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REVIEW ARTICLE

The oxidative stress-inducible cystine/glutamate antiporter, system x_c^-: cystine supplier and beyond

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Abstract The oxidative stress-inducible cystine/glutamate exchange system, system x_c^-, transports one molecule of cystine, the oxidized form of cysteine, into cells and thereby releases one molecule of glutamate into the extracellular space. It consists of two protein components, the 4F2 heavy chain, necessary for membrane location of the heterodimer, and the xCT protein, responsible for transport activity. Previously, system x_c^- has been regarded to be a mere supplier of cysteine to cells for the synthesis of proteins and the antioxidant glutathione (GSH). In that sense, oxygen, electrophilic agents, and bacterial lipopolysaccharide trigger xCT expression to accommodate with increased oxidative stress by stimulating GSH biosynthesis. However, emerging evidence established that system x_c^- may act on its own as a GSH-independent redox system by sustaining a redox cycle over the plasma membrane. Hallmarks of this cycle are cystine uptake, intracellular reduction to cysteine and secretion of the surplus of cysteine into the extracellular space. Consequently, increased levels of extracellular cysteine provide a reducing microenvironment required for proper cell signaling and communication, e.g. as already shown for the mechanism of T cell activation. By contrast, the enhanced release of glutamate in exchange with cystine may trigger neurodegeneration due to glutamate-induced cytotoxic processes. This review aims to provide a comprehensive picture from the early days of system x_c^- research up to now.

Keywords 4F2 · Cystine/cysteine redox cycle · Glutamate · Glutathione · *Slc7a11* · xCT

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Introduction

Cystine, a disulfide formed between two cysteine molecules, is the predominant form in the extracellular space, whereas cysteine is the prevailing form in cells due to highly reducing conditions. These amino acids are important not only as precursors for protein and antioxidant glutathione (GSH) biosynthesis, but also for the maintenance of physiological redox conditions inside/outside of the cell. Although cysteine may be synthesized from methionine by the transsulfuration pathway in some cells (McBean 2011), most cells use different transport systems that take up either of the forms from the extracellular space to cope with the intracellular demand. For those unfamiliar with transport system nomenclature, please refer to the editorial of this issue by Had-Aissouni (2011a). Transport of cysteine across the plasma membrane is mediated by system ASC (alanine, serine, cysteine-preferring) and one member of system L (leucine-preferring), LAT-2 (large amino acid transporter 2) (Verrey et al. 2004). In neuronal cells, EAAC-1 (excitatory amino acid carrier 1), a member of system X_{AG}^- (sodium-dependent acidic amino acid transporters with high affinity for glutamate and aspartate),

contributes to cysteine uptake (Aoyama et al. 2006; Zerangue and Kavanaugh 1996) while the members of system X_{AG}^- expressed by glial cells may also transport cystine (Hayes et al. 2005). The most active cystine transporter in the plasma membrane is expressed in the brush border membrane of kidney and small intestine. First described in blastocysts, it is designated as system $b^{0,+}$, as it recognizes large neutral amino acids, cationic amino acids and cystine (Chairoungdua et al. 1999; Pfeiffer et al. 1999; Rajan et al. 1999; Van Winkle et al. 1988); the mutation of this transporter causes cystinuria (Feliubadalo et al. 1999). Another cystine transporter, designated as system x_c^- (sodium-independent acidic amino acid transporter with high affinity for cystine) (Bannai et al. 1984), is detected in most types of mammalian cultured cells and has unique characteristics as discussed below. A similar activity of this transporter is detected in avian cultured cells, but not in insect-derived cultured cells (unpublished data). Interestingly, the component of the cystine transporter is also functioning as the virus receptor for Kaposi's sarcoma-associated herpesvirus (Kaleeba and Berger 2006). On the other hand, the possibility that inhibitors of this transporter such as sulfasalazine can be candidates for chemotherapy for certain cancers is emerging (Lo et al. 2008b). In this review, we will provide basic features and recent implications of this cystine transporter in physiology and disease development. For more specific details of the contribution of system X_{AG}^- and system x_c^- in maintenance of antioxidant defenses of neuronal cells, please refer to the reviews by Aoyama et al. (2011) and Lewerenz et al. (2011). For glial cells, see the reviews by Gras et al. (2011), Had-Aissouni (2011b), and Persson and Rönnbäck (2011).

System x_c^-: basic features

Discovery of system x_c^- and its functions observed in cultured cells

Thirty years ago, Bannai and Kitamura (1980) reported on a cystine transport system whose activity was inhibited by glutamate in mammalian cultured cells. The activity of system x_c^- is Na^+-independent and an exchange agency. The anionic form of cystine is transported in exchange for glutamate with a molar ratio of 1:1 (Bannai 1986). Concentration of the different ionic forms of cystine is pH dependent; hence, the uptake of cystine via system x_c^- is pH sensitive, whereas the uptake of glutamate is not (Bannai and Kitamura 1981). Although the primary function of system x_c^- is to transport cystine into cells, the physiological function of the transport system is obvious at least in vitro. Cultured cells die when just cystine is removed from the normal culture medium (Fig. 1), and this

