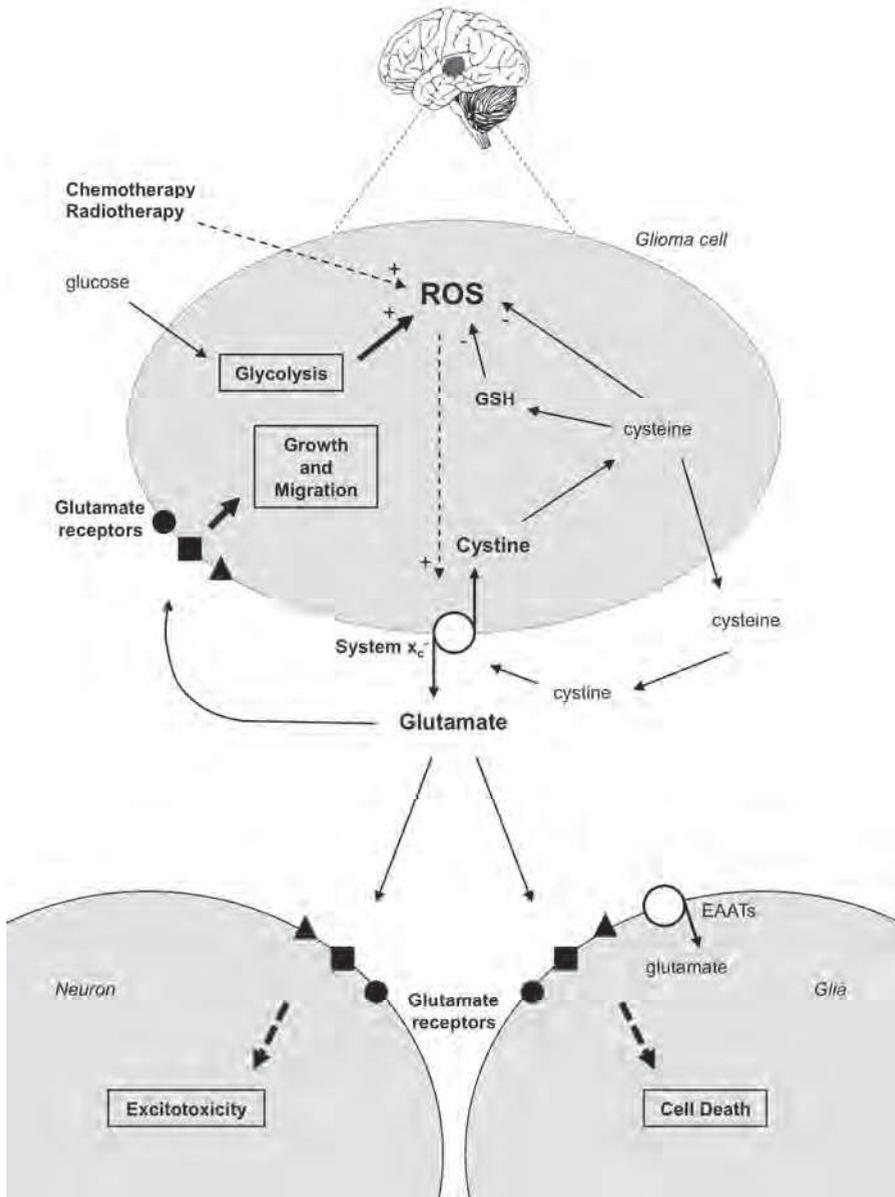


Fig. 1. Summary concept model demonstrating the impact of altered cancer cell metabolism and cytotoxic treatments on cellular ROS, the upregulation of system x_c^- , and the consequent import of cystine and export of glutamate. Cystine import allows the synthesis of GSH and the cycling of the cystine/cysteine redox cycle. The export of glutamate has cytotoxic effects on brain cells within the tumour microenvironment, and autocrine and paracrine effects on the glioma initiating growth and increased migration.

