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The x_c^- Cystine/Glutamate Antiporter: A Potential Target for Therapy of Cancer and Other Diseases

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The x_c^- cystine/glutamate antiporter is a major plasma membrane transporter for the cellular uptake of cystine in exchange for intracellular glutamate. Its main functions in the body are mediation of cellular cystine uptake for synthesis of glutathione essential for cellular protection from oxidative stress and maintenance of a cystine:cysteine redox balance in the extracellular compartment. In the past decade it has become evident that the x_c^- transporter plays an important role in various aspects of cancer, including: (i) growth and progression of cancers that have a critical growth requirement for extracellular cystine/cysteine, (ii) glutathione-based drug resistance, (iii) excitotoxicity due to excessive release of glutamate, and (iv) uptake of herpesvirus 8, a causative agent of Kaposi's sarcoma. The x_c^- transporter also plays a role in certain CNS and eye diseases. This review focuses on the expression and function of the x_c^- transporter in cells and tissues with particular emphasis on its role in disease pathogenesis. The potential use of x_c^- inhibitors (e.g., sulfasalazine) for arresting tumor growth and/or sensitizing cancers is discussed.

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Plasma membrane amino acid transporters allow regulated bidirectional transfer of specific amino acids across the plasma membrane. As such they have essential roles in the maintenance and proper functioning of numerous amino acid-dependent cellular processes, including protein synthesis, energy metabolism, and cell preservation (Christensen, 1990; Chillaron et al., 2001). These transporters are essential for cells that cannot sufficiently synthesize certain amino acids and hence require their uptake from the extracellular space for growth and viability. Normal and malignant lymphoid cells, for example, have a critical requirement for extracellular cystine or cysteine, the reduced form of the amino acid (Eagle et al., 1966; Iglehart et al., 1977; Ishii et al., 1981b; Gmunder et al., 1990). While cysteine is required for general protein biosynthesis, it is particularly important as a rate-limiting precursor in the biosynthesis of glutathione, a tripeptide thiol consisting of glutamate, cysteine, and glycine, which plays a critical role in cellular defenses against oxidative stress as a free radical scavenger and detoxifying agent (Griffith, 1999). In cancer cells glutathione content is particularly relevant in regulating DNA synthesis, growth, and multidrug and radiation resistance. As such, glutathione is considered an important target in cancer therapy (Estrela et al., 2006). Since intracellular glutathione has a short half-life, cysteine deficiency can readily lead to glutathione depletion followed by growth arrest and reduced therapy resistance. Cystine/cysteine starvation of target cells has therefore been suggested for use in therapy of a variety of cancers (Uren and Lazarus, 1979; Gout et al., 1997; Chung et al., 2005; Doxsee et al., 2007).

This review focuses on the x_c^- cystine/glutamate antiporter, a major plasma membrane transporter for cystine and glutamate, and its role in various diseases, particularly cancer. This transporter is essential for maintenance of a variety of experimental cancers that require extracellular cystine/cysteine for growth by mediating (i) the maintenance of cysteine levels in their extracellular compartment and/or (ii) their uptake of cystine. Inhibition of the x_c^- transporter could hence be useful for generating cystine/cysteine starvation of such cancers (Gout et al., 1997, 2001; Narang et al., 2003; Chung et al., 2005; Doxsee et al., 2007). Furthermore, the x_c^- transporter appears to be critically involved in glutathione-based drug resistance (Okuno et al., 2003; Huang et al., 2005; Kagami et al., 2007; Narang et al., 2007), glioma-induced excitotoxicity (Sontheimer, 2003), and promotion of glioma cell invasion (Lyons et al., 2007). Quite recently, the xCT subunit of the x_c^- transporter has been reported to act as a cellular receptor for human herpesvirus 8, a causative agent of Kaposi's sarcoma and other lymphoproliferative syndromes (Kaleeba and Berger, 2006). The x_c^- transporter is also reported to play a role in various CNS and eye diseases. In view of this, it appears to represent an important potential target for therapy of a variety of disorders.

The x_c^- Cystine/Glutamate Antiporter

Function and structure

The x_c^- transporter was first described in 1980 by Bannai and Kitamura as an Na^+-independent transport system for L-cystine and L-glutamate in human fibroblasts (Bannai and Kitamura, 1980). The x_c^- designation was subsequently assigned by Makowske and Christensen (1982). The x_c^- transporter is an

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obligate, electroneutral, anionic amino acid antiporter. The anionic form of extracellular cystine is transported into cells in exchange for intracellular glutamate with a stoichiometry of 1:1 (Fig. 1) (Bannai, 1986; Christensen, 1990; Chillaron et al., 2001). Once inside a cell, cystine is rapidly reduced to cysteine, which can then enter glutathione and protein biosynthetic pathways. As a result the intracellular levels of cystine are much lower than its extracellular levels. In contrast, the intracellular levels of glutamate are in general much higher than its extracellular levels, a result of glutamine uptake via the alanine-serine-cysteine (ASC) transporter system. This glutamate concentration gradient is thought to provide, at least in part, the driving force of the cystine/glutamate exchange by x_c^- (Bannai and Ishii, 1988; Christensen, 1990). The x_c^- transporter can also mediate cellular uptake of L-glutamate, and x_c^- activity can be quantified using either L-cystine or L-glutamate as a substrate; in both instances, the uptake is Cl^--dependent and Na^+-independent (Patel et al., 2004). In view of similar affinities of cystine and glutamate for x_c^-, L-cystine uptake by this exchange system is potently inhibited by L-glutamate (e.g., monosodium glutamate) and vice versa (Bannai, 1986).

The x_c^- antiporter has two major functions. It mediates cellular uptake of cystine in particular for maintenance of intracellular levels of glutathione essential for protection of cells from oxidative stress and xenobiotics. Furthermore, it is instrumental in maintaining the redox balance between extracellular cystine and cysteine. In the extracellular milieu cysteine is readily oxidized to cystine which consequently is the predominant form of the amino acid in the circulation and particularly in culture media (Toohey, 1975); intracellularly, cysteine is the predominant form (Bannai and Ishii, 1988; Christensen, 1990). Whereas cysteine can be readily taken up by mammalian cells via, for example, the ubiquitous ASC transport system (Christensen, 1990), cystine transporters are not universally expressed by cells (Gmunder et al., 1991; Gout et al., 2001). However, somatic cells, such as fibroblasts, activated macrophages, and dendritic cells, express the x_c^- transporter and take up extracellular cystine, reduce it internally to cysteine and secrete cysteine into the extracellular compartment, thus closing the loop in the redox cycling of the amino acid (Bannai and Ishii, 1988; Eck and Droge, 1989; Christensen, 1990; Gmunder et al., 1990; Angelini et al., 2002; Edinger and Thompson, 2002).