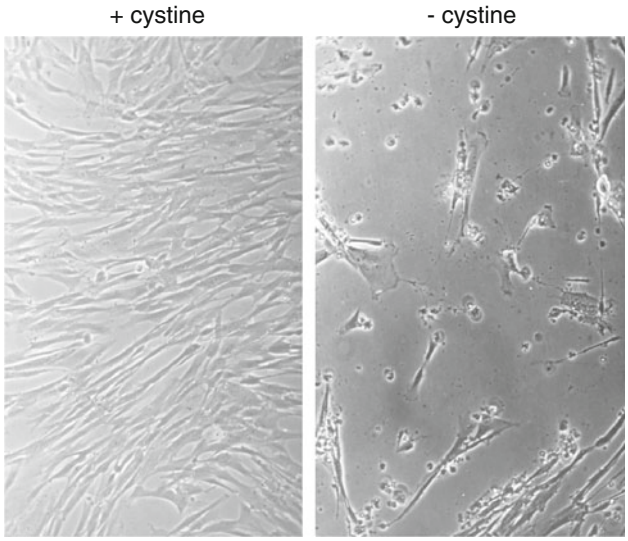

Fig. 1 Effect of cystine deficiency in human fibroblasts. Cells were cultured for 24 h in normal culture medium with or without cystine. Bannai made the early observation that cells rapidly die when cultured in medium just lacking cystine (unpublished observation)

phenomenon is caused by the deprivation of intracellular GSH, the most abundant endogenous antioxidant. In general, cysteine, the rate-limiting precursor for the synthesis of GSH (Bannai and Tateishi 1986), is hardly detectable in normal culture medium, because it is rapidly oxidized to cystine under normoxic conditions. Most cultured cells do not express the proper enzymatic make-up to ensure appropriate endogenous cysteine levels. Therefore, the supply of cysteine for the maintenance of intracellular GSH level in cultured cells depends on extracellular cystine. The expression of system x_c^- is essential for maintaining intracellular GSH at least for cells cultured under routine cell culture conditions.

Another function of system x_c^- is to provide a reducing extracellular microenvironment by maintaining a constant flow of cysteine into the extracellular space. Cystine taken up into the cell by system x_c^- is rapidly reduced to cysteine (we shall discuss this later), which, in turn, is used for the synthesis of GSH and proteins. Some part of cysteine is released into the extracellular space via constitutively expressed neutral amino acid transport systems. There, cysteine is rapidly oxidized to cystine, and consequently taken up by system x_c^- (Fig. 2). Therefore, when cells express the activity of system x_c^-, a cystine/cysteine cycle, comprised of system x_c^-, neutral amino acid transport system(s), and intracellular and extracellular redox reactions, is established in the cell culture system. During cell culture, this cystine/cysteine cycle reaches steady-state conditions and cysteine becomes detectable in the culture medium. Interestingly, the ratio of cystine and cysteine in the medium gradually resembles to that in plasma as cell