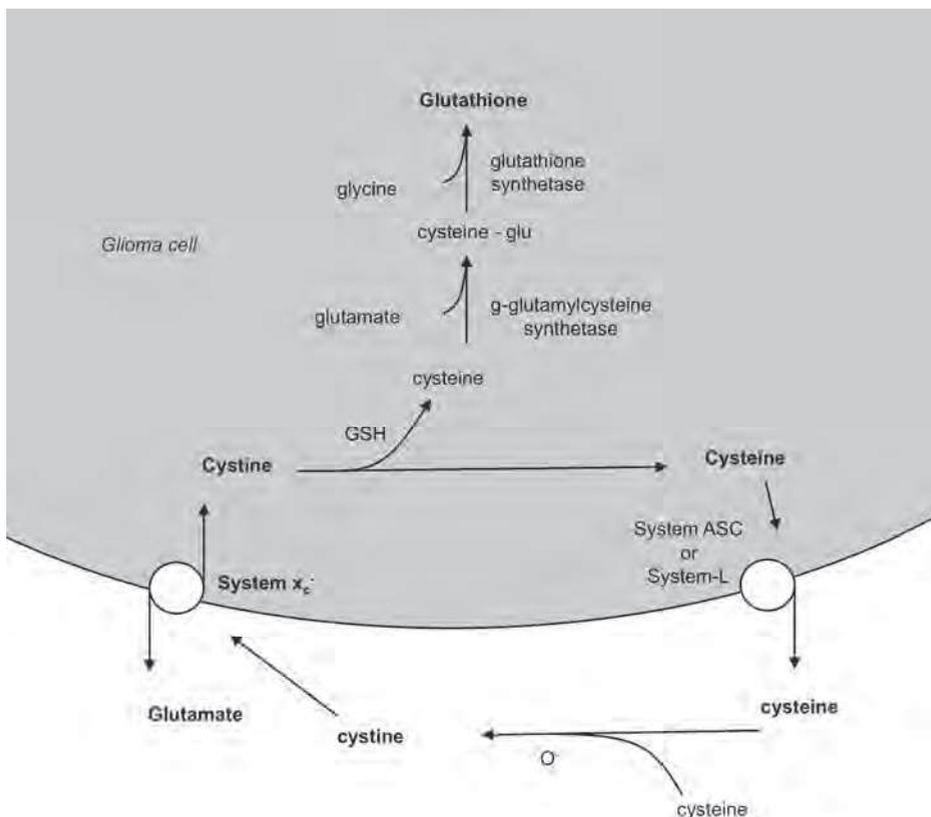


Fig. 2. Glutathione biosynthesis and the cystine/cysteine redox cycle as driven by amino acid transporters in glioma. Glutamate is secreted by system x_c^- which requires both substrates to function.

common in circulation (Bannai & Ishii, 1988). Not all cells possess the molecular machinery to import cystine, however, many brain cells and consequently, glioma with their high expression of system x_c^- and abundance of intracellular glutamate have both the mechanisms and the gradient to drive cystine transport. Once inside the cell, cystine is reduced to cysteine where it can be incorporated into polypeptide synthesis including the synthesis of GSH (Savaskan & Eyüpoglu, 2010). System x_c^- is one of many cystine transporters in the CNS, however it has been identified as the only cystine transporter expressed in glioma (Chung et al., 2005). Many cancers including glioma have demonstrated increased basal levels of intracellular GSH (Louw et al., 1997). Pharmacological inhibition of system x_c^- , and therefore limitation or elimination of available intracellular cysteine is able to deplete intracellular GSH almost entirely in a dose and time-dependent manner in glioma cell lines (Chung et al., 2005; Chung & Sontheimer, 2009; Pham et al., 2010). The negative effects of this GSH limitation on cell growth can be rescued entirely by the introduction of membrane permeable exogenous GSH, suggesting that cysteine availability for GSH production is critical for glioma cell growth (Chung & Sontheimer, 2009).

4.2 System x_c^- drives the cystine/cysteine redox cycle

As cysteine is rate-limiting in GSH synthesis, it is well expected that increased availability of the amino acid from system x_c^- upregulation would have the observed positive impact on GSH levels. Increased cystine import in glioma has also been demonstrated to drive the cystine/cysteine redox cycle across the cell membrane, which acts independently of GSH to counter ROS. To cycle the amino acid, cystine is imported by system x_c^- , where it is promptly reduced in the cytoplasm, likely by GSH, and conversely cysteine is exported by the amino acid transporters system-L or system ASC to the extracellular environment where it is promptly oxidized (Conrad & Sato, 2011). It was discovered in xCT induced lymphoma cells that the cystine/cysteine cycle raised concentrations of extracellular cysteine and acted as an effective antioxidant even in cases of GSH depletion (Banjac et al., 2008). A subsequent study found that in cells negative for γ -glutamylcysteine synthetase and therefore unable to produce GSH, the cystine/cysteine cycle was sufficient to maintain oxidative stress protection (Mandal et al., 2010). This suggests that alternative redox systems can compensate for each other to the point of redundancy, and in this case, both cycles are driven by the import of cystine (Mandal et al., 2010). Both this redox cycle and GSH synthesis are enabled by the actions of system x_c^- and in glioma, both confer protection from oxidative stress to the cell.