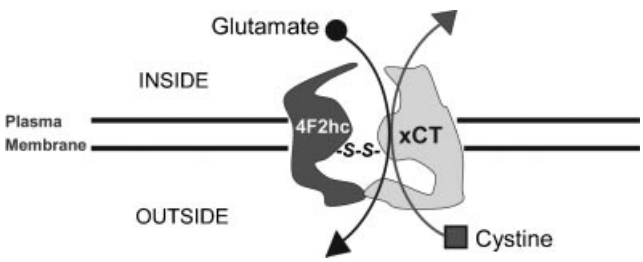

Fig. 1. Schematic diagram showing the function of the plasma membrane x_c^- cystine/glutamate transporter, an obligate, electroneutral, anionic amino acid antiporter. It is a heteromeric protein composed of two subunits coupled via a disulfide bridge, that is, a heavy subunit, 4F2hc, involved in trafficking of the heterodimer to the plasma membrane, and a light subunit, xCT, conferring transport and substrate specificity. The anionic form of extracellular cystine is transported into cells in exchange for intracellular glutamate with a stoichiometry of 1:1.

Structurally, the x_c^- transporter is a member of a family of heteromeric amino acid transporters (HATs). These transporters are composed of a heavy subunit (HSHAT) and a light subunit (LSHAT) coupled via a disulfide bridge. The heavy subunit is involved in trafficking of the heterodimer to the plasma membrane, whereas the light subunit confers transport and substrate specificity. In the case of the human x_c^- transporter, a heavy subunit, designated 4F2hc (also termed CD98), is coupled to xCT, a member of the human LSHAT family conferring specificity for cystine. The 4F2hc subunit is a type II membrane glycoprotein commonly expressed in cells since it acts as a subunit for various amino acid transporters (Chillaron et al., 2001; Verrey et al., 2004). It may be noted that the 4F2hc subunit of x_c^- can be replaced by rBAT, another HSHAT, with retention of x_c^- activity (Wang et al., 2003; Fernandez et al., 2006).

The molecular nature of the x_c^- transporter was elucidated in 1999 by Sato and co-workers (Sato et al., 1999). They isolated cDNA encoding the mouse x_c^- transporter from mouse peritoneal macrophages treated with diethylmaleate and lipopolysaccharide, two potent inducers of x_c^- activity. Expression of mouse x_c^- activity in *Xenopus* oocytes was found to require two cDNA transcripts, one coding for 4F2hc and the other for a protein, designated xCT, consisting of 502 amino acids and 12 putative transmembrane domains as indicated by its hydrophobicity profile. Subsequently, cDNA for human xCT (hxCT) was identified (Sato et al., 2000; Bassi et al., 2001; Kim et al., 2001). The full-length *hxCT* gene was isolated by RT-PCR from an undifferentiated human teratocarcinoma cell line; the nucleotide sequence translated into a 501-amino acid predicted protein product with 89% identity and 93% similarity to mouse xCT (Bassi et al., 2001). To achieve expression of x_c^- activity in *Xenopus* oocytes, expression of both hxCT and 4F2hc subunits was required (Sato et al., 1999; Bassi et al., 2001; Kim et al., 2001). Similarly, cystine/glutamate transport function of cloned hxCT in human retinal cells was shown to be dependent on co-expression of 4F2hc (Bridges et al., 2001). Variants of hxCT cDNA have been reported (Sato et al., 2000; Kim et al., 2001), including a functional splice variant, hxCTb, showing differences in the C-terminal region and degree of expression in a variety of tissues (Kim et al., 2001). The Human Genome Mapping Workshop (HGMW)-approved name for *hxCT* is Solute Carrier Family 7, member 11 (*SLC7A11*). Thus far, ten members of the LSHAT family have been identified (*SLC7A5-11, Asc-2, AGT-1,* and *arpAT*) (Verrey et al., 2004; Fernandez et al., 2005).

Extensive research has been conducted on x_c^- membrane topology and substrate-binding sites (Gasol et al., 2004; Jimenez-Vidal et al., 2004). The studies focused on the xCT subunit considered responsible for transport of cystine and glutamate; the 4F2hc subunit, predicted to have only a single transmembrane domain is presumably incapable of transport activity by itself (Palacin et al., 2000; Jimenez-Vidal et al., 2004). Glutamate transport by 4F2hc-hxCT heterodimers in *Xenopus* oocytes was found to be markedly inhibited by thiol-modifying mercurial reagents suggesting a role for cysteine residues in xCT function. Using heterodimers formed by 4F2hc with hxCT cysteine mutants generated via site-directed mutagenesis, it was found that Cys^{327} in the 8th putative transmembrane domain of xCT is a functionally important residue, accessible from the aqueous extracellular compartment and structurally linked to the permeation pathway and/or substrate-binding site (Jimenez-Vidal et al., 2004). Based on accessibility of single xCT cysteines to 3-(*N*-maleimidyl-propionyl)biocytin, a topological model was proposed for xCT of 12 transmembrane domains with both the N- and C-termini located inside the cell; the intracellular locations of the termini were confirmed by immunofluorescence. Furthermore, a re-entrant loop with substrate-restricted accessibility was revealed within intracellular loops 2 and 3 (Gasol et al., 2004).

Dependence on pH

As found with cultured fibroblasts or *Xenopus* oocytes injected with 4F2hc and xCT cRNA, L-cystine transport showed a marked dependence on pH with an optimum of about 7.5. In contrast, L-glutamate transport was almost independent of pH. Whereas glutamate inhibited cystine uptake independently of pH, cystine inhibited glutamate uptake in a pH-dependent manner (Bannai and Kitamura, 1981; Bassi et al., 2001).

Inhibitors

Monosodium glutamate is the main exchange substrate in the x_c^--mediated uptake of cystine, with an affinity for x_c^- similar to that of cystine; as such it is a potent, highly specific inhibitor of cystine uptake. Likewise, cystine competitively inhibits glutamate uptake via the x_c^- transporter (Bannai and Kitamura, 1980). By specifically inhibiting the x_c^- transporter, monosodium glutamate can severely inhibit or completely arrest in vitro proliferation of malignant cells that depend for growth on x_c^--mediated uptake of cystine (Gout et al., 1997); however, glutamate cannot be used as a therapeutic to inhibit cellular uptake of cystine in vivo since it is neurotoxic (Choi, 1988). Other potent inhibitors of the x_c^- transporter include α-aminoadipate, α-aminopimelate, homocysteate (Bannai and Kitamura, 1981), (S)-4-carboxyphenylglycine, L-serine-O-sulphate, ibotenate, (RS)-4-bromohomoibotenate, and quisqualate (Patel et al., 2004). In addition, x_c^--mediated cystine uptake can be inhibited by UV-B irradiation, as reported for human HaCaT keratinocytes (Zhu and Bowden, 2004), and by L-lactate, as observed with cultured rat astrocytes (Koyama et al., 2000). Certain anti-inflammatory drugs have also been reported to have x_c^--inhibitory activity (Bannai and Kasuga, 1985). In a search for x_c^- inhibitors potentially useful as novel anticancer agents, it was first observed in our laboratory that sulfasalazine, a disease-modifying anti-rheumatic drug (DMARD), is a potent and quite specific inhibitor of the transporter (Gout et al., 2001).