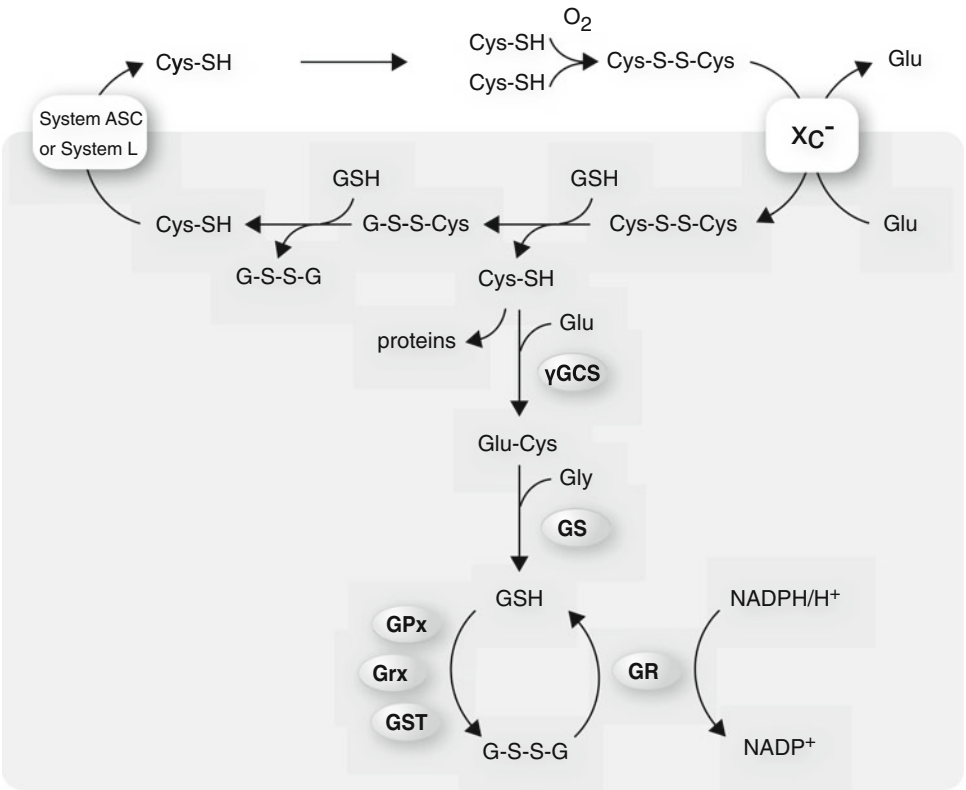

Fig. 2 The cystine/cysteine redox cycle driven by system x_c^-. System x_c^- sustains a redox cycle at the membrane, consisting of cystine (Cys-S–S-Cys) uptake, intracellular reduction of cystine most likely by forming mixed disulfides with glutathione (GSH) and secretion of cysteine into the extracellular space where, eventually, cysteine is oxidized to cystine. Cysteine is also used intracellularly for both protein and GSH synthesis. GSH is synthesized in two steps by the rate-limiting enzyme γ-glutamylcysteine synthetase (γ-GCS, EC 6.3.2.2) and glutathione synthetase (GS, EC 6.3.2.3). GSH itself is used as a cofactor for glutathione peroxidases (GPx, EC 1.11.1.9), glutaredoxins (Grx, EC 1.20.4.1) and glutathione-S-transferases (GST, EC 2.5.1.18) and other GSH dependent functions such as Fe–S cluster biogenesis. Oxidized glutathione (G-S–S-G) is recycled back to GSH by glutathione reductase (EC 1.6.4.2) at the expense of $NADPH/H^+$

culturing is continued (Bannai 1984b). Even when cells do not display the system x_c^- activity in vivo, it is swiftly induced when they are transferred into the cell culture system (Takada and Bannai 1984; Watanabe and Bannai 1987). Therefore, the expression of system x_c^- may be one of the main cellular adaptations in the cell culture system. Cells like lymphocytes, which are incapable to induce system x_c^- even under cell culture condition, require a reducing agent like β-mercaptoethanol (β-ME) to be stably maintained in normal cell culture (Ishii et al. 1981a). The reducing agent reacts with cystine in the culture medium and forms a mixed disulfide, and thereby releases cysteine. These reaction products go into cells via neutral amino acid transporters and contribute to the maintenance of adequate GSH levels in the cells.

Cloning of the Slc7a11 gene

For a better understanding of the physiological functions of system x_c^- in vivo, we set out to clone the gene(s) for system x_c^- by an expression cloning approach using the Xenopus oocytes. In the early 1990s, several cDNAs for amino acid transporters were cloned by this method and this technique finally allowed us to get access to the molecular identity for system x_c^- (Sato et al. 1999). To our surprise, system x_c^- appeared to consist of two protein components, a novel protein, designated xCT, and 4F2 heavy chain, which had been known as one of cell surface antigens (CD98), and later found to be shared by several amino acid transporters.

Various considerations were taken into account to be able to successfully clone multiple cDNAs from the library simultaneously. First, we generated a cDNA library from thioglycollate-induced peritoneal macrophages, which had been stimulated with diethyl maleate and a very low amount of bacterial lipopolysaccharide (LPS). Since the activity of system x_c^- in the cells is strongly induced under these conditions, this treatment probably contributed to a strong accumulation of *xCT* mRNA in the starting material for the synthesis of the cDNA library. Second, when we measured the uptake of cystine in the oocytes injected with cRNA, it was performed at 30°C to increase the sensitivity

of the measurement (Ishii et al. 1991). To achieve this, each oocyte was individually isolated without using collagenase, because the oocytes treated with collagenase were too weak to tolerate the uptake experiment at 30°C. In this way, we finally succeeded to isolate the two clones.

xCT is composed of 502 (mouse) and 501 (human) amino acids, respectively (Sato et al. 1999, 2000). xCT is predicted to have 12 putative transmembrane domains, and the N and C termini are located inside the cell (Gasol et al. 2004). xCT is thought to be linked with 4F2 heavy chain via a disulfide bond at the second cysteine residue from the N-terminus (Cys^{158}), which is conserved in other amino acids transporters like LAT and y^+LAT (Wagner et al. 2001). The activity of system x_c^- exclusively depends on xCT. 4F2 heavy chain is the common counterpart of some other amino acid transporters and functions as a chaperone-like protein to guide the counterpart protein to the plasma membrane (Verrey et al. 2004).

Regulation of xCT expression

One of most important features of system x_c^- is that the activity of system x_c^- is highly inducible by various stimuli, including electrophilic agents like diethyl maleate (Bannai 1984a), cystine deprivation in the culture medium (Bannai and Kitamura 1982), oxygen (Bannai et al. 1989), oxidative stress like hydrogen peroxide (Bannai et al. 1991) and oxidized low density lipoprotein (Sato et al. 1995b), LPS (Sato et al. 1995a), TNF-α (Tumor Necrosis Factor-alpha) (Sato et al. 1995a), HIV tat (Human Immunodeficiency Virus trans-activation of transcription) protein (Bridges et al. 2004), amyloid-β (Aβ) (Qin et al. 2006), and erythropoietin (Sims et al. 2010). On the other hand, stimuli that suppress the expression of system x_c^- have not been reported so far. Interferon-γ reduces the induction of the activity of system x_c^- by LPS (Sato et al. 1995a). Yet, various pharmacological agents are known which inhibit the transport activity of system x_c^- (Patel et al. 2004).