4.3 Consequences of ROS resistance

The upregulation of antioxidant defences in glioma cells confers proliferation and survival benefits to glioma above those of normal cells without which, glioma could not thrive in their self-induced oxidative environment. The ability of glioma to upregulate antioxidant production in the face of ROS has long been suspected to contribute to the chemotherapy and radiation-resistance that is devastatingly common in the treatment of glioma, a condition already characterized by poor prognoses (Sontheimer, 2008). A large scale microarray to coordinate transporter gene expression in 60 cancer cell lines with the activity of 1400 anticancer drugs revealed 39 drugs that positively correlate with SLC7a11 (xCT) expression and 296 that negatively correlate (Huang & Sadée, 2006). An example of a positively correlating drug is L-alanosine, an amino acid analogue whose uptake is mediated by system x_c^- . The authors demonstrated that pharmacologic system x_c^- inhibition reduced the efficacy of L-alanosine by impeding its system x_c^- mediated uptake. A negatively correlating drug is geldanamycin, an antibiotic that targets heat shock protein 90 (Hsp90). System x_c^- inhibition increased the efficacy of geldanamycin through a reduction of intracellular GSH which reduced cellular resistance to the drug's cytotoxicity (Huang et al., 2005). Celestrol is another Hsp90 targeting drug that has demonstrated antitumoral properties specifically in glioma, and is also very negatively correlated with SLC7a11 expression (Huang et al., 2008). Inhibition of system x_c^- in celestrol-resistant glioma cells reduced chemoresistance to celestrol treatment, as did other negative modulators of GSH synthesis, indicating that celestrol resistance in glioma is at least in part mediated through the availability of GSH (Pham et al., 2010).

5. System x_c^- and glutamate

The corollary effect of system x_c^- mediated cystine uptake is the necessary secretion of glutamate into the extracellular space, without which system x_c^- cannot function. It has been demonstrated that glioma cells secrete amounts of glutamate *via* this mechanism that are significant enough to mediate excitotoxic cell death in the brain (Sontheimer, 2003; Takano

et al., 2001; Ye & Sontheimer, 1999). The amino acid glutamate is most well known as the primary excitatory neurotransmitter in the CNS, however it also functions as a growth factor and motogen to different cell types in the brain (de Groot & Sontheimer, 2010), and mediates critical cell signalling in many non-neuronal tissues (Hinoi et al., 2004).

5.1 Glutamate release

The normal brain usually does not harbour extracellular glutamate in excess of 1-3 μ M, likely due to the glutamate reuptake mechanisms of glia (de Groot & Sontheimer, 2010). *In vitro*, astrocyte cultures demonstrate the ability to reduce extracellular glutamate concentrations to near 1 μ M from 92 μ M within 3 hours, while conversely several glioma cell lines raised extracellular glutamate to 400-500 μ M in a 12-hour period (Ye & Sontheimer, 1999). When neurons were grown in co-culture or treated with media from independent glioma cultures, neurons died from glutamate-mediated excitotoxicity (Ye & Sontheimer, 1999). In normal brain, glutamate released into the extracellular space is rapidly removed, either back into the presynaptic nerve terminal, or, more commonly, by glial cells *via* one of the excitatory amino acid transporters (EAAT1 or EAAT2) (Danbolt, 2001). Glutamate reuptake is a key feature of normal glial cells that surround the synaptic cleft, a mechanism that contributes to neuron protection and signal consistency (de Groot & Sontheimer, 2010). It has been demonstrated by microarray that EAAT2 expression in glioma is negatively correlated with tumour progression, and that induction of glioma with EAAT2 expression dose-dependently limits cell growth, suggesting that the loss of EAAT function in glioma cells may play a role in the accumulation of extracellular glutamate (de Groot et al., 2005). Glutamate release from glioma was confirmed *in vivo* through glioma cells implanted into rat brain. Glutamate was measured to be highest in peritumoral regions, significantly higher than in the normal brain and the tumour itself (Behrens et al., 2000; Takano et al., 2001). Cells of the same type cloned as to not release glutamate grew significantly smaller tumours than their glutamate-releasing counterparts (Takano et al., 2001). In glioma patients, despite conflicting reports, it appears that glutamate concentrations are significantly elevated in glioma in both the tumour (Behrens et al., 2000) and the peritumoural region (Roslin et al., 2003).

5.2 Consequences of glutamate release

Glutamate release into the peritumoral environment has a number of cytotoxic and cell signalling effects whose results are advantageous to glioma and seriously deleterious to the host. It has been suggested that glutamate release confers an adaptive advantage upon glioma (Sontheimer, 2003), but it is also possible that the release in great quantities of such a ubiquitous signalling molecule into a tissue that is highly sensitive to such molecules exerts a disruptive influence simply as a side-effect. This has also been demonstrated as a feature of glutamate-releasing cancers metastasized to bone, a tissue where glutamate is an important intercellular communication molecule (Seidlitz et al., 2010).

Glioma exist in an environment physically constrained to the cavity of the cranium, a space consumed by 85% tissue and 15% cerebrospinal fluid (CSF). To grow, glioma must create space to occupy, as compression cannot occur in a vessel filled with fluid. Glutamate-induced excitotoxic cell death is thought to be principally responsible for the clearance of brain cells along the tumour borders that allows glioma progression. Indicating the susceptibility of brain tissues to glutamatergic disruption is that no cell type in the brain is without receptors for glutamate. The inhibition of system x_c^- in glioma significantly reduces extracellular glutamate levels, as well as neurodegeneration and cellular edema both *in vitro*

and *in vivo*, indicating the role of system x_c^- in the induction of these morbidities (Savaskan et al., 2008). Excitotoxicity is thought to be initiated as a result of excessive activation of glutamate receptors resulting in the uncontrolled increase of intracellular Ca^{2+} which stimulates the activation of cytotoxic enzymes (Choi, 1988). Neurons possess both the ionotropic α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate acid (AMPA) glutamate receptors, and N-methyl-D-aspartate (NMDA) glutamate receptors for excitatory glutamate signal transmission. Neurons in coculture and *in vivo* were shown to be highly sensitive to excitotoxic cell death when exposed to glutamate release from glioma (Takano et al., 2001). Treatment with the NMDA receptor antagonist MK801 reduced but did not entirely eliminate this excitotoxicity (Takano et al., 2001).

Normal glial cells are also highly receptive to glutamate. Oligodendrocytes demonstrate a similar low tolerance to glutamate exposure as neurons, while astrocytes can tolerate much higher concentrations (Oka et al., 1993). Astrocytes normally function to remove glutamate from the extracellular space, so their tolerance to high glutamate concentrations is not surprising; however they too are eventually killed by an expanding glioma. Whether this cell death is also mediated by exposure to glutamate is not yet understood (de Groot & Sontheimer, 2010).

It has also been reported that glutamate may have an autocrine or paracrine signalling effect on glioma cells. AMPA, NMDA, Kainate, and the metabotropic glutamate receptors mGluR3 and mGluR5 have all been identified in glioma, and growth-effects have been demonstrated through manipulation of both AMPA and NMDA receptors. Most glioma express AMPA receptors that are permeable to Ca^{2+} upon activation by glutamate (Ishiuchi et al., 2007). Induced expression of the GluR2 receptor subunit which renders AMPA receptors Ca^{2+} impermeable sensitized glioma cells to apoptosis and reduced tumour growth *in vivo*, suggesting the ability of glioma-derived glutamate signalling through AMPA receptors to act in an autocrine/paracrine manner to stimulate cell growth (Ishiuchi et al., 2007).

Exogenous glutamate has a stimulatory effect on growth when applied to glioma cells, and conversely, antiproliferative effects on glioma have been demonstrated individually with several AMPA receptor antagonists and several NMDA receptor antagonists (Rzeski et al., 2001). Inhibition of mGlu2/3 receptors with the antagonist LY341495 in glioma cells positive for both receptors also was able to reduce glioma cell growth both *in vitro* and *in vivo* (Arcella et al., 2005). Taken together, these results obtained through the blockade of nearly all glutamate receptors expressed in glioma suggest a significant autocrine/paracrine effect on growth of glioma-derived glutamate.

6. Experimental therapeutics

The myriad consequences originating from the upregulation of xCT in glioma have uncovered several novel possibilities for treatment of glioma. Any therapeutic targeting of the mechanisms of antioxidant production and glutamate release could prove to be critical in the treatment of glioma, as current therapies are limited in efficacy and often become redundant through acquired cell-resistance (Sontheimer, 2008). Symptom management may also arise from treating glutamate release, as it is hypothesized that frequent seizures, a morbidity that affects over 80% of glioma sufferers could be related to glutamate-induced hyperexcitability in the CNS, possibly in advance of neuron excitotoxic death and possibly an early indication of the cancer (de Groot & Sontheimer, 2010).

6.1 Targeting glutamate receptors

AMPA receptor targeting has emerged as the most prolific avenue of interest for treatment from glioma glutamate-release work. An AMPA antagonist called talampanel is currently the most likely candidate for glioma treatment in this manner in large part because it does not exhibit the side-effects of most glutamate receptor antagonists in the CNS, and it has been shown to increase the lifespan of mice xenografted with human glioma (Goudar et al., 2004). Two clinical trials have developed from these findings. The first, begun in 2009, was a phase II trial designed to examine the efficacy of talampanel in conjunction with standard radiation and temozolomide treatments in improving survival in adults with newly diagnosed glioblastoma (Grossman et al., 2009). This trial concluded that patients treated with talampanel demonstrated significantly longer survival than those who received standard care alone (Grossman et al., 2010). While this is promising and certainly demands further investigation, this study alone cannot be deemed conclusive. The second trial, a smaller phase II trial, examined the effects of talampanel alone on 6-month survival of patients with recurrent malignant glioma (Iwamoto et al., 2010). This trial determined that talampanel alone conferred no obvious advantage on patient survival, but the drug was tolerated well with no severe side-effects (Iwamoto et al., 2010).

Inhibitors of other glutamate receptors have not yet been clinically evaluated, however the preliminary success of animal models of glioma treatment as mentioned above will certainly lead to trials of other glutamate receptor antagonists in the near future. Significant promise is held by these inhibitors as both candidate adjuvant therapies capable of supplementing treatment cytotoxicity or of mediating the effects of glutamate on the brain.

6.2 System x_c^- Inhibition

While the above-mentioned therapies for mediating the excess glutamate released by glioma are promising, certainly the most attractive potential therapies to arise from these studies are those that involve the inhibition of system x_c^- . Rather than mediate the consequences of destructive glutamate release and treatment-resistance, system x_c^- inhibition could eliminate the function of the transporter responsible for the excess glutamate, and consequently limit the multiple morbidities of glutamate release rather than manage its downstream effects. In addition, system x_c^- inhibition would limit cysteine availability to the glioma cell and therefore inhibit its antioxidative capabilities by way of both limiting glutathione synthesis and halting the drive of the cystine/cysteine redox cycle. There are many chemical inhibitors of system x_c^- ; of these, the cyclic glutamate analogue S-(4)-carboxyphenylglycine has emerged as the most potent inhibitor (Patel et al., 2004), and the FDA approved anti-inflammatory drug sulfasalazine has garnered the most clinical interest. Sulfasalazine has been demonstrated in animal models to effectively slow the growth of glioma and reduce levels of both intracellular GSH and extracellular glutamate (Chung et al., 2005; Chung & Sontheimer, 2009). A phase I clinical trial of sulfasalazine to evaluate drug safety and effects on tumour growth in the treatment of grade 3 glioma in a small number of patients was prematurely terminated due to several adverse effects during treatment (Robe et al., 2009; 2006). This study was initiated on the basis on sulfasalazine acting as an inhibitor of NF κ B, however treatment did not differ from that required for system x_c^- inhibition. Although the poor outcomes from this trial are unfortunate, they do little to dampen the potential of sulfasalazine for glioma treatment or system x_c^- as a therapeutic target. Another phase I clinical trial has just recently been initiated with the intent to examine the effects of sulfasalazine on glutamate release in the brain, and on seizures in low-grade, newly-

diagnosed glioma patients (de Groot & Sontheimer, 2010). The upcoming results of this trial will be the first clinical evidence of treatments directed at system x_c^- inhibition, and will contribute greatly to the establishment of the role of this critical transporter in glioma morbidity and treatment.

7. Conclusion

Glioma exist in conditions of high oxidative stress as a result of the metabolic shift away from the TCA cycle and towards increased rates of glycolysis. This metabolic shift, the Warburg effect, is characteristic of cancer cells and confers unique benefits to the cell which allow survival and proliferation in conditions of rapid growth, division and variable access to blood vasculature. However, a result of this reliance on glycolysis is the increased prevalence of ROS in the cancer cell. To survive these conditions, cancer cells must possess upregulated mechanisms of antioxidation. Glioma exhibit an oxidative stress-mediated upregulation of the xCT coding gene SLC7a11, which, along with the membrane-anchoring protein 4F2hc comprise the two subunits of the Na^+ independent electroneutral cystine/glutamate antiporter called system x_c^- . This antiporter drives the molecular turnover that is ultimately responsible for several of the features of morbidity in glioma, as well as its characteristic chemo- and radiation-resistance. The import of cystine into the cell increases the availability of cysteine for the GSH synthesis pathway and for the cystine/cysteine redox cycle. These two antioxidant pathways function to relieve the glioma cell of significant oxidative stress, allowing increased proliferation and survival of the cancer cells. This also allows resistance of glioma to oxidative stress-inducing radiation and chemotherapies. Conversely, the export of glutamate results in the neurotoxic death of neurons and glial cells in the vicinity of the tumour, and acts in an autocrine/paracrine manner to stimulate glioma proliferation and migration. Therapies are currently under development with these mechanisms in mind. Glutamate receptor antagonists have been demonstrated to limit brain cell death and to inhibit tumour growth *in vitro* and in xenograft animal models of glioma. System x_c^- inhibitors that prevent the import of cystine for antioxidant purposes and also prevent the release of glutamate into the extracellular environment have also demonstrated success *in vitro* and *in vivo*, and a clinical trial is currently underway with the inhibitor sulfasalazine. This work opens new pathways for investigation in a condition well known for poor prognoses and limited treatment options. The evidence of system x_c^- in other cancers in addition to glioma suggests that this mechanism may soon become of great importance to cancer treatment.

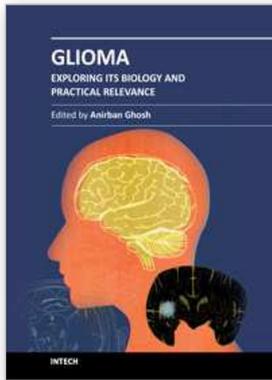
8. References

- Arcella, A., Carpinelli, G., Battaglia, G., D'Onofrio, M., Santoro, F., Ngomba, R. T., et al. (2005). Pharmacological blockade of group II metabotropic glutamate receptors reduces the growth of glioma cells *in vivo*. *Neuro-oncology*, 7(3), 236-45.
- Balendiran, G. K., Dabur, R., & Fraser, D. (2004). The role of glutathione in cancer. *Cell biochemistry and function*, 22(6), 343-52.
- Banjac, A., Perisic, T., Sato, H., Seiler, A., Bannai, S., Weiss, N., et al. (2008). The cystine/cysteine cycle: a redox cycle regulating susceptibility versus resistance to cell death. *Oncogene*, 27, 1618-1628.

- Bannai, S., & Ishii, T. (1988). A novel function of glutamine in cell culture: utilization of glutamine for the uptake of cystine in human fibroblasts. *Journal of cellular physiology*, 137(2), 360-6.
- Bannai, S., & Kitamura, E. (1980). Transport interaction of L-cystine and L-glutamate in human diploid fibroblasts in culture. *The Journal of biological chemistry*, 255(6), 2372-6.
- Bannai, S., & Kitamura, E. (1982). Adaptive enhancement of cystine and glutamate uptake in human diploid fibroblasts in culture. *Biochimica et biophysica acta*, 721(1), 1-10.
- Bannai, S., Sato, H., Ishii, T., & Taketani, S. (1991). Enhancement of glutathione levels in mouse peritoneal macrophages by sodium arsenite, cadmium chloride and glucose/glucose oxidase. *Biochimica et biophysica acta*, 1092(2), 175-9.
- Behrens, P. F., Langemann, H., Strohschein, R., Draeger, J., & Hennig, J. (2000). Extracellular glutamate and other metabolites in and around RG2 rat glioma: an intracerebral microdialysis study. *Journal of neuro-oncology*, 47(1), 11-22.
- Burdo, J., Dargusch, R., & Schubert, D. (2006). Distribution of the cystine/glutamate antiporter system xc- in the brain, kidney, and duodenum. *Journal of Histochemistry and Cytochemistry*, 54(5), 549-557.
- Chillarón, J., Roca, R., Valencia, A., Zorzano, A., & Palacín, M. (2001). Heteromeric amino acid transporters: biochemistry, genetics, and physiology. *American journal of physiology. Renal physiology*, 281(6), 995-1018.
- Choi, D. W. (1988). Glutamate neurotoxicity and diseases of the nervous system. *Neuron*, 1(8), 623-34.
- Chung, W J, Lyons, S A, Nelson, G. M., Hamza, H., Gladson, C. L., Gillespie, G. Y., et al. (2005). Inhibition of cystine uptake disrupts the growth of primary brain tumors. *Journal of Neuroscience*, 25(31), 7101-7110.
- Chung, W J, & Sontheimer, H. (2009). Sulfasalazine inhibits the growth of primary brain tumors independent of Nuclear factor-kappaB. *Journal of Neurochemistry*, 110, 182-193.
- Conrad, Marcus, & Sato, Hideyo. (2011). The oxidative stress-inducible cystine/glutamate antiporter, system x (c) (-) : cystine supplier and beyond. *Amino acids*. Electronic Advance Copy.
- deGroot, J., & Sontheimer, Harald. (2010). Glutamate and the biology of gliomas. *Glia*. Electronic Advance Copy.
- Goudar, R. K., Keir, S. T., Bigner, D. D., & Friedman, H. S. (2004). NMDA and AMPA glutamate receptor antagonists in the treatment of human malignant glioma xenografts. *AACR Meeting Abstracts*, 2004(1), 1053.
- Grossman, S. A., Ye, X., Chamberlain, M., Mikkelsen, T., Batchelor, T., Desideri, S., et al. (2009). Talampanel with standard radiation and temozolomide in patients with newly diagnosed glioblastoma: a multicenter phase II trial. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*, 27(25), 4155-61.
- Grossman, S. A., Ye, X., Piantadosi, S., Desideri, S., Nabors, L. B., Rosenfeld, M., et al. (2010). Survival of patients with newly diagnosed glioblastoma treated with radiation and temozolomide in research studies in the United States. *Clinical cancer research : an official journal of the American Association for Cancer Research*, 16(8), 2443-9.
- Halliwel, B. (2007). Oxidative stress and cancer: have we moved forward? *The Biochemical journal*, 401(1), 1-11.
- Hinoi, E., Takarada, T., Ueshima, T., Tsuchihashi, Y., & Yoneda, Y. (2004). Glutamate signaling in peripheral tissues. *European Journal of Biochemistry*, 271, 1-13.
- Huang, Ying, Dai, Z., Barbacioru, C., & Sadée, W. (2005). Cystine-glutamate transporter SLC7A11 in cancer chemosensitivity and chemoresistance. *Cancer research*, 65(16), 7446-54.

- Huang, Ying, & Sadée, W. (2006). Membrane transporters and channels in chemoresistance and -sensitivity of tumor cells. *Cancer letters*, 239(2), 168-82.
- Huang, Yulun, Zhou, Y., Fan, Y., & Zhou, D. (2008). Celestrol inhibits the growth of human glioma xenografts in nude mice through suppressing VEGFR expression. *Cancer letters*, 264(1), 101-6.
- Ishii, T., Sato, H, Miura, K., Sagara, J., & Bannai, S. (1992). Induction of cystine transport activity by stress. *Annals of the New York Academy of Sciences*, 663, 497-8.
- Ishii, T., Sugita, Y., & Bannai, S. (1987). Regulation of glutathione levels in mouse spleen lymphocytes by transport of cysteine. *Journal of cellular physiology*, 133(2), 330-6.
- Ishiuchi, S., Yoshida, Y., Sugawara, K., Aihara, M., Ohtani, T., Watanabe, T., et al. (2007). Ca²⁺-permeable AMPA receptors regulate growth of human glioblastoma via Akt activation. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 27(30), 7987-8001.
- Iwamoto, F. M., Kreisl, T. N., Kim, L., Duic, J. P., Butman, J. A., Albert, P. S., et al. (2010). Phase 2 trial of talampanel, a glutamate receptor inhibitor, for adults with recurrent malignant gliomas. *Cancer*, 116(7), 1776-82.
- Kim, J. Y., Kanai, Y, Chairoungdua, A., Cha, S. H., Matsuo, H., Kim, D. K., et al. (2001). Human cystine/glutamate transporter: cDNA cloning and upregulation by oxidative stress in glioma cells. *Biochimica et biophysica acta*, 1512(2), 335-44.
- Kim, J.-whan, & Dang, C. V. (2006). Cancer's molecular sweet tooth and the Warburg effect. *Cancer research*, 66(18), 8927-30.
- Kroemer, G., & Pouyssegur, J. (2008). Tumor cell metabolism: cancer's Achilles' heel. *Cancer cell*, 13(6), 472-82.
- Le, A., Cooper, C. R., Gouw, A. M., Dinavahi, R., Maitra, A., Deck, L. M., et al. (2010). Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression. *Proceedings of the National Academy of Sciences of the United States of America*, 107(5), 2037-42.
- Lim, J. C., & Donaldson, P. J. (2010). Focus On Molecules: The cystine/ glutamate exchanger (System x(c)(-)). *Experimental eye research*. 92(3),162-3
- Lo, M., Wang, Y. Z., & Gout, P W. (2008). The x(c) (-) cystine/ glutamate antiporter: A potential target for therapy of cancer and other diseases. *Journal of Cellular Physiology*, 215(1097-4652).
- Louw, D. F., Bose, R., Sima, A. A., & Sutherland, G. R. (1997). Evidence for a high free radical state in low-grade astrocytomas. *Neurosurgery*, 41(5), 1146-50.
- Lyons, Susan A, Chung, W Joon, Weaver, A. K., Ogunrinu, T., & Sontheimer, Harald. (2007). Autocrine glutamate signaling promotes glioma cell invasion. *Cancer research*, 67(19), 9463-71.
- Makowske, M., & Christensen, H. N. (1982). Contrasts in transport systems for anionic amino acids in hepatocytes and a hepatoma cell line HTC. *The Journal of biological chemistry*, 257(10), 5663-70.
- Mandal, P. K., Seiler, Alexander, Perisic, Tamara, Koelle, P., Banjac Canak, A., Foester, H., et al. (2010). System xC- and thioredoxin reductase 1 cooperatively rescue glutathione deficiency. *The Journal of biological chemistry*.
- Meister, A. (1995). Glutathione metabolism. *Methods in enzymology*, 251, 3-7.
- Oka, A., Belliveau, M. J., Rosenberg, P. A., & Volpe, J. J. (1993). Vulnerability of oligodendroglia to glutamate: pharmacology, mechanisms, and prevention. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 13(4), 1441-53.

- Patel, S. A., Warren, B. A., Rhoderick, J. F., & Bridges, R. J. (2004). Differentiation of substrate and non-substrate inhibitors of transport system xc(-): an obligate exchanger of L-glutamate and L-cystine. *Neuropharmacology*, 46(2), 273-84.
- Pham, A.-N., Blower, P. E., Alvarado, O., Ravula, R., Gout, Peter W., & Huang, Ying. (2010). Pharmacogenomic approach reveals a role for the x(c)- cystine/glutamate antiporter in growth and celastrol resistance of glioma cell lines. *The Journal of pharmacology and experimental therapeutics*, 332(3), 949-58.
- Robe, P. A., Martin, D. H., Nguyen-Khac, M. T., Artesi, M., Deprez, M., Albert, A., et al. (2009). Early termination of ISRCTN45828668, a phase 1/2 prospective, randomized study of sulfasalazine for the treatment of progressing malignant gliomas in adults. *BMC cancer*, 9, 372.
- Robe, P. A., Martin, D., Albert, A., Deprez, M., Chariot, A., & Bours, V. (2006). A phase 1-2, prospective, double blind, randomized study of the safety and efficacy of Sulfasalazine for the treatment of progressing malignant gliomas: study protocol of [ISRCTN45828668]. *BMC cancer*, 6, 29.
- Roslin, M., Henriksson, R., Bergström, P., Ungerstedt, U., & Bergenheim, A. T. (2003). Baseline levels of glucose metabolites, glutamate and glycerol in malignant glioma assessed by stereotactic microdialysis. *Journal of neuro-oncology*, 61(2), 151-60.
- Rzeski, W., Turski, L., & Ikonomidou, C. (2001). Glutamate antagonists limit tumor growth. *Proceedings of the National Academy of Sciences of the United States of America*, 98(11), 6372-7.
- Savaskan, Nic E, & Eyüpoglu, I. Y. (2010). xCT modulation in gliomas: Relevance to energy metabolism and tumor microenvironment normalization. *Annals of anatomy = Anatomischer Anzeiger : official organ of the Anatomische Gesellschaft*.
- Savaskan, Nicolai E, Heckel, A., Hahnen, E., Engelhorn, T., Doerfler, A., Ganslandt, O., et al. (2008). Small interfering RNA-mediated xCT silencing in gliomas inhibits neurodegeneration and alleviates brain edema. *Nature medicine*, 14(6), 629-32.
- Seidlitz, E. P., Sharma, M. K., & Singh, G. (2010). Extracellular glutamate alters mature osteoclast and osteoblast functions. *Canadian journal of physiology and pharmacology*, 88(9), 929-36.
- Shih, A. Y., Erb, H., Sun, X., Toda, S., Kalivas, P. W., & Murphy, T. H. (2006). Cystine/glutamate exchange modulates glutathione supply for neuroprotection from oxidative stress and cell proliferation. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 26(41), 10514-23.
- Sontheimer, Harald. (2003). Malignant gliomas: perverting glutamate and ion homeostasis for selective advantage. *Trends in neurosciences*, 26(10), 543-9.
- Sontheimer, Harald. (2008). A role for glutamate in growth and invasion of primary brain tumors. *Journal of neurochemistry*, 105(2), 287-95.
- Takano, T., Lin, J. H., Arcuino, G., Gao, Q., Yang, J., & Nedergaard, M. (2001). Glutamate release promotes growth of malignant gliomas. *Nature medicine*, 7(9), 1010-5.
- Verrey, F., Closs, E. I., Wagner, C. A., Palacin, M., Endou, Hitoshi, & Kanai, Yoshikatsu. (2004). CATs and HATs: the SLC7 family of amino acid transporters. *Pflügers Archiv : European journal of physiology*, 447(5), 532-42.
- Wang, H., Tamba, M., Kimata, M., Sakamoto, K., Bannai, Shiro, & Sato, Hideyo. (2003). Expression of the activity of cystine/glutamate exchange transporter, system x(c)(-), by xCT and rBAT. *Biochemical and biophysical research communications*, 305(3), 611-8.
- Warburg, O., Wind, F., & Negelein, E. (1927). The metabolism of tumours in the body. *The Journal of general physiology*, 8(6), 519-30.
- Ye, Z. C., & Sontheimer, H. (1999). Glioma cells release excitotoxic concentrations of glutamate. *Cancer research*, 59(17), 4383-91.



Glioma - Exploring Its Biology and Practical Relevance

Edited by Dr. Anirban Ghosh

ISBN 978-953-307-379-8

Hard cover, 486 pages

Publisher InTech

Published online 02, November, 2011

Published in print edition November, 2011

The title 'Glioma - Exploring Its Biology and Practical Relevance' is indicative of its content. This volume contains 21 chapters basically intended to explore glioma biology and discussing the experimental model systems for the purpose. It is hoped that the present volume will provide supportive and relevant awareness and understanding on the fundamental advances of the subject to the professionals from any sphere interested about glioma.

How to reference

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

Robert Ungard and Gurmit Singh (2011). Oxidative Stress and Glutamate Release in Glioma, Glioma - Exploring Its Biology and Practical Relevance, Dr. Anirban Ghosh (Ed.), ISBN: 978-953-307-379-8, InTech, Available from: <http://www.intechopen.com/books/glioma-exploring-its-biology-and-practical-relevance/oxidative-stress-and-glutamate-release-in-glioma>

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

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 is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.