The pharmacology and kinetic properties of system x_c^- have been extensively studied by Patel and co-workers (Patel et al., 2004) using rat astrocytoma cells, L-[^{3}H]-glutamate to determine cellular uptake of this amino acid and glutamate efflux as a measure of substrate activity. A wide variety of cystine, aspartate, and glutamate analogues was examined for glutamate uptake-inhibitory activity. In addition to identifying a number of competitive inhibitors, such uptake blockers could be further classified as either alternative substrates (e.g., ibotenate) or non-substrate inhibitors [e.g., (S)-4-carboxyphenylglycine]. Interestingly, the latter compound, a cyclic glutamate analogue with very little substrate activity, was one of the most potent competitive inhibitors, suggesting that distinct structural features of the transporter may control the actual binding and transport of a substrate into the cell. It is noteworthy that substrate inhibitors of the x_c^- transporter, while inhibiting cystine/glutamate uptake, could potentially increase the likelihood of excitotoxic injury by way of an x_c^- exchange-mediated glutamate efflux leading to increased extracellular levels of glutamate (Patel et al., 2004). In view of this, non-substrate x_c^- inhibitors, inducing intracellular glutathione depletion without glutamate efflux, could be more useful for therapeutic applications aimed at reducing growth and/or drug resistance of malignant cells dependent on x_c^- function.

Induction of the x_c^- system

Expression of x_c^- coupled to increases in glutathione levels can be obtained in a variety of cell systems (e.g., macrophages, retinal, endothelial cells) by numerous stimuli, including electrophilic agents such as diethylmaleate (Bannai, 1984a; Kim et al., 2001; Tomi et al., 2002, 2003), oxygen (Bannai et al., 1989), bacterial lipopolysaccharide (Sato et al., 1995), nitric oxide (Watanabe and Bannai, 1987; Li et al., 1999; Sato et al., 1999; Bridges et al., 2001; Dun et al., 2006), *Nrf2* overexpression (Shih et al., 2003), and by the Tat transactivator protein (Bridges et al., 2004). It should be noted that the induction of glutathione synthesis can depend on the concentration of the stimulating agent. Thus diethylmaleate at low concentrations (0.1 mM) can increase x_c^- transporter expression and glutathione levels, but at higher concentrations (1 mM) can act as an oxidative stressor depleting glutathione levels, as seen in human fibroblasts (Bannai, 1984a). Increased xCT expression can also result from deprivation of cystine or other amino acids, involving transcriptional control mediated by amino acid response elements (Sato et al., 2004). Increased expression of both x_c^- subunits has been reported for astrocytes following incubation with dibutyryl-cAMP (Gochenauer and Robinson, 2001).

Regulators of x_c^- function

The response of the *xCT* gene to oxidative stress or electrophiles is mediated by a *cis*-acting transcriptional regulatory element in its promoter region designated "Antioxidant Response Element" (ARE) or "Electrophile Response Element" (EpRE). Mutational analysis of the ARE/EpRE has shown that it is critically involved in the response to agents such as diethylmaleate, arsenite, cadmium, and hydroquinone (Sasaki et al., 2002). The ARE/EpRE is also essential for expression of many other antioxidant/detoxification genes, and treatment of mammalian cells with e.g., electrophilic agents, can trigger a coordinated expression of a family of phase II detoxification enzymes (Mann et al., 2007). The inducible expression of the *xCT* gene and antioxidant/detoxification genes is primarily regulated by binding to the ARE of the Cap "n" Collar transcription factor, Nuclear factor erythroid 2-related factor-2 (Nrf-2) (Lee and Johnson, 2004). Thus overexpression of *Nrf2* in astrocytes in vitro resulted in a marked increase in xCT-mRNA levels and expression of the x_c^- cystine transporter, as well as a coordinated upregulation of proteins/enzymes involved in the biosynthesis of glutathione (γ-glutamyl cysteine synthetase, glutathione synthase), function of glutathione (glutathione-S-transferase, glutathione reductase), and export of the tripeptide thiol (multidrug resistance-associated Protein-1) (Shih et al., 2003). In another study it was found that astrocytes infected with ts1, a mutant of the Moloney murine leukemia virus, mobilized their thiol redox defenses by upregulating levels of Nrf2 protein as well as its targets, including the x_c^- cystine/glutamate antiporter (Qiang et al., 2004). Under basal conditions, Nrf2 protein is sequestered in the cytoplasm by Kelch-like ECH-associated protein-1 (Keap1/INrf2), a negative regulator protein associated with the actin cytoskeleton. Upon induction, involving oxidation of cysteine residues, Nrf2 dissociates from Keap1, translocates to the nucleus, and binds to ARE sequences which leads to transcriptional activation of antioxidant/detoxifying genes (Mann et al., 2007). ARE-mediated gene expression can be negatively regulated by transcription factors such as c-Maf (Dhakshinamoorthy and Jaiswal, 2002) and Bach1 (Dhakshinamoorthy et al., 2005). Activation of *Nrf2* has been implicated in conferring protection against many human diseases and a proper understanding of *Nrf2* regulation appears crucial in the development of drugs for therapeutic intervention (Zhang, 2006). On the other hand, targeting *Nrf-2* may assist in decreasing expression of the x_c^- transporter and glutathione biosynthetic genes in malignant cells thereby lowering their drug resistance.

Expression of the x_c^- transporter in normal cells and tissues

The x_c^- transporter is expressed almost ubiquitously in cultured mammalian cell lines. It should be noted that the expression of the transporter by cultured cells may not reflect the native status of the cells, since it can be induced upon culturing (Ishii et al., 1992). In some cells/tissues, xCT mRNA has been determined by Northern analysis or quantitative real time RT-PCR. xCT protein has been identified via anti-xCT antibodies of which more preparations have recently become available (Kim et al., 2001; Burdo et al., 2006; Kaleeba and Berger, 2006; Shih et al., 2006; La Bella et al., 2007). In *mice*, xCT mRNA expression has been detected in the brain (Sato et al., 2002; Dave et al., 2004), cultured chondrocytes (Wang et al., 2006), endothelial cell line (MBEC4) as a blood–brain barrier model (Hosoya et al., 2002), kidney (Bassi et al., 2001), stomach (Dave et al., 2004), intestine (Bassi et al., 2001; Dave et al., 2004), ovary (Bassi et al., 2001), retina (Bridges et al., 2004; Dun et al., 2006), cultured melanocytes (Chintala et al., 2005), spleen (Bassi et al., 2001), macrophages (Sato et al., 2001), and cultured mesenchymal C3H10T1/2 stem cells (Iemata et al., 2007); xCT protein was detected in the brain (Burdo et al., 2006). In *rats*, x_c^- transporter function was observed in freshly isolated fetal brain cells (Sagara et al., 1993), primary cultures of cortical neurons (Murphy et al., 1990) and cultured pancreatic cells (Sato et al., 1998); xCT protein was detected in the brain (Shih et al., 2006; La Bella et al., 2007) and in the lens of the eye (Li et al., 2007); xCT mRNA expression was observed in the lens (Lim et al., 2005), immortalized retinal endothelial cells (Tomi et al., 2002), in cultured retinal Muller cells (Tomi et al., 2003), chondrocytes (Wang et al., 2006), and microglia (Qin et al., 2006). In *monkeys*, xCT protein distribution was shown in the kidney and duodenum (Burdo et al., 2006). In *humans*, xCT mRNA expression was found in the spinal cord (Kim et al., 2001), brain, and pancreas (Bassi et al., 2001; Kim et al., 2001); xCT protein was also observed in the brain (Burdo et al., 2006) and x_c^- transporter function was detected in fibroblasts (Bannai, 1986), monocytes (Eck and Droge, 1989), macrophages (Rimaniol et al., 2001), and antigen-presenting dendritic cells (Angelini et al., 2002). It may be noted that of a wide variety of human tissues and cells examined, the x_c^- transporter is predominantly expressed in the brain, pancreatic islets, and stromal and immune cells. Of particular interest are studies with the following normal tissues.

Brain. Brain cells, and in particular neurons, consume high levels of oxygen for excitatory processes and therefore generate more oxidative stress than any other cell type. Glutathione is thought to protect the brain by detoxifying reactive oxygen species continuously generated during oxidative metabolism (Dringen, 2000). Astrocytes (glial cells) are thought to protect neurons from oxidative stress by releasing glutathione into the extracellular compartment (Sagara et al., 1996; Wang and Cynader, 2000; Shih et al., 2006), a process dependent on preceding uptake of cystine via the x_c^- transporter (Cho and Bannai, 1990) and *Nrf*2 upregulation (Shih et al., 2003). Subsequently, a thiol/disulfide exchange reaction between the extracellular glutathione and cystine is thought to produce cysteine which can be readily taken up by the neuronal cells for intracellular glutathione biosynthesis (Wang and Cynader, 2000). Alternatively, brain cells may directly take up cystine via the x_c^- transporter for production of glutathione, and specific inhibition of the transporter can lead to cell death of primary neuronal cultures due to accumulation of cellular oxidants (Murphy et al., 1989).

A number of x_c^- localization studies have been reported. In a recent study using anti-xCT antibodies, xCT protein was found to be enriched in fetal and adult rat brain at the CSF–brain barrier (i.e., meninges) and also expressed in the cortex, hippocampus, striatum, and cerebellum (Shih et al., 2006). Subcellular fractionation of rat brain cells showed that xCT concentrated mainly in the microsomal-mitochondrial fraction, consistent with its structure as a transmembrane protein (La Bella et al., 2007). In another recent study, xCT protein in mouse and human brain was localized predominantly to neurons, with some labeling of glial cells; border areas between the brain proper and its periphery, including vascular endothelial cells, ependymal cells, choroid plexus, and leptomeninges, were also highly positive (Burdo et al., 2006). Previously, xCT mRNA expression was found in similar sections of the normal mouse brain with expression detected in the meninges and some circumventricular organs suggesting that the x_c^- transporter may contribute to the maintenance of the redox state (i.e., cysteine/cystine ratio) in the cerebral spinal fluid (Sato et al., 2002). xCT mRNA was also expressed in mouse brain endothelial MBEC4 cells (Hosoya et al., 2002). Together, the data suggest that the x_c^- cystine transporter contributes to the maintenance of intracellular cysteine and glutathione levels in many areas of the brain and hence to its cellular health.

In addition to its role in cystine uptake, the x_c^- transporter also mediates the export of glutamate, the exchange substrate for cystine. Glutamate is a major excitatory neurotransmitter in the mammalian brain and possesses excitotoxic properties; it is a potent neurotoxic molecule both in vivo and in vitro (Choi, 1988; Meldrum and Garthwaite, 1990). In view of this, the expression/activity of glutamate-related transporters, including the x_c^- transporter, must be precisely regulated in brain cells for normal cellular function to occur (Burdo et al., 2006; Augustin et al., 2007). Seizures and neuronal cell death can be induced by an increase in the levels of extracellular glutamate. This is normally prevented by astrocytes through glutamate uptake via Na^+-dependent glutamate receptors such as GLT-1 and GLAST (Ye et al., 1999). Indeed, glutamate has been implicated as the proximate cause of many CNS pathologies, including cerebral ischemia (stroke), seizures, hypoxia, trauma, and hypoglycemia (Choi, 1988).

In Alzheimer's disease, microglia can enhance the toxicity of neurotoxic amyloid-β aggregates by releasing glutamate via the x_c^- transporter (Qin et al., 2006). Alzheimer's disease has been linked to elevated concentrations of aluminum in the brain and in this context it is of interest that the x_c^- transporter has been implicated in the uptake of aluminum citrate by cultured rat endothelial brain cells (Nagasawa et al., 2005). While neuronal death may be induced by glioma cells releasing glutamate, macrophages may inflict neuronal damage in a similar manner (Piani and Fontana, 1994). Neurons can be sensitized by quisqualic acid (a known glutamate receptor agonist) to depolarization by certain excitatory amino acids and the x_c^- transporter has been implicated in the cellular internalization of quisqualic acid thought to be required for the sensitization (Chase et al., 2001). There is also evidence that the x_c^- transporter plays a role in virus-induced encephalopathy (Espey et al., 1998), periventricular leukomalacia (Oka et al., 1993), and cocaine relapse (Baker et al., 2003; Lu et al., 2004).

It is becoming increasingly evident that the x_c^- cystine/glutamate antiporter plays an important role in many CNS pathologies. Further knowledge of the molecular mechanism of action of this transporter could lead to development of novel therapeutics aimed at brain diseases associated with depletion of glutathione, including Parkinson's disease and amyotrophic lateral sclerosis (Schulz et al., 2000).

Pancreas. Two distinct mechanisms for L-cystine uptake have been reported to exist in the normal human pancreatic ductal cell line PaTu 8902. Specifically, 50–60% of cystine uptake by this cell line was found to be mediated by the x_c^- transporter, with the remainder being mediated via the γ-glutamyl cycle by γ-glutamyl transpeptidase, an enzyme located on the outer

surface of the plasma membrane (Sweiry et al., 1995). Incubation of cultured pancreatic AR42J acinar and βTC3 islet cells with diethylmaleate, an agent known to activate cellular antioxidant responses, led to cystine uptake which was predominantly mediated by the x_c^- transporter. Upregulation of x_c^- activity would likely contribute to cellular antioxidant defenses in pancreatic disease (Sato et al., 1998).

Ocular tissue. Oxidative damage of eye proteins is thought to underlie major eye diseases. Glutathione may prevent the development of such diseases by protecting thiol groups of proteins and hence minimize oxidation-induced protein aggregate formation via non-scheduled disulfide bond cross-linkages. Glutathione appears to play a major role in maintaining lens transparency and in the prevention of diseases such as age-related macular degeneration, diabetic retinopathy, and cataracts (Bridges et al., 2001; Lim et al., 2005; Li et al., 2007). Recent studies have demonstrated that transporters for cystine (x_c^-), glycine (GLYT1), and glutamate (ASCT2), the three amino acids required for biosynthesis of glutathione, are expressed in the rat lens. Their molecular identification and particular localization in the eye tissues are consistent with a specific requirement for glutathione in the lens cortex and core (Lim et al., 2005, 2006). In studies of non-infectious AIDS retinopathy it was found that the HIV-1 genome-encoded transactivator protein, Tat, decreased glutathione levels in the mouse retina and upregulated the x_c^- transporter. This led to enhanced release of glutamate into the extracellular space of the retina and excitotoxicity. It is speculated that this upregulation of the x_c^- transporter may underlie the pathogenesis of AIDS retinopathy (Bridges et al., 2004).

Skin. In melanocytes, cysteine is an important precursor of melanogenesis by reacting non-enzymatically with dopaquinone to form sulfur-containing pheomelanin, the red/yellow pigment that, together with eumelanin, determines the color of the skin in mammals (Wakamatsu and Ito, 2002). Melanogenesis studies of a mouse pigmentation mutant, designated subtle gray (*sut*), revealed a mutation in the *SLC7A11* gene encoding the xCT subunit of the x_c^- transporter (Chintala et al., 2005). Cultured *sut*-mutant melanocytes showed markedly reduced uptake of extracellular cystine and undetectable levels of glutathione. Similarly, the levels of pheomelanin in hair from *sut* mutant mice were substantially depressed. The study suggests that the *SLC7A11* gene is a major genetic regulator of pheomelanin in hair and melanocytes with minimal effects on eumelanin (Chintala et al., 2005). In view of this, the x_c^- transporter may have yet undefined roles in melanin-related normal and pathological processes.

Immune and stromal cells. Activation of T-lymphocytes by antigen-presenting cells (activated macrophages, dendritic cells) has been reported to involve expression of the x_c^- transporter in the antigen-presenting cells. This allows such cells to take up cystine from their micro-environment, reduce it to cysteine and secrete cysteine which can be readily taken up by the lymphocytes (e.g., via the ASC transport system); indeed, this process is apparently essential for lymphocyte clonal expansion (Gmunder et al., 1990; Sido et al., 2000; Angelini et al., 2002; Edinger and Thompson, 2002). As such, activated macrophages and dendritic cells may serve as local suppliers of cysteine which in general is readily taken up by cells in contrast to cystine (Eck and Droge, 1989; Gmunder et al., 1990, 1991). Similarly, stromal cells, such as fibroblasts, can serve as cysteine suppliers by continuously secreting cysteine. In fact, this ability was initially applied in vitro to support growth of lymphoma cells in co-cultures with fibroblasts (Ishii et al., 1981b). Such growth-supporting, cysteine-secreting somatic cells could later be replaced by 2-mercaptoethanol (50–100 μM), a thiol which allows cellular uptake of cystine via the L transport system in the form of a mixed cysteine-2-mercaptoethanol disulfide. Once inside the cell, this disulfide is split into cysteine and 2-mercaptoethanol after which the latter diffuses out of the cell. Hence 2-mercaptoethanol provides a pathway for shuttling cystine into cells while circumventing the x_c^- transporter (Ishii et al., 1981a). Use of 2-mercaptoethanol enabled establishment of a prolactin-dependent rat Nb2 lymphoma cell line (Gout et al., 1980) that serves as a novel, specific, and sensitive in vitro bioassay for lactogenic hormones (Tanaka et al., 1980) and forms the basis of studies showing potential usefulness of the x_c^- transporter as a target for cancer therapy (Gout et al., 1997).

Other cystine or glutamate transporters

Other systems mediating uptake of cystine in mammalian cells have been reported. One, termed system $b^{0,+}$, exists as a heterodimer consisting of either 4F2hc (SLC3A2) or rBAT (SLC3A1) as the heavy chain and $b^{0,+}$AT (SLC7A9) as the light chain. Uptake by this transporter is not confined to only cystine but includes several cationic and neutral amino acids and is also Na^+-independent. Its expression, however, is limited mostly to the small intestines and kidney (Rajan et al., 2000; Bridges et al., 2001). A role for γ-glutamyl transpeptidase in the transport of cystine has been reported (Sweiry et al., 1995). Additional systems mediating influx of glutamate include the Na^+-dependent X_{AG}^- family, commonly known as the excitatory amino acid transporters (EAAT1-5) of glutamate and aspartate (Kanai and Hediger, 2004). The X_{AG}^- glutamate transporters can also mediate cellular uptake of cystine (McBean and Flynn, 2001). The x_c^- transporter, however, has been found to be highly dominant in a variety of systems, including brain and pancreatic cells (Bassi et al., 2001), activated macrophages (Gmunder et al., 1990), dendritic cells (Angelini et al., 2002), and fibroblasts (Bannai and Ishii, 1988). It may be noted that although the x_c^- transporter can be vital to cellular health, especially in vitro, it is dispensable during mammalian development, as demonstrated with the generation of healthy and fertile x_c^- knock-out mice (Sato et al., 2005).

Role of the x_c^- Cystine/Glutamate Antiporter in Cancer

Growth requirement of cancers for extracellular cystine/cysteine: Leukemias and lymphomas

Cysteine is traditionally viewed as a nutritionally non-essential amino acid since it is synthesized in the body, primarily by the liver, from L-methionine via the transsulfuration pathway (Rosado et al., 2007). Certain cancers, including leukemias and lymphomas, are incapable of synthesizing cysteine (Iglehart et al., 1977; Gout et al., 1997), probably due to a deficiency in γ-cystathionase, the last enzyme in the transsulfuration pathway (Uren and Lazarus, 1979). Cystine/cysteine is thus an essential amino acid for such cancers and its uptake from the micro-environment is vital for their growth and viability. A growth requirement for extracellular cystine can be readily demonstrated for cultured cells by transferring them to culture medium specifically deficient in the amino acid and monitoring their growth (Doxsee et al., 2007) or by omitting cystine uptake enhancers (e.g., 2-mercaptoethanol) from the medium (Gout et al., 1997). Blood plasma contains relatively high concentrations of cystine (100–200 μM half-cystine), but only 10–20 μM cysteine; such cysteine concentrations are extremely low in comparison with the plasma concentrations of other protein-forming amino acids (Saetre and Rabenstein, 1978; Chawla et al., 1984; Gmunder et al., 1991). Lymphoid cells generally have a low uptake capability for cystine due to lack of cystine transporter expression and, in the absence of endogenous cysteine-synthetic ability, are mainly dependent on uptake of extracellular cysteine (Gmunder et al., 1991; Gout et al., 1997). In view of the relatively very low cysteine

concentrations in the circulation it is thought that—in vivo—such cells acquire the necessary amounts of cysteine for their functions from transient increases in the levels of this amino acid in their micro-environment as it is secreted by somatic cells in their vicinity (e.g., fibroblasts, activated macrophages, or dendritic cells)—a process based on x_c^--mediated cystine uptake (Gmunder et al., 1990; Sido et al., 2000; Angelini et al., 2002; Edinger and Thompson, 2002). Furthermore, tumor-associated macrophages (TAMs) have been reported to promote cancer growth via secretion of a variety of factors, including cytokines and angiogenic factors (Bingle et al., 2002). Similarly, such stromal cells could promote growth and therapy resistance of certain cancers via secretion of cysteine essential for glutathione/protein biosynthesis. On the other hand, cancer cells expressing the x_c^- transporter can take up cystine directly. The apparent importance of the x_c^- transporter in the supply of cysteine by somatic cells and in the uptake of cystine by target cells led to an early suggestion that the x_c^- transporter represents a potential target for therapy of cancers that are critically dependent on uptake of the amino acid for growth and viability (Gout, 1997; Gout et al., 1997).

Expression of x_c^- in cancer cell lines and tissues

The x_c^- transporter has been demonstrated in numerous cultured cancer cell lines, including human/rat hepatoma cells (Makowske and Christensen, 1982; Bannai, 1984b; Maechler and Wollheim, 1999), advanced rat and human lymphoma cells (Gout et al., 2001), human glioma cells (Chung et al., 2005) and human colon (Bassi et al., 2001), breast (Narang et al., 2003), prostate (Doxsee et al., 2007), and pancreatic (Lo et al., 2006) cancer cells. In some cases, the presence of the x_c^- transporter in cultured cell lines reflects its regular expression in their tissues of origin, such as normal pancreatic tissue (Bassi et al., 2001). However, the presence of the transporter in cultured cells, when the tissues of origin do not normally express it, may be a manifestation of cells circumventing cystine starvation. Whereas, in vivo, cells have access not only to cystine but also to cysteine, culture media such as Minimum Essential Medium and Fischer's medium only provide cystine [cysteine would be rapidly converted to cystine via autoxidation (Toohey, 1975)]. Establishment in such media of cell lines from cancer tissue, requiring extracellular cystine/cysteine but lacking cystine transporters, may therefore be due to cellular adaptation leading to x_c^- expression or to outgrowth of an existing subpopulation of x_c^--expressing cells, as reported for lymphoma cells (Hishinuma et al., 1986; Gout, 1987) and a variety of normal mammalian cells (Ishii et al., 1992). Consistent with this notion is the recent observation that human peripheral neutrophils did not express x_c^- activity until the cells were established in vitro (Sakakura et al., 2007). It may be noted that certain culture media (e.g., RPMI-1640) contain cystine at levels markedly exceeding those in for example, Minimal Essential Medium or human plasma, to enhance cystine uptake and hence boost their culture growth sustaining ability. Cellular uptake of cystine can also be enhanced by additives such as 2-mercaptoethanol (50–100 μM) (Ishii et al., 1981a). Lymphoid/lymphoma cell cultures infrequently express the x_c^- transporter when they have been established in medium containing 2-mercaptoethanol; advanced lymphoma cells expressing the transporter have no requirement for cystine uptake enhancement to sustain their growth in vitro (Ishii et al., 1981b; Gout et al., 1997, 2001).

The existence of x_c^--expressing cancers in patients has recently been established. Thus, as shown by Western blot analysis, both 4F2hc and xCT subunits were prominently expressed in all glioma samples acutely derived from five patients (Lyons et al., 2007).

Brain cancer

The discovery that the x_c^- transporter is substantially expressed in normal brain (Bassi et al., 2001; Kim et al., 2001; Burdo et al., 2006) raises the possibility that it plays a role in the growth and maintenance of brain tumors. Indeed, expression and activity of the x_c^- transporter have been reported in glioma cell lines, patient-derived non-malignant brain tissues and glioma tissues (Ye and Sontheimer, 1999; Ye et al., 1999; Chung et al., 2005; Lyons et al., 2007). In glioma cells the transporter was found to represent the only viable pathway for cystine uptake to sustain glutathione synthesis and growth. This dependence on cystine uptake was exploited in inhibiting experimental glioma growth in vitro and in vivo (Chung et al., 2005). Gliomas are aggressive cancers for which no effective treatment exists. Although their growth is limited by the bony cavity of the skull and spinal canal, they are able to overcome this physical limitation by killing neurons in their vicinity, thus vacating space; gliomas are therefore often associated with seizures (Sontheimer, 2003; Chung et al., 2005). The destruction of neuronal tissue apparently occurs, at least in part, by release of excessive amounts of glutamate (Ye and Sontheimer, 1999; Takano et al., 2001; Sontheimer, 2003), a process which has been linked to x_c^- transporter activity, since over 50% of glutamate release by glioma cell lines was found to be mediated by the x_c^- transporter (Ye et al., 1999). The glutamate release in vivo is an obligatory byproduct in the x_c^--mediated exchange of intracellular glutamate for extracellular cystine, as the latter is required by the glioma cells for glutathione-based protection against reactive oxygen species (Chung et al., 2005). However, the elevated extracellular glutamate concentrations are not only due to enhanced x_c^- transport activity of gliomas, but also to their reduced glutamate uptake capability relative to normal glial cells (Murphy et al., 1990; Ye et al., 1999; Schubert and Piasecki, 2001; Sontheimer, 2003; Lewerenz et al., 2006; Domercq et al., 2007). Recent evidence has indicated that glutamate, released by malignant glioma cells via the x_c^- transporter, can also act as an autocrine/paracrine signal promoting glioma cell invasion and that this process can be inhibited with potent blockers of the x_c^- system such as sulfasalazine and (S)-4-carboxyphenylglycine (Lyons et al., 2007).

Pancreatic cancer

Recent studies in our laboratory have shown that the x_c^- transporter is markedly expressed in a number of human pancreatic cancer cell lines (Lo et al., 2006), a finding not unexpected since x_c^- is substantially expressed in normal pancreatic tissue (Bassi et al., 2001; Kim et al., 2001). The growth of these cell lines is critically dependent on uptake of extracellular cystine and can be arrested by cystine starvation (Lo et al., 2006).

Kaposi's sarcoma

Recently, the xCT subunit of the x_c^- transporter was found to play a role in Kaposi's sarcoma, a connective tissue cancer commonly found in HIV/AIDS patients. Thus, xCT was found to be the predominant receptor in host cells mediating the fusion and entry of Kaposi's Sarcoma-associated Herpesvirus (KSHV) 8 (Kaleeba and Berger, 2006). Interestingly, inhibition of the transporter, resulting from its interaction with the virus, could lead to depletion of intracellular glutathione which, in turn, would be expected to result in upregulation of the transporter (Bridges et al., 2004; Qiang et al., 2004) thereby providing more receptors for viral entry of the host and enhancing KSHV infectivity (Kaleeba and Berger, 2006). This discovery of an important role for the x_c^- transporter in KSHV-induced disease could lead to novel x_c^--targeted therapy of the disease.

The x_c^- transporter in tumor progression

Early evidence for a role for the x_c^- transporter in tumor progression came from a rat prolactin-dependent Nb2 lymphoma cell line and its sublines, used as an in vitro and in vivo model for the malignant progression of lymphomas to growth autonomy (Gout et al., 1994). The sublines had clonally developed from the parent line with an increase in the number of chromosomal alterations at each progression step (Horsman et al., 1991). The emergence of x_c^- expression, manifested in the Nb2-SFJCD1 subline and contrasting with the lack of x_c^- activity in the parent line, augmented the cystine uptake and glutathione-generating capability of this subline thereby enhancing its growth autonomy (Gout et al., 1997) and resistance against oxidative stress (Meyer et al., 1998), typical features of malignant progression.

Role of x_c^- in multidrug resistance

The x_c^- transporter can contribute to drug resistance of cells by mediating cellular uptake of cystine to enhance biosynthesis of glutathione, as found for ovarian (Okuno et al., 2003) and lung (Huang et al., 2005) cancer cells. This tripeptide thiol has a major role in the protection of cells from drug-induced oxidative stress by mediating cellular detoxification of drugs and their extrusion via multidrug resistance proteins (Haimeur et al., 2002; Filipits et al., 2005; Yang et al., 2006; Yadav et al., 2007). Apparently, glutathione induces a conformational change within the multidrug resistance-associated protein-1 (MRP1) which is essential for interaction of this efflux protein with a drug and extrusion of the latter (Uchino et al., 2002). Conversely, depletion of intracellular glutathione levels, as induced by treatment with for example, L-buthionine-(S,R)-sulfoximine (BSO), an inhibitor of glutathione biosynthesis, can cause marked inhibition of cell growth, induction of apoptosis (Schnelldorfer et al., 2000), and reduced drug resistance (Vanhoefer et al., 1996). In view of this, inhibition of the x_c^- transporter leading to glutathione depletion would provide an alternative avenue for overcoming drug resistance, as previously suggested (Gout et al., 2003).

In a pharmacogenomics approach, microarrays have been used to analyze gene expressions of membrane transporters in 60 human cancer cell lines, used by the National Cancer Institute for drug screening (NCI-60), and the expression of the x_c^- transporter was linked with the potencies of 1,400 candidate anticancer drugs (Huang and Sadee, 2006). The study revealed 39 drugs showing positive expression correlations with the x_c^- transporter (e.g., the amino acid analogue, L-alanosine), whereas 296 drugs exhibited negative correlations (e.g., geldanamycin). These findings highlight the possibility of using L-alanosine-related drugs as a potential therapy for cancers expressing the x_c^- transporter, while resistance may be expected with drugs structurally similar to geldanamycin (Huang and Sadee, 2006). Importantly, such pharmacogenomic studies may be used to predict mechanisms of drug sensitivity and resistance and provide insights for selecting optimal drug regimens for combination chemotherapy (Dai et al., 2007).

Cystine/cysteine starvation of target cells via inhibition of the x_c^- cystine transporter: Use of sulfasalazine

Development of more effective strategies for treating cancers is of major importance in cancer management and many different approaches have been initiated, including nutrient starvation of target cells. Thus asparaginase treatment, aimed at depletion of L-asparagine in the circulation, has been used for decades in clinical combination chemotherapy of acute lymphocytic leukemia, a cancer that has a critical growth requirement for the amino acid (Keating et al., 1993). The finding that lymphoma and leukemia cells critically depend on extracellular cystine/cysteine for growth (Iglehart et al., 1977) suggested that depletion of this amino acid in the circulation provided a useful therapeutic approach for such malignancies. In fact, a therapeutic regimen was developed based on an L-cysteine-degrading enzyme in combination with an inhibitor of cysteine biosynthesis. Clinical trials were precluded, however, by rapid plasma clearance of the enzyme (Uren and Lazarus, 1979). In exploring an alternative method to attain cystine/cysteine starvation of lymphomas, it was recognized by one of us (PWG) that this could probably be achieved by specifically blocking the function of the x_c^- transporter and hence inhibiting cysteine supply by somatic cells as well as uptake of cystine by target cells (Gout, 1997), as illustrated in Figure 2. Support for this approach was obtained in vitro. Monosodium glutamate (10 mM), a highly specific inhibitor of the x_c^- transporter (Bannai and Kitamura, 1980), markedly reduced cystine uptake by Nb2-SFJCD1 lymphoma cells and almost completely arrested (98%) their proliferation in Fischer's medium. This growth arrest was based on specific inhibition of cystine uptake since it could be essentially completely prevented by inclusion in the medium of 50 μM 2-mercaptoethanol aimed at supplying the cells with cysteine via a route circumventing the x_c^- transporter (Gout et al., 1997). Unfortunately, monosodium glutamate is a neurotoxin which precludes its use as a therapeutic agent (Choi, 1988). In a search for a compound that inhibited the transporter and could be used therapeutically, it was discovered in our laboratory that the anti-inflammatory drug, sulfasalazine, potently inhibited x_c^--mediated uptake of cystine by Nb2-SFJCD1 lymphoma cells (Gout et al., 2001). In vitro, 0.1–0.3 mM sulfasalazine, a concentration range found in patients' sera, markedly arrested the proliferation of rat Nb2-SFJCD1 and human DoHH2 lymphoma cells whose growth in vitro depends on proper x_c^- functioning. The growth

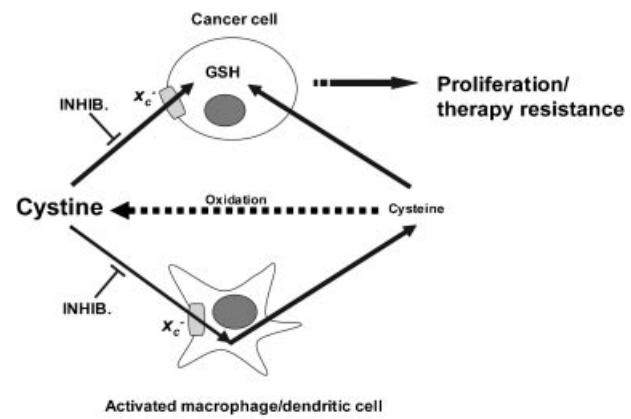

Fig. 2. The x_c^- transporter as a target for induction of cystine/cysteine starvation of cancer cells that depend on uptake of the amino acid from their micro-environment for synthesis of glutathione (GSH) required for growth and/or therapy resistance. In the extracellular milieu cysteine is quickly oxidized to cystine, leading to cystine predominance and low cysteine levels. Cysteine is readily taken up by cells, due to the expression of ubiquitous cysteine transporters; in contrast, cystine is not, since cystine transporters are not expressed by all cell types. Tumor-associated somatic cells, such as activated macrophages and dendritic cells (involved in the immune response), can regenerate cysteine from cystine, by taking up cystine via the x_c^- transporter and secreting it as cysteine back into the micro-environment. By using a specific x_c^- inhibitor (INHIB), the uptake of cystine by the somatic cells can be inhibited, leading to reduced levels of cysteine in the environment of the cancer cells. The inhibitor will also reduce cystine uptake by cancer cells when they express the x_c^- transporter. INHIB-induced cysteine deficiency of the cancer cells would readily lead to intracellular GSH depletion, subsequent growth arrest, and/or reduced drug resistance.

arrest and subsequent cell lysis were primarily due to cystine starvation since they could be largely prevented by 2-mercaptoethanol (60 μM). Sulfasalazine (0.15 mM) also inhibited secretion by fibroblast feeder layers of cysteine, critical for the growth of co-cultured Nb2 lymphoma cells lacking the x_c^- transporter; this led to growth arrest of the lymphoma cells whereas the proliferation of the fibroblasts was not inhibited. Importantly, intraperitoneal administration of sulfasalazine to rats (b.i.d.; 250 mg/kg body weight; 7 days) led to substantial growth arrest of well-developed, rapidly growing Nb2-U17 lymphoma allografts without major toxicity to the rat hosts; it may be noted that the Nb2-U17 cells do not express the x_c^- transporter (Gout et al., 2001, 2003). Subsequently we found that sulfasalazine induced marked glutathione depletion (>90%) in human DU-145 and PC-3 prostate and Mia PaCa-2 and Panc-1 pancreatic cancer cells leading to growth arrest; intraperitoneal administration of the drug substantially reduced growth of xenografts of these cell lines in SCID mice (Lo et al., 2006; Doxsee et al., 2007). Furthermore, sulfasalazine significantly enhanced the growth-inhibitory activity of doxorubicin against human MDA-MB-231 mammary cancer cells in vitro (Narang et al., 2007) and PC-3 prostate cancer xenografts (Kagami et al., 2007). Taken together, the above observations suggest that inhibition of the x_c^- cystine/glutamate antiporter by sulfasalazine, an FDA-approved drug, could form a viable strategy for chemotherapy of a variety of cancers, especially in combination with conventional drugs. For example, acute lymphocytic leukemia may, in view of its lymphoid origin, respond to cystine/cysteine starvation and its treatment might be improved by using asparaginase in combination with an x_c^- inhibitor such as sulfasalazine.

Sulfasalazine has also been used as an anticancer agent by other researchers. Quite recently Robe et al. (2004) showed, using xenograft animal models for human gliomas, that sulfasalazine could significantly inhibit glioma growth in vivo. A tumor-sensitizing effect of sulfasalazine was reported for human pancreatic cancer xenografts by Muerkoster et al. (2003) and recently for human lung adenocarcinoma xenografts by Lay et al. (2007). The anticancer effects of sulfasalazine in these studies were thought to be based on its inhibitory effect on activation of the transcription factor, NFκB (Wahl et al., 1998), considered an important target for cancer growth and chemoresistance (Arlt et al., 2003). Other researchers have previously reported drug efficacy enhancement by sulfasalazine, thought to act via inhibition of glutathione S-transferase, a detoxification enzyme (Awasthi et al., 1994), and a limited clinical trial, using sulfasalazine in combination with melphalan, showed some success for treatment of ovarian cancer (Gupta et al., 1995).

In contrast to the studies by Robe et al. (2004), more recent studies by Chung et al. showed that experimental human glioma growth could be inhibited by treatment with sulfasalazine, both in vitro and in a xenograft model, but rather as a consequence of x_c^- inhibition leading to reduced glutathione levels (Chung et al., 2005). These findings are in line with our observations that the growth-inhibitory effects of sulfasalazine (0.05–0.4 mM) in vitro are primarily due to glutathione depletion resulting from cystine/cysteine starvation induced by inhibition of the x_c^- transporter. In fact, sulfasalazine-induced growth arrest could be prevented by specifically enhancing cellular uptake of cystine via a route circumventing the x_c^- transporter (Gout et al., 2001; Narang et al., 2003; Lo et al., 2006; Doxsee et al., 2007). While sulfasalazine-induced cell death of glioma cells has been associated with apoptosis (Chung et al., 2005), recent evidence in our laboratory indicates that sulfasalazine-induced death of human PC-3 prostatic cancer cells can involve autophagy (Kagami et al., unpublished work). Importantly, a phase I/II trial is currently underway by Robe et al. (2006) for use of sulfasalazine as a single anticancer agent against glioblastomas and a SPORE clinical trial has been initiated by Sontheimer et al. (2006) on potential use of sulfasalazine for preventing neurotoxic glutamate release by gliomas.

Concluding Remarks

The x_c^- cystine/glutamate antiporter has, as a mediator of cellular cystine uptake, an important role in maintaining elevated intracellular levels of glutathione and hence preserving health of both normal and malignant cells. With regard to the latter there is an increasing body of evidence that short-term, specific inhibition of the x_c^- transporter can result in inhibited growth and/or reduced drug resistance of a variety of cancers without major side effects to the host. Accordingly, relatively non-toxic x_c^--inhibitory drugs such as sulfasalazine may prove useful in clinical cancer therapy, particularly in combination with conventional chemotherapeutics. The mediation of glutamate efflux by the antiporter is apparently a contributing factor in excitotoxicity associated with a variety of brain disorders, including Alzheimer's disease and malignant gliomas, and specific x_c^- inhibitors may therefore also be therapeutically useful in this area. Of recent interest is the finding that the x_c^- transporter can act as a cellular receptor mediating the fusion and entry of Kaposi's Sarcoma-associated Herpesvirus 8. The x_c^- transporter therefore appears to provide a potential therapeutic target for a number of diverse diseases.

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