By promoter analysis several regulatory mechanisms for the induction of *xCT* expression have been described mainly in the 5′ flanking region of *xCT* gene. At least 4 putative elements similar to the electrophile response element (EpRE, also called antioxidant response element) are present in the upstream promoter region of the *xCT* gene (Sasaki et al. 2002). At least one of the elements functions as an EpRE, which is recognized by the transcription factor Nrf2 (nuclear factor E2-related factor 2). Nrf2 is activated by electrophilic agents and up-regulates the expression of many genes including stress-related genes (Itoh et al. 2004). Hence, the induction of xCT by electrophilic agents including diethyl maleate and arsenite is mediated by EpRE (Sasaki et al. 2002).

Expression of some genes such as asparagine synthetase (Barbosa-Tessmann et al. 2000) and CCAAT/enhancer-binding protein homologous protein (CHOP) (Bruhat et al. 2000) are regulated by the amino acid response element (AARE). The induction of xCT by amino acid deprivation, including cystine deprivation, is regulated by two AAREs found in the 3′ flanking region of the functional EpRE of the *xCT* gene (Sato et al. 2004). A characteristic structure of the AAREs in the *xCT* gene is that the two AAREs are located in the opposite direction with a 9 bp intervening sequence meaning they are arranged symmetrically on the DNA double helix. It was also shown that the transcription factor ATF4 (Activating transcription factor 4) mediates the induction of xCT by amino acid deprivation (Sato et al. 2004).

The regulatory mechanism for xCT induction by LPS has not been clarified. Toll-like receptor 4 (TLR4) mediates the signal transduction by LPS (Cohen 2002). Since even little amounts of LPS are sufficient to strongly induce xCT expression without activating NF-κB (Nuclear Factor kappa B) (Sato et al. 2001), the signal pathway downstream from TLR4 is most likely different from the NF-κB-mediated pathway. It might also well be that the IRF3 (Interferon regulatory factor 3)-mediated pathway is involved in the induction of xCT by a very small amount of LPS (Akira and Takeda 2004). Interestingly, the induction of xCT by LPS in peritoneal macrophages requires the presence of oxygen (Sato et al. 2001), and oxygen itself had been shown to induce the activity of system x_c^- (Bannai et al. 1989). Hence, the possible cross-talk between the signaling pathways of oxygen and LPS certainly merits further investigations.

Expression of xCT in vivo

Constitutive expression of *xCT* mRNA in vivo is detected in brain, thymus, and spleen of mice (Sato et al. 2002; Taguchi et al. 2007). In the brain, *xCT* mRNA is mainly detected in meninges, area postrema, subfornical organ, habenular nucleus, hypothalamic area, and some ependymal cells of the lateral wall of the third ventricle. However, it has not been demonstrated, which types of cells express xCT in the cerebrum. In addition, we have recently detected the expression of *xCT* mRNA in Peyer's patch and mesenteric lymph node (unpublished data). Our preliminary data suggest that *xCT* mRNA is expressed also in bone marrow. Thus, constitutive expression of xCT in vivo seems to be restricted to the central nervous system and the immune system (Fig. 3).

Yet, it has not been fully elucidated that xCT harbors similar functions in vivo as to those observed in vitro, i.e., maintaining intracellular GSH and maintaining redox balance in the extracellular space. Under pathological

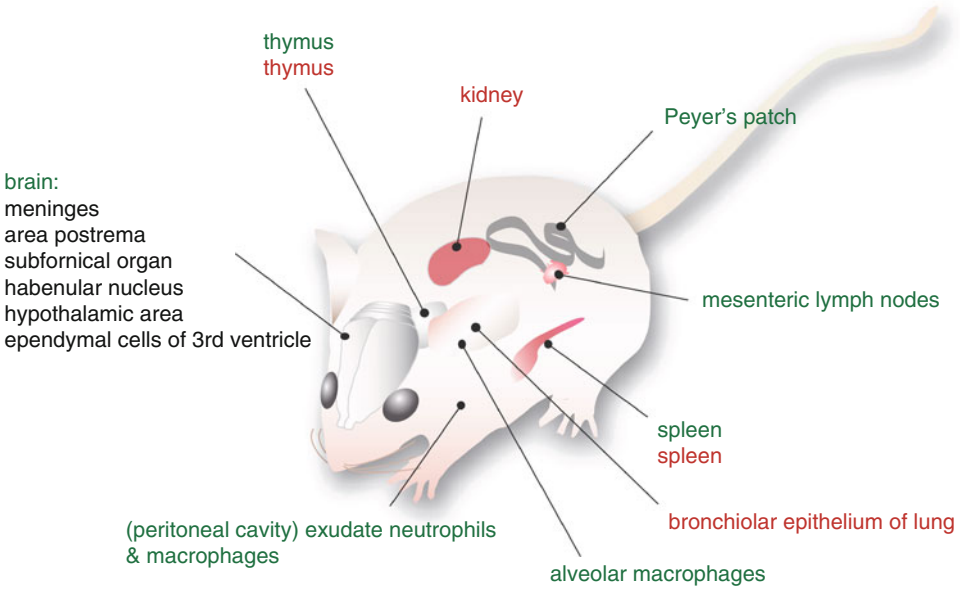

Fig. 3 Expression of xCT in murine tissues and organs. Constitutive and inducible expression of xCT in vivo is shown in *green* and *red* color, respectively

conditions, xCT can be induced in vivo. For instance, thioglycollate-stimulated peritoneal exudated neutrophils already show xCT expression and a significant activity of cystine uptake can be detected (Sakakura et al. 2007). In some experimental models of inflammatory conditions, xCT is expressed in the kidney (Shibasaki et al. 2009) and in the infiltrating neutrophils and macrophages into the inflammatory site (Nabeyama et al. 2010). Administration of LPS further enhances the expression of *xCT* mRNA in thymus and spleen in a dose- and time-dependent manner (Taguchi et al. 2007). Administration of a lethal dose of LPS also induces strong expression of *xCT* mRNA in the bronchiolar epithelium of the lung (Taguchi et al. 2007), while low expression of *xCT* mRNA is detectable in the lung of normal mice (manuscript in preparation).

System x_c^- as the driver of the cystine/cysteine redox cycle

Increased expression of xCT, either in an Nrf2-dependent or -independent manner (Ishii et al. 2000), and hence higher availability of cysteine for stimulating GSH biosynthesis has long been regarded as the main mechanism of system x_c^- to respond to oxidative stress. However, various studies mainly during the last few years have suggested that the steady-state redox potential (E_h) of extracellular cystine/cysteine is regulated independently of intracellular E_h GSH/GSSG. In other words, the cystine/cysteine redox couple may act independently of GSH, and early work by Jones' laboratory substantiated this hypothesis (Jones et al. 2004). For instance, pharmacological depletion of intracellular GSH by the highly specific and irreversible γ-glutamylcysteine synthetase (γ-GCS, EC 6.3.2.2) inhibitor l-buthionine [S,R]-sulfoximine (BSO) causes a sharp drop of intracellular GSH along with a strong oxidation of the GSH/GSSG redox couple (40 mV), whereas the cystine/cysteine redox couple remains largely unaffected (Jones et al. 2004). Variations in the extracellular cystine/cysteine redox couple (more oxidized) also enhance the adhesion of monocytes to endothelial cells via the increased expression of cell adhesion molecules, such as ICAM1, PECAM1 and P-selectin (at E_h 0 mV, compared to −150 mV, adhesion is increased approximately twofold due to increased expression of ICAM-1, PECAM-1 and P-selectin) (Go and Jones 2005).

Similar findings for a GSH-independent role for the cystine/cysteine couple were seen in lung injury models. Intraperitoneal treatment of mice with LPS leads to pathophysiological changes similar to that seen in patients suffering from acute lung injury. LPS treatment in mice is associated with a fast drop in plasma cysteine levels and concomitant oxidation of the cystine/cysteine redox couple (approx. 2–6 h after treatment) (Iyer et al. 2009a). A decrease of the GSH/GSSG redox couple is also observed in LPS-treated mice; however, this appeared rather to be due to a decline in food consumption than a direct oxidative effect of LPS treatment (Iyer et al. 2009a). Intra-tracheal administration of bleomycin, another widely used model for lung fibrosis in mice, again causes distinctive and temporal alterations in the GSH/cysteine pools and in their oxidation status (Iyer et al. 2009b). While prominent oxidation of E_h GSH/GSSG occurs already seven days post-treatment (i.e. the proinflammatory phase of lung injury) and recovers to normal values 14 days after bleomycin administration, the cystine/cysteine redox state is considerably more oxidized at the fibrotic phase (14 days

post treatment). In this context it should be mentioned that the E_h cystine/cysteine is more oxidized in association with ageing and smoking, both well known risk factors of idiopathic pulmonary fibrosis.

The mechanism of how the extracellular cystine/cysteine couple is regulated and maintained has been elucidated only quite recently. Previously, it was thought that secreted GSH may react with cystine by forming mixed disulfides, and thereby maintains the extracellular cystine/cysteine balance. To study the regulation of the extracellular cystine/cysteine couple, the rate of reduction of extracellular cystine was monitored in BSO treated HT-29 cells (Anderson et al. 2007). While BSO treatment was shown to induce the well known GSH depleting effect, the rate of extracellular cystine reduction appeared not to be affected (Anderson et al. 2007). Further manipulations of the extracellular cysteine and cystine levels led to the conclusion that specific transport systems for cystine/cysteine and/or oxidoreductases must exist, which ensure the balance of extracellular E_h cystine/cysteine (Anderson et al. 2007).

Direct evidence for the involvement of system x_c^- in the maintenance of a reduced microenvironment over the membrane was provided in a parallel study using stable expression of xCT in Burkitt's Lymphoma (BL) cells (Banjac et al. 2008). BL cells are characterized by limited uptake capacity for cystine and are therefore highly sensitive to oxidative stress, e.g. imposed by seeding at low cell densities (Brielmeier et al. 1998; Falk et al. 1998). Along this line, oxidative stress-induced cell death of BL cells can be prevented by co-culturing with irradiated fibroblasts, which provide cysteine to the BL cells (Falk et al. 1998), or by antioxidant supplementation (Brielmeier et al. 1998). Consequently, stable expression of xCT in BL cells protects BL cells from seeding at low cell concentrations, hydrogen peroxide and GSH depletion by BSO (Banjac et al. 2008). Somehow this was expected as it had been initially hypothesized that higher cystine uptake mediated by system x_c^- would spark GSH biosynthesis and render xCT-overexpressing cells much more resistant to oxidative stress and GSH depletion. Nevertheless, HPLC measurements of intracellular GSH levels showed a different picture (Banjac et al. 2008). Rather, the increased cystine uptake in xCT-overexpressing cells only hardly affected the intracellular GSH pool. Although, xCT-overexpressing cells were much resistant to oxidative stress and GSH deprivation, this effect appeared to be independent of intracellular GSH levels. A striking difference between xCT overexpressing and control cells was the remarkably higher extracellular cysteine levels in xCT expressing cells, which is responsible for the rescuing effect. Hence, sustained system x_c^- activity drives a redox cycle over the plasma membrane, which consists of increased cystine uptake, intracellular reduction and secretion of the surplus of cysteine into the medium (Fig. 2) (Banjac et al. 2008).

Most intriguingly, in γ-GCS knockout cells (Shi et al. 2000), stable expression of xCT allows these cells to proliferate in the absence of exogenous GSH or *N*-acetyL-cysteine (NAC) supplementation (Mandal et al. 2010). As γ-GCS null cells lack endogenous GSH biosynthesis, the high intracellular and remarkably high extracellular cysteine levels are sufficient to provide growth advantage to the knockout cells and in co-cultures to the neighboring knockout cells, which do not express xCT. This also implies that the major GSH function can be replaced by the cystine/cysteine redox cycle. In this study it was also shown that in the absence of intracellular GSH, the cytosolic thioredoxin reductase system is able to deal with the excess cystine transported into cells (Mandal et al. 2010), thus establishing a link between system x_c^-, the GSH system and the thioredoxin dependent system.

Uptake of nitric oxide (NO) from the vascular system is also controlled by extracellular cysteine and GSH. NO diffusion in vessels is limited due to the rapid binding to haemoglobin in erythrocytes. After reaction with oxygen, NO can lead to the formation of S-nitrosothiols in a process called S-nitrosation (Hess et al. 2005). Hence, S-nitrosohemoglobin and S-nitrosoalbumin may serve as a reservoir for deliverable NO. Thus, transnitrosation of NO from S-nitrosoalbumin to extracellular cysteine would lead to formation of S-nitroso-L-cysteine, which is then taken up by cells via the L amino acid transporter family (Li and Whorton 2005). Since the transnitrosation pathway, however, requires cysteine as an acceptor, it was not known before how sufficient cysteine becomes available in the extracellular space. Work by Whorton's laboratory finally demonstrated that the cystine/cysteine cycle in vascular smooth muscle cells is responsible to provide sufficient extracellular cysteine for NO uptake in form of S-nitroso-L-cysteine. Moreover, this cycle enhances the inhibitory effect of S-nitrosoalbumin towards epidermal growth factor signaling (Zhu et al. 2008).

Another mystery can be explained on the basis of the cystine/cysteine redox cycle. Selenite (SeO_3^{2-}) toxicity, most likely through the intracellular generation of nonstoichometric amounts of superoxide and disulfides, has been known for a long time. In addition, an inverse relationship between the sensitivity of malignant cells towards selenite-induced toxicity and resistance to cytotoxic drugs has been proposed (Bjorkhem-Bergman et al. 2002), but it was not explainable on the basis of different intracellular traits. In this context, it is notable that already in the 1960s selenite in the nanomolar range was used in patients to localize tumors as tumors display a high affinity for selenite. In an elegant study, Olm et al. (2009) recently showed that selenite cytotoxicity is determined by its cellular uptake and

not by intrinsic cellular differences. Apparently, selenite uptake, and consequently its cytotoxic effect, is dependent on the extracellular redox state. Modulation of the extracellular state by oxidizing or reducing compounds is able to sensitize or de-sensitize cells towards selenite. Moreover, like oxidizing conditions, extracellular glutamate is able to lower the sensitivity of the malignant cells to selenite, indicating that the high extracellular cysteine levels in the selenite-sensitive cells must be linked with high system x_c^- activity. Higher extracellular cysteine concentrations thus facilitate the reduction of selenite to selenide (H_2Se), which is rapidly taken up into cells and thus increases its cytotoxicity (Olm et al. 2009).

Although some recent efforts have yielded intriguing insights where the cystine/cysteine shuttle is involved, it will be interesting to see in which other cellular processes the cystine/cysteine redox cycle is also implicated.

The role of system x_c^- in physiology and disease development

Brain

The brain is one among the few organs, where system x_c^- is constitutively expressed (see above). In a way this is not really unexpected as neurons are considered to be highly vulnerable to oxidative stress (Lin and Beal 2006), and are thus privileged sites for the steady supply of cystine/cysteine for proper GSH synthesis. On the other hand, cystine uptake via system x_c^- by neurons or nearby cells must be properly controlled since it is ultimately coupled to the release of glutamate, which may cause oxidative glutamate toxicity in neuronal cells (Murphy et al. 1989). In fact, excess glutamate inhibits GSH synthesis due to impaired cystine uptake leading to oxidative stress and neuronal cell death of neuronal cell cultures and immature cortical neurons (Murphy et al. 1989, 1990). Released glutamate may also trigger excitotoxic neuronal death by overstimulation of glutamate receptors. For a full description of the different ways in which extracellular glutamate may be toxic to brain cells, see the review by Had-Aissouni (2011b).

However, as discussed in the following it is striking that there are more reports in the literature describing the role of glutamate released by system x_c^- in brain disease than about protective effects of increased cystine availability, and thus GSH synthesis. It is also worth mentioning that the different brain cell populations appear to have different routes for cysteine and cystine uptake. While astrocytes express various transporters for cysteine and cystine and mature neurons transport cysteine mainly via the cysteine-permeable Na^+-dependent glutamate transporter, immature neurons exclusively use system x_c^- for cystine uptake (Murphy et al. 1990). Furthermore, as shown by Shih et al. (2006), there are also remarkable differences regarding the uptake capacity of cystine between meningeal cells and astrocytes. Due to higher cystine uptake, meningeal cells appear to be much more neuroprotective than astrocytes in neuronal co-culture experiments. Similar neuroprotective effects are also evident in co-culture experiments with immature cortical neurons when xCT is ectopically expressed in astroctyes or even in fibroblasts (Shih et al. 2006). Along this line, loss of function of xCT (*sut*/*sut* mice; outlined below) causes brain atrophy in mice around 3–4 months after birth as evidenced by cortical thinning, reduction in striatal area and enlarged ventricles (Shih et al. 2006). It is also noteworthy that mice lacking EAAC1, a high-affinity Na^+-dependent L-glutamate/D, L-aspartate transporter with cysteine transport activity, develop age-dependent neurodegeneration approximately 11 months after birth (Aoyama et al. 2006). The underlying mechanism appears to be decreased GSH levels and increased oxidative stress. Whether activation of 12-lipoxygenase (EC 1.13.11.31), an arachidonic acid metabolizing enzyme, mediates the effects of GSH depletion remains to be elucidated in EAAC1 null mice, although it seems pretty likely (Li et al. 1997). Glutamate- or BSO-mediated GSH depletion was previously shown to trigger neuronal cell death via the activation of 12-lipoxygenase and lipid peroxide generation, which could be efficiently prevented by 12-lipoxygenase inhibitors (Li et al. 1997). Earlier, similar protective effects had been recognized for vitamin E and other antioxidants being strong inhibitors of oxidative glutamate toxicity (Murphy et al. 1989; Schubert et al. 1992).

Recently, we demonstrated that the activation of 12/15-lipoxygenase is controlled by glutathione peroxidase 4 (Gpx4, EC 1.11.1.9) and its major cofactor GSH (Conrad 2009; Loscalzo 2008; Seiler et al. 2008). Like BSO-mediated GSH depletion, Gpx4 disruption sparks a cell death pathway entailing 12/15-lipoxygenase activation, 12/15-lipoxygenase-derived lipid peroxidation and apoptosis-inducing-factor (AIF)-mediated cell death (Loscalzo 2008; Seiler et al. 2008). A further refinement of this pathway revealed that activation (=truncation) of the pro-apoptotic protein Bid by the initial 12/15-lipoxygenase-derived lipid peroxide signal, causes translocation of truncated Bid (tBid) to mitochondria, a secondary ROS boost and finally AIF activation (Tobaben et al. 2011). Consistent with the involvement of 12/15-lipoxygenase in this cell death pathway, pharmacological inhibition of 12/15-lipoxygenase (or genetic knockout of 12/15-lipoxygenase) was shown to protect brain tissue from the deleterious effects of cerebral ischemia/reperfusion (van Leyen et al. 2006).

System x_c^- is also expressed in microglia, the resident macrophages of the brain and spinal cord, and particularly highly expressed in transgenic mouse models of Alzheimer's disease (Qin et al. 2006). The xCT expression in microglial cells can also be strongly induced by intracranial injection of Aβ into the hippocampus, suggesting an important role for system x_c^- in Aβ processing by microglia. Yet, as for glutamate toxicity, stimulation of microglial cells with increasing amounts of Aβ is neurotoxic due to system x_c^- dependent glutamate secretion. Conversely, when system x_c^- mediated glutamate toxicity is inhibited, microglial cells are neuroprotective through the secretion of apolipoprotein E (Qin et al. 2006), which may confer an antioxidant function via the scavenging of lipid peroxidation products (Miyata and Smith 1996).

A similar adverse implication of system x_c^- in neuronal injury was described in mice lacking interleukin-1 receptor type I (IL-1RI) (Fogal et al. 2007). IL-1RI-deficient mice subjected to transient middle cerebral artery occlusion (MCAO) have strongly reduced infarct size (Fogal et al. 2007). Apparently, interleukin-1β (IL-1β) potentiates the toxic effects of secreted glutamate of mixed cortical cell cultures under hypoxia, whereas system x_c^- antagonism prevents the IL-1β-enhanced hypoxic neuronal injury (Fogal et al. 2007).

A very recent study also showed that transgenic mice lacking xCT (as discussed below) are strongly protected from 6-hydroxydopamine-induced degeneration of nigrostriatal neurons (Massie et al. 2010). Nigral dopaminergic neuronal loss is one of the hallmarks of Parkinson's disease. These and other studies described here strongly argue for pharmacological targeting of system x_c^- to alleviate glutamate toxicity (Albrecht et al. 2010).

Yet, it is somehow astonishing that system x_c^- activity has been mainly linked to glutamate toxicity and to a far lesser extent to the protection of neuronal cells from oxidative stress. Experimental support that extracellular glutamate levels, e.g. released as a consequence of increased system x_c^- activity, are balanced by glutamate transporters have been provided by using HT22 neuronal cells selected for increased resistance to millimolar concentrations of glutamate (Lewerenz et al. 2006). Not only become cells more resistant to millimolar concentrations of glutamate (>40 mM) and hydrogen peroxide, glutamate-induced GSH depletion is also markedly reduced. The underlying molecular changes in resistant cell lines is increased xCT expression and cystine uptake along with increased expression of the excitatory amino acid transporters (EAATs) 1, 2 and 3, which are all involved in glutamate uptake. Accordingly, EAATs inhibition exacerbates glutamate-induced cell death, whereas combined expression of xCT and EAAT3 in HT22 cell lines prevents glutamate-induced cell death (Lewerenz et al. 2006). Therefore, the cooperative action of system x_c^- and EAATs may ensure optimal protection of neuronal cells from oxidative stress by increased cystine uptake, GSH biosynthesis and properly controlled extracellular glutamate concentrations.

Primary brain tumors

Most primary brain tumors arise from glial cells or their progenitors and are therefore named gliomas. Gliomas are among the most difficult tumors to treat, for their steady growth and diffuse invasion. Due to their highly metabolic turnover, gliomas are considered to produce large amounts of oxygen radicals, and thus maintain high levels of GSH and other antioxidant systems. Since glioma growth is physically limited by the cranial space they may make way for their growth by releasing toxic amounts of glutamate to induce cell death of adjacent cells (Sontheimer 2003, 2008; Ye and Sontheimer 1999). Glia-derived tumor cells indeed display high expression of xCT (Cho and Bannai 1990) and, in contrast to other brain cells, almost exclusively transport cystine via system x_c^- (Chung et al. 2005). Consequently, cystine uptake of glioma cell lines and glioma primary cultures is highly sensitive to sulfasalazine (Chung et al. 2005), and system x_c^- inhibition causes rapid GSH depletion (within 24 h after treatment), growth inhibition and apoptotic cell death of glioma cells in vitro. Importantly, the growth of intracranially transplanted glioma cells in mice can also be reduced by sulfasalazine treatment in vivo (Chung et al. 2005). Gliomas therefore respond in a similar manner to sulfasalazine treatment, like tumors of different genesis such as lymphoma, mammary carcinoma, prostate and pancreatic cancer (see below).

Independent and affirmative evidence to inhibit glioma growth was provided by siRNA-mediated silencing of xCT in gliomas (Savaskan et al. 2008). Knockdown of xCT causes a significant reduction of extracellular glutamate levels of glioma cell culture-conditioned medium and in the peritumoral regions of glioma implanted into rats. In contrast to control glioma cells, the xCT knockdown-mediated reduction of glutamate secretion alleviates the extent of neuronal cell death due to a decline of perifocal edema formation (Savaskan et al. 2008). In addition, the mean survival time of rats implanted with xCT-silenced gliomas is prolonged in a manner similar to glioma-bearing rats treated with the system x_c^- antagonist S-4-carboxyphenylglycine (S-4-CPG).

Enthused by these highly promising in vivo findings, a phase 1/2, prospective, double blind, randomized clinical study of sulfasalazine for the treatment of progressing malignant gliomas had been initiated a few years ago (Robe et al. 2006). But sadly enough, this study had to be discontinued due to the lack of clinical responses, severe toxic side effects and the death of two patients who died

under treatment or shortly thereafter (Robe et al. 2009). Hence, the authors of this study concluded NOT to recommend the use of sulfasalazine in the routine treatment of glioblastoma patients or even in other cancer patients.

Immune system and T cell activation

As mentioned above, lymphocytes lack an efficient uptake system for cystine, whereas they easily transport cysteine (Droge et al. 1991; Ishii et al. 1981b, 1987). By contrast, LPS- or TNF-stimulated macrophages consume cystine and release around equivalent amounts of acid-soluble thiol into the medium (Gmunder et al. 1990). Initial indications that T cell activation is dependent on a reduced extracellular milieu were provided by Rubartelli's laboratory (Angelini et al. 2002). Human dendritic cells (DCs) release quite significant amounts of thiols mainly in the form of cysteine. While LPS treatment is able to stimulate the release of cysteine to some extent, the strongest cysteine secretion can be observed only after co-culturing DCs with alloreactive T cells (Angelini et al. 2002). DC and T cell co-cultures not only trigger the release of cysteine but also thioredoxin, a relatively small redox protein involved in numerous intra- and extracellular functions (Lillig and Holmgren 2007). Importantly, the cysteine release induced by LPS or co-culture experiments was shown to be sensitive to 2 mM glutamate and the thioredoxin reductase inhibitor DCNB (1,2 dichloro-4-nitrobenzