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Review

The role of glutathione in brain tumor drug resistance

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ABSTRACT

Chemotherapy is central to the current treatment modality for primary human brain tumors, but despite high-dose and intensive treatment regimens there has been little improvement in patient outcome. The development of tumor chemoresistance has been proposed as a major contributor to this lack of response. While there have been some improvements in our understanding of the molecular mechanisms underlying brain tumor drug resistance over the past decade, the contribution of glutathione (GSH) and the GSH-related enzymes to drug resistance in brain tumors have been largely overlooked. GSH constitutes a major antioxidant defense system in the brain and together with the GSH-related enzymes plays an important role in protecting cells against free radical damage and dictating tumor cell response to adjuvant cancer therapies, including irradiation and chemotherapy. Glutamate cysteine ligase (GCL), glutathione synthetase (GS), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferases (GST), and GSH complex export transporters (GS-X pumps) are major components of the GSH-dependent enzyme system that function in a dynamic cascade to maintain redox homeostasis. In many tumors, the GSH system is often dysregulated, resulting in a more drug resistant phenotype. This is commonly associated with GST-mediated GSH conjugation of various anticancer agents leading to the formation of less toxic GSH–drug complexes, which can be readily exported from the cell. Advances in our understanding of the mechanisms of drug resistance and patient selection based on biomarker profiles will be crucial to adapt therapeutic strategies and improve outcomes for patients with primary malignant brain tumors.

Contents

Abbreviations: GSH, glutathione; GCL, glutamate cysteine ligase; GS, glutathione synthetase; GPx, glutathione peroxidase; GR, glutathione reductase; GST, glutathione-S-transferase; ROS, reactive oxygen species; Mrp, multidrug resistance-associated protein; γ-GC, γ-glutamylcysteine; GSSG, glutathione disulfide; GCLC, GCL catalytic subunit; GCLM, GCL modulatory subunit; EAAT, excitatory amino acid transporter; BSO, L-buthionine-S,R-sulfoximine; CPA, cyclophosphamide; γGT, γ-glutamyltranspeptidase; EAAC, excitatory amino acid carrier; X_c^-, glutamate-cystine antiporter; CNS, central nervous system; GBM, glioblastoma multiforme; NAC, N-acetyl cysteine; CPT, camptothecin; CENU, chloroethylnitrosourea; 4-HC, hydroperoxy-CPA; ACNU, nimustine hydrochloride; BCNU, bis-chloroethylnitrosourea; PNET, primitive neuroectodermal tumor; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; EGFR-TK, epidermal growth factor receptor tyrosine kinase.

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1. Introduction

In the basal state, the average adult human brain accounts for a large percentage of the total oxygen consumed by the body and, as a result, generates a disproportionate amount of reactive oxygen species (ROS) as compared with other tissues [1]. ROS are continuously produced during oxidative metabolism and can have deleterious effects on cell function and viability due to their ability to induce lipid peroxidation, protein modification, and DNA damage [2,3]. Neural tissue (Fig. 1A) also exhibits higher vulnerability to oxygen and glucose deprivation, which are required to support normal function through glycolysis and oxidative phosphorylation, than any other tissue or organ [4]. Mammalian cells have developed a number of antioxidant defense systems to counter the deleterious effects of endogenous production and/or accumulation of ROS, thereby protecting against cellular oxidative damage [1].

Glutathione (GSH) is an important cellular antioxidant in the brain, where it plays a critical role in suppressing oxidative stress and maintaining cellular redox homeostasis [5,6]. GSH is a tripeptide composed of glutamate, cysteine, and glycine and the antioxidant and conjugation properties of GSH are derivative of the sulfhydryl moiety of the cysteine residue. GSH has the ability to directly scavenge cellular ROS in a non-enzymatic manner as well as serve as a co-factor for GSH peroxidase (GPx) in the reduction of H_2O_2 and other peroxide species. GSH can also be utilized in disulfide exchange reactions resulting in formation of mixed protein-glutathione disulfides, and the direct post-translational modification of proteins via glutathionylation of protein sulfhydryl groups is gaining recognition as an important signal transduction mechanism for regulating various cellular processes [7–9]. In addition, GSH can function as a storage depot for both cysteine and glutamate and this serves an important cytoprotective function in the brain by preventing the inherent cytotoxicity of free cysteine and glutamate-dependent neuronal excitotoxicity [10]. While maintenance of intracellular GSH homeostasis is essential to protect against oxidative damage, GSH is also intimately involved in the detoxification of numerous xenobiotics. The glutathione-S-transferase (GST) family of enzymes utilize GSH as a co-factor in the Phase II metabolism of various chemotherapeutic agents, resulting in the formation of GSH–drug conjugates that are more water soluble than the parent compound and subject to transporter-mediated efflux [11–14]. It is by this detoxification mechanism that elevated intracellular GSH levels and the over-expression and/or unregulated activation of one or more of the GSH metabolic enzymes are thought to contribute to the development of tumor cell chemoresistance [13–17]. This review article attempts to summarize the GSH biosynthetic pathway and the major GSH-related mechanisms of anticancer drug resistance, their interrelated action, and their role in conferring clinical non-responsiveness to chemotherapy in different brain tumor types. Most studies examining drug resistance in CNS neoplasms have focused on those with the highest incidence: gliomas in adults and medulloblastomas among pediatric age groups. Accordingly, the discussion will focus mainly on these tumor types.

GSH can detoxify ROS both non-enzymatically and enzymatically by serving as an electron donor in the reduction of peroxides by glutathione peroxidase (GPx) [18–20]. GPx leads to the oxidation of GSH resulting in the generation of glutathione disulfide (GSSG). Reduced GSH can be salvaged from GSSG via the activity of glutathione reductase (GR), which utilizes NADPH as an electron donor [20]. GR activity and GSH:GSSG ratios are sensitive to cellular NADPH levels, which is derived from the pentose phosphate pathway [21,22]. Therefore, an intact functional pentose phosphate pathway is essential in maintaining GSH redox homeostasis [22]. GR activity is sufficient to efficiently reduce basal GSSG produced in non-stressed cells. However, GR activity can become limiting during periods of oxidative stress leading to the accumulation of GSSG (50–70% of total GSH) and a dramatic shift in GSH:GSSG ratio [20,23]. Thus, there can be a loss in redox homeostasis due to the loss of the GR-mediated salvage of GSH from GSSG even when total cellular GSH levels are relatively

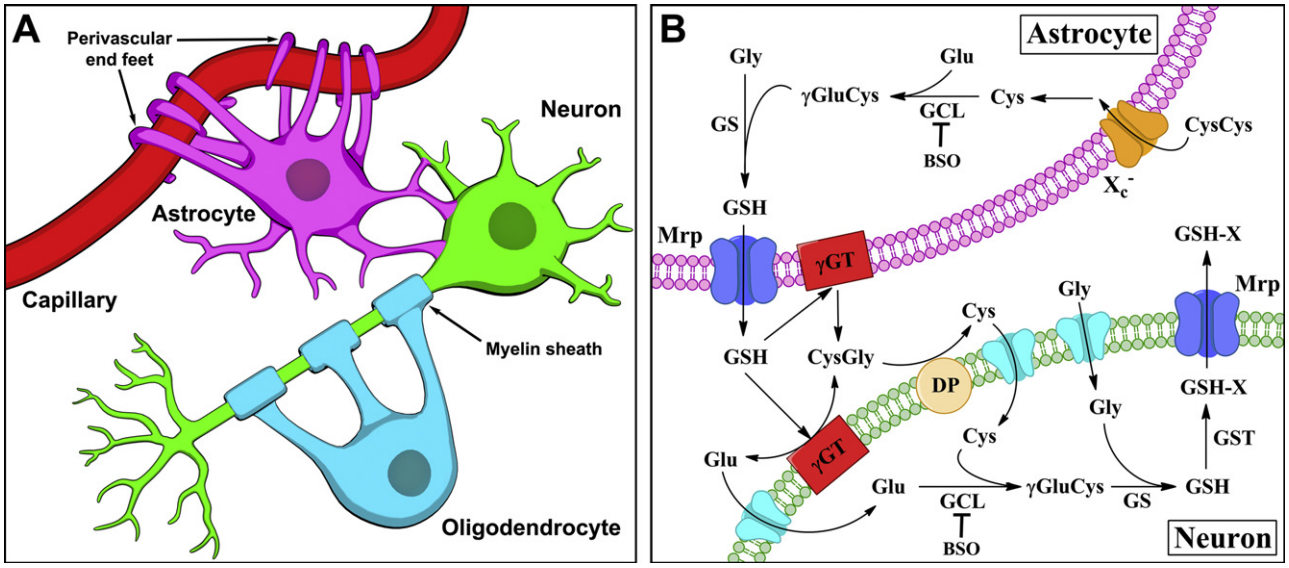

Fig. 1. The major cell populations present in neural tissue and a scheme of the GSH-mediated interactions between astrocytes and neurons. (A) Most brain tumors arise from three main cell types or their progenitors: neurons (green), oligodendrocytes (cyan), and astrocytes (magenta). (B) Glutathione (GSH) biosynthesis consists of two ATP-dependent reactions: the glutamate cysteine ligase (GCL)-mediated formation of γ-glutamylcysteine (γGluCys) from glutamate (Glu) and cysteine (Cys) and the glutathione synthetase (GS)-catalyzed formation of GSH from γGluCys and glycine (Gly). Buthionine sulfoximine (BSO) is a specific and irreversible inhibitor of GCL enzymatic activity. Detoxification of chemotherapeutic compounds proceeds via the glutathione-S-transferase (GST)-mediated formation of GSH–drug conjugates (GSH-X) followed by efflux by multidrug resistance-associated protein (Mrp) transporters. Mrp transporters are also capable of GSH efflux into the extracellular space which is broken down by γ-glutamyl transpeptidase (γGT) into Glu and cysteinylglycine (CysGly). CysGly is further hydrolyzed by cellular dipeptidases (DP) followed by the transporter-mediated uptake of the constituent amino acids that can serve as substrates for either cellular GSH production or protein synthesis. Additional Cys may also be obtained via the glutamate-cystine antiporter (X_c^-)-mediated uptake of extracellular cystine (CysCys) which is rapidly reduced to Cys within the intracellular milieu.

normal. In addition to the GR-mediated salvage pathway, GSSG levels and GSH:GSSG ratios can be readily reestablished via the active efflux of GSSG, which is mediated by the ATP-dependent multi drug resistance-associated protein-1 (Mrp1) transporter in cultured astrocytes and neurons [24–26]. In the absence of such mechanisms, GSSG levels can become significantly elevated leading to cytotoxicity [1].

2. The GSH antioxidant defense system in the brain

GSH homeostasis in both glial and neuronal cell types is dependent on the dynamic regulation of three distinct processes: GSH synthesis, utilization, and export [6]. A major route of chemotherapeutic metabolism is via the direct conjugation of the xenobiotic with GSH. The GSH conjugates are readily exported from the cell in a transporter-dependent fashion. This leads to the depletion of cellular GSH and the disruption of cellular redox balance. Restoration of normal cellular GSH homeostasis, therefore, requires *de novo* biosynthesis, as cells are not capable of readily importing intact GSH. GSH biosynthesis proceeds via two stepwise ATP-dependent reactions. The first and rate-limiting step is the formation of γ-glutamylcysteine (γGC) catalyzed by glutamate cysteine ligase (GCL), a heterodimeric holoenzyme composed of a catalytic (GCLC) and a modulatory (GCLM) subunit [27,28]. Cellular GSH content is highly sensitive to GCL activity, which is dependent on relative enzyme subunit expression, substrate availability, and negative feedback inhibition by GSH [29,30]. The GCL subunits are regulated at the transcriptional and post-transcriptional levels in response to oxidative stress, with the Nrf1/2, AP-1, and Nf-κB transcription factors regulating the constitutive and inducible levels of both subunits [28]. The Nrf2 transcription factor mediates the induction of multiple cytoprotective enzymes in astrocytes, including enzymes involved in GSH biosynthesis, utilization, and export [31,32]. Interestingly, Nrf2-dependent upregulation of glial cell GSH biosynthesis is necessary and sufficient to protect co-cultured neurons from oxidative stress by providing cysteine for neuronal GSH biosynthesis [33,34]. Furthermore, Nrf2 activation may coordinate astrocyte release of GSH and neuronal GSH biosynthesis via transcriptional upregulation of astrocyte GCL and neuronal excitatory amino acid transporter 3 (EAAT3) expression, respectively [35]. Although the GCL subunits are often coordinately induced, GCLM appears to contribute more significantly to the inducible increase in neuronal GCL activity [36]. Both GCL subunits are essential for the viability of cultured primary cortical neurons and overexpression of GCLC protects against glutamate- and nitric oxide-induced apoptosis [37]. Like other GSH metabolic enzymes, the GCL subunits are overexpressed in many tumor cell types and may play a central role in the development of chemoresistance [28].

Consistent with a role for GCL in the development of tumor cell drug resistance, a functionally significant polymorphic GAG/CTC trinucleotide repeat within the 5′ untranslated region of GCLC has been shown to correlate with drug sensitivities in an NCI panel of 50 human tumor cell lines [38,39]. Chemoresistance to bis-chloroethylnitrosourea (BCNU) and other chemotherapeutics also correlate with elevated cellular GSH levels in a host of brain tumor cell lines [40–43]. Such findings suggest that depletion of GSH levels may reverse the resistant phenotype and enhance sensitivity to various chemotherapeutics. In this regard, L-buthionine-*S*,*R*-sulfoximine (BSO), an inhibitor of GCL enzymatic activity that leads to the depletion of cellular GSH levels [29], reverses chemoresistance to cyclophosphamide (CPA) or hydroperoxy-CPA in medulloblastoma [44], and enhances melphalan cytotoxicity in human medulloblastoma and glioma xenografts [45–48]. BSO and melphalan have also been reported to exhibit synergistic toxicity and overcome melphalan-resistance in neuroblastoma cell lines derived after disease progression following myeloablative therapy [7,33,34,48,49].

GSH synthetase (GS) mediates the second ATP-dependent reaction in GSH synthesis, coupling γGC to glycine [28]. While GS activity has been measured in the brain, little is known concerning its potential role in regulating brain GSH levels. GS is transcriptionally regulated [28], yet there has been little effort to determine whether GS levels are elevated in cancer. Interestingly, the induction of GS gene expression has been shown to confer resistance to cisplatin in human ovarian cancer cells [50,51]. However, most pharmacological approaches to deplete GSH via inhibition of GSH biosynthesis have been directed at GCL, which is the rate-limiting in GSH synthesis, as deficiencies in GS leads to metabolic acidosis due to the catabolism of γGC to oxoproline [52].

The inability of most cell types to effectively import intact GSH highlights the importance of precursor availability and uptake, as well as subsequent *de novo* GSH biosynthesis, to the maintenance of neuronal GSH homeostasis. Substrate availability is a major determinant of GSH content in the brain and cysteine is typically the rate-limiting substrate for neuronal GSH biosynthesis [53]. The brain provides a rather unique model of cell-to-cell metabolic interaction in which astrocytes provide neighboring cells with the precursor amino acids necessary for GSH biosynthesis [54]. This significant degree of metabolic trafficking between astrocytes and neurons (Fig. 1B) plays a critical role in neuroprotection against oxidative stress by maintaining neuronal GSH levels [55]. Astrocytes provide neurons with lactate for ATP production, cysteine for GSH synthesis, and assist with the removal of glutamate from the synaptic cleft of glutamatergic neurons [55]. The cysteine utilized for neuronal GSH synthesis is derived from GSH that is exported from astrocytes and broken down into its constituent amino acids (Fig. 1B) [53,56]. The γ-glutamyl moiety is released by γ-glutamyltranspeptidase (γGT) activity and cysteine and glycine are released from the resulting CysGly dipeptide by neuronal dipeptidase activity [53]. Interestingly, neuronal cells utilize cysteine for GSH biosynthesis, while glial cells can use cysteine or cystine, the dipeptide formed via the oxidation of two cysteine molecules [56]. While cysteine can be derived from methionine via the trans-sulfuration pathway for astrocytic GSH synthesis, it does not appear to be a major source of cysteine for neuronal GSH biosynthesis [1,57]. Various transporters have been implicated in the uptake of cysteine and cystine for GSH biosynthesis. While the glutamate excitatory amino acid transporters, EAAT2 and EAAT3, contribute to neuronal cysteine uptake [10,58], most cysteine is imported via the Na^+-dependent EAAC1 glutamate transporter [59]. EAAC1 expression levels are transcriptionally regulated, but EAAC1 activity can also be rapidly induced via translocation of the transporter to the plasma membrane without *de novo* protein synthesis [60]. Several signal transduction pathways that induce this trafficking are upregulated in tumor cells, including Akt/PI3K and PKC [1]. In contrast, cystine is imported into astrocytes in exchange for glutamate via the Na^+-independent glutamate-cystine antiporter (X_c^-) [61,62].

The export of GSH from astrocytes plays an important cytoprotective role in providing cysteine for neuronal GSH biosynthesis and the export of GSH-conjugates from neurons is critical in the ultimate removal of conjugated cytotoxic xenobiotics (Fig. 1B). The multidrug resistance-associated proteins can function as a GSH, GSSG, and GSH conjugate export pump (GS-X) as well as active anticancer agent efflux pumps [13,14]. These transport proteins regulate intracellular GSH and drug levels via ATP-dependent efflux of GSH–drug conjugates and may contribute to chemoresistance by mediating the export of these species. In addition, the expulsion of these GSH-conjugated drug metabolites may dramatically alter the relative intracellular levels of GSH, displacing GSH:GSSG redox homeostasis and cellular antioxidant

capacity, and promote *de novo* GSH synthesis and GSH salvage pathways. Mrps are thought to play an important role in the development of resistance to various chemotherapeutics in most human CNS tumors [9]. Mrp overexpression correlates directly with drug resistance in gliomas [47,63] and inversely with clinical outcome in neuroblastoma [64].

3. Primary human brain tumors

Malignant gliomas account for approximately 40% of newly diagnosed primary brain cancers each year in the United States [65]. The current standard of care for the treatment of malignant glioma consists of surgical resection, when feasible, followed by radiotherapy with concomitant and/or adjuvant chemotherapy. However, despite intensive treatment regimens the prognosis for patients diagnosed with malignant glioma remains poor with average 5-year survival ranging from 22% for anaplastic astrocytomas to only 2% for glioblastoma multiforme (GBM), the most common and aggressive type of primary malignant glioma [65,66]. Adjuvant chemotherapy with nitrosoureas was the mainstay of treatment for three decades prior to the introduction of the alkylating agent temozolomide in 1999, which is now the primary chemotherapeutic agent prescribed for newly diagnosed GBM and recurrent anaplastic astrocytoma [67–69]. Tumor relapse after apparent successful treatment is a common occurrence in gliomas and typically results in greater overall tumor resistance to subsequent therapy. In these instances, clinicians will intensify the dose regimen of the chemotherapeutic agent to overcome drug resistance mechanisms or incorporate alternative and/or experimental therapeutics. It is thought that both intrinsic and acquired drug resistance mechanisms are responsible for the poor response of primary and recurrent glial tumors to chemotherapy [70].

GBM, anaplastic astrocytoma and the lower grade astrocytomas arise from the astrocytes, glial cells in the brain and spinal cord that support both neurons and the endothelial cells that form the blood–brain barrier (Fig. 1A). Cultured astrocytes contain high levels of reduced GSH (~8 mM) [71], substantially higher than typically reported neuronal levels [72]. The intracellular concentration of GSH generally dictates the rate and amount of GSH released from astrocytes [73] and conditions that modify astrocyte GSH homeostasis not only result in altered astrocytic GSH export, but also directly impact neuronal GSH biosynthetic capacity due to changes in cysteine availability. Astrocytes have a robust GSH biosynthetic and efflux capacity and the astrocyte-neuron antioxidant coupling plays a particularly important role in both maintaining neuronal GSH homeostasis and protecting neurons from various xenobiotics and oxidative stress [74]. The inherent cellular GSH machinery likely remains intact after neoplastic transformation, resulting in the characteristic resistance to radiation and many chemotherapeutics observed in GBM and the astrocytomas.

Oligodendrocytes are glial cells responsible for the formation and maintenance of the myelin sheath insulating neuronal axons (Fig. 1A). Oligodendrogliomas, the third most common type of glioma, are diffusely infiltrating brain tumors arising from oligodendrocytes or oligodendrocyte precursors and represent 3–5% of primary brain tumors [75]. The majority (60–90%) of oligodendrogliomas exhibit combined chromosome loss of 1p and 19q, which predicts a less aggressive tumor phenotype and enhanced sensitivity to chemotherapy [76–78]. Cultured oligodendrocyte precursors have high levels of iron, lower levels of cellular GSH and higher levels of baseline oxidative stress compared with astrocytes or neurons [79,80]. In addition, oligodendrocyte precursors are more sensitive to oxidative stress-induced death in response to GSH depletion than their mature counterparts [81]. Interestingly, while mature oligodendrocytes also contain high levels of iron and low levels of cellular GSH, they exhibit enhanced metabolism of hydrogen peroxide due to increased enzymatic activity of GPx, GR, and catalase [82,83]. Although functionally relevant GST polymorphisms have been found to correlate with survival [84,85], the low level of cellular GSH in these cells likely plays a central role in the enhanced susceptibility of oligodendrogliomas to chemotherapy due to decreased GSH availability for GST-mediated detoxification. The high level of iron in these cells also makes them exquisitely sensitive to chemotherapeutic-mediated generation of ROS [86].

Medulloblastoma is a highly malignant brain tumor arising from the posterior fossa (usually the cerebellum) that is the most common type of CNS malignancy diagnosed in children under 20 and is the second leading cause of cancer-related death in children [75]. Currently, 5-year survival stands at roughly 60% although clinical outcomes vary with age, histological subtype, and metastatic status [75,87,88]. In contrast to gliomas, medulloblastomas are thought to originate from immature neuronal progenitors (Fig. 1A), with tumor cells exhibiting less differentiated and more stem cell-like characteristics, including robust drug resistance [89,90]. The GSH detoxification system has been implicated in the chemoresistance of medulloblastoma [12]. Treatment with GSH or N-acetyl cysteine (NAC) protects medulloblastoma cells against camptothecin (CPT)-induced apoptosis [91]. Furthermore, while cyclophosphamide-resistant medulloblastoma cell lines did not demonstrate altered GST expression or activity compared with non-resistant parental lines, they did exhibit enhanced cellular GSH levels and γGT expression [44]. Interestingly, individuals with GST null polymorphisms were associated with increased risk of adverse events in medulloblastoma patients during therapy, including ototoxicity, myelosuppression, nephrotoxicity, and cognitive impairment, as well as lower rates of survival due to the global nature of the null phenotype and the resulting enhancement of chemotherapy-associated toxicity [92].

4. The contribution of GSH and GSH-related enzymes to chemoresistance

The GSTs are classical Phase II metabolic enzymes that detoxify xenobiotics via conjugation of reduced GSH with the electrophilic center of a large spectrum of hydrophilic molecules [18,93–97]. GSTs comprise a family of widely distributed, heterogeneous cellular enzymes encoded by structurally different genes located on different chromosomes [95,98,99]. It is generally thought that GSTs play an important role in cancer susceptibility, development of chemoresistance, and clinical outcome due to their ability to metabolize both carcinogens and cancer chemotherapeutics. The GSH conjugates of chloroethylnitrosoureas (CENUs), platinum compounds and a number of other alkylating agents, including melphalan, cyclophosphamide, chlorambucil, doxorubicin and nitrogen mustards are more polar and less active than their parent compounds and are substrates for transporter-mediated export from the cell [12,16,41]. Therefore, GSH conjugation may be a contributing factor in the drug-resistant phenotype and clinical non-responsiveness of brain tumors to alkylating agent-based therapy.

Several studies have indicated that drug resistance mediated by the GSH/GST system in brain tumors may occur as a result of an alteration in a GSH-linked enzyme system and/or elevated GSH content [42–44]. GSH levels and GST expression have been shown to be inversely related to tumor response to nitrogen mustard therapy in brain neoplasms [43]. Increased levels of GSH are also associated with significant inactivation of BCNU, as demonstrated by a decreased induction of interstrand DNA crosslinks and an increased survival of the tumor cells [42]. Elevated levels of GSH have also been shown to directly correlate with resistance to CPA

and 4-hydroperoxy-CPA (4-HC) in a panel of medulloblastoma cell lines [44].

4.1. The expression of GST isoforms in normal brain and brain tumor tissue

GSTs are ubiquitously expressed throughout the brain, however specific isoforms are expressed at elevated levels in many primary brain malignancies [100]. Seven cytosolic GST family members have been identified, with four major isoforms present in the brain: α, μ, π and θ, all of which display distinct expression profiles and substrate specificities [9,102]. The GST π isoform is the major isoenzyme contributing to GST activity in normal brain tissue and is elevated in tumor tissue [100]. The GST π class is also the most highly expressed in human cancers and appears to be the most relevant isoform in brain tumor drug resistance [9]. The upregulation of GST π appears to be a common feature in numerous malignancies and a consistent finding in these tumors is that the expression levels of GST π inversely correlate with clinical response to chemotherapy and patient outcome [103–107]. Patients diagnosed with malignant glioma that have a more favorable outcome after irradiation and chemotherapy have a lower level of GST expression compared with patients with a poor clinical outcome [108]. In a series of 168 cerebral glioblastomas, GST π expression was associated with a significantly shorter patient survival time [109]. The expression of GST π has also been assessed as an independent prognostic indicator of patient outcome irrespective of chemotherapy. In a cohort of 61 surgically treated primary gliomas GST π levels correlated with tumor histopathology and inversely with patient survival [110]. These findings suggest that elevated expression of GST π in glioma cells is related to more aggressive and/or chemoresistant tumors and is a strong predictor of poor prognosis.

Immunohistochemical studies have confirmed that GST π is the predominant isoform in both malignant and physiological brain tissues. In the normal brain, GST π is present in astrocytes and endothelial cells, but not in neurons or oligodendrocytes [111]. GST π was found to be expressed in 49 out of 53 astrocytomas ranging from grade 1 to grade 4, with grade 2 tumors exhibiting the highest percentage of GST-positive cells [108]. An examination of GST π expression in human gliomas demonstrated that physiological expression is low in astroglia and at marginal levels in low-grade tumors [47,63,112–114]. GST π expression significantly increased in high grade gliomas and this expression correlated with increasing malignancy grade [47,114]. This association between tumor grade and GST π expression has also been extended to pediatric astrocytic tumors [113]. GST π activity has been shown to correlate with GST π protein expression and mRNA levels, suggesting that transcriptional upregulation of GST may primarily be responsible for the elevated activity of this enzyme in human brain tumors [63]. Glioma cells resistant to nimustine hydrochloride (ACNU) had expression levels of GST π mRNA and protein that were 1.3–3-fold higher than that of their sensitive counterparts [112], which was in agreement with another study that demonstrated a direct correlation between GST π expression and BCNU resistance in a series of malignant glioma cell lines [40].

The level and expression profile of the GST isoforms in brain tumors may also be related to tumor histology, with some tumor types expressing high levels of GST and some only marginally elevated or even diminished levels of GST compared with physiological CNS tissue. The expression and activity profile of GST varies significantly among different histological types of brain tumor [116]. The GST π enzyme is active in benign tumors, such as meningioma and neurinoma, with levels of GST activity ~2–3-fold higher than that of physiological brain tissue [116,117]. The activity of GST in grade 2 and 3 astrocytomas appears to be only weakly elevated compared with normal brain and GST activity in GBMs has been reported to be below that of non-neoplastic brain tissue [116]. In addition, some studies have also found significantly reduced levels of GSH in GBM compared with normal brain tissue [118]. These observations are in contrast with the results of other studies [47,100,111,113,114], indicating that GSH levels and GST expression in individual brain tumor classes remains a controversial topic. However, the GST enzymes represent only a part of the GSH metabolic pathway and the role of other GSH-linked enzymes in chemoresistance should not be overlooked.

4.2. The expression of GST genetic polymorphisms in brain tumor tissue

The susceptibility of human cancers to chemotherapy and their ability to detoxify anticancer agents are likely to be dependent on the expression of distinct gene variants of individual GST classes as well as the enzymes involved in *de novo* GSH synthesis and the GSH salvage pathways. The GST gene family is highly polymorphic and numerous correlations between allelic variants of specific GSTs and tumor drug resistance have been identified [101]. Human genetic polymorphisms for the GST μ class, GST-M1*A, GST-M1*B, and GST-M1*0, and θ null phenotype at the T1 locus, GST-T1*0, have been shown to be either a product of gene deletion or due to a specific allelic variation resulting in a catalytically active enzyme with altered charge properties [16,119,120]. The expression of the null phenotype of these GST isozymes in a tumor may influence the metabolism of some cytotoxic drugs and their response to certain chemotherapeutics, rendering these tumors vulnerable to treatment. The human GST π locus is polymorphic in human brain tumors, resulting in functionally active yet distinct GST π proteins [121–123]. Three closely related, full-length GST π cDNA variants, hGSTP1*A, hGSTP1*B, and hGSTP1*C have been isolated, with the hGSTP1*C variant being expressed at a higher frequency in gliomas than in normal cells [16,119,120]. Despite advances in understanding the molecular nature of the GST π gene in human cells, the role of individual variants in xenobiotic metabolism in brain tumors has yet to be established. In addition, the importance of polymorphisms of enzymes involved in *de novo* synthesis or salvage of GSH in drug resistance is currently unknown.

4.3. The induction of GST expression by chemotherapy

The expression of GST in human brain tumors is also inducible in response to treatment with certain anticancer agents. Although the GST π gene was overexpressed in 38% of a panel of 67 non-chemotherapy-treated human brain tumors [124], the expression and activities of both GST π and μ increased after treatment with ACNU [115,125]. The increased expression of GSTs in brain tumors after chemotherapy suggests that GST-based drug resistance of brain tumors may represent an acquired phenomenon. Furthermore, CPT-resistant glioma cells have increased levels of intracellular GSH compared with CPT-sensitive glioma cells [126]. This increase in GSH levels in brain tumors in response to chemotherapy may also represent an additional adaptive mechanism through which the GSH/GST detoxification system may respond when confronted with anticancer regimens.

4.4. Regulation of apoptotic signal transduction pathways

In addition to their role in GSH-conjugation reactions, GSTs can regulate pro-apoptotic signal transduction pathways via direct protein-protein interaction [127]. GSTP1 and GSTM1 bind and prevent JNK and ASK1 protein kinase activation, respectively [11,124]. This regulatory mechanism may play a role in drug resistance as dissociation of the GSTP1-JNK complex and

polymerization of GSTP1 is required for optimal etoposide-induced apoptosis in etoposide-resistant human neuroblastoma cells [8]. GSTP1 is also activated via post-translational phosphorylation by PKA, PKC, and the epidermal growth factor receptor tyrosine kinase (EGFR-TK), which results in increased metabolism and resistance against cisplatin in human glioblastoma cells [128–130]. Inhibition of EGFR-TK activity reversed this EGF-induced cisplatin resistance [129], suggesting that combined therapy may prove to be effective in tumors with elevated GSTP1 and inappropriately activated EGF receptor.

5. Conclusion

In summary, GSH and the GSH enzyme-linked system may be a determining factor for the sensitivity of some brain tumors to various chemotherapeutic agents. The GST π isoform has been the most extensively studied enzyme in the GSH metabolic pathway as a relevant parameter for chemotherapy response in brain tumors. The expression and activity of GST π among different histopathological groups of brain neoplasms and among individual tumors within a certain tumor class may prove to be a useful biomarker for selecting tumors that may potentially respond to a particular chemotherapeutic regimen. The interplay between GSH/GST-mediated drug detoxification and Mrp-facilitated efflux of the GSH–drug conjugate may have an important role in conferring drug resistance in primary brain tumors and may even constitute a favorable target for therapeutic strategies directed at selectively modulating drug sensitivity [12–14]. Approaches using BSO to directly deplete GSH as a means of enhancing the efficacy of chemotherapy are currently being explored for brain tumors [44,50,131,132]. However, a major drawback to BSO is its potential to enhance toxicity in normal tissue due to a lack of selectivity for tumor cells. GST may also be considered as a potential target to modulate chemosensitivity in human brain tumors. Due to the association between the expression and activity of GST and chemoresistance, there have been efforts to develop GST-directed therapeutics and GST-activated prodrugs as novel anticancer agents [133]. However, these agents have not shown any significant beneficial response to date in clinical trials [134].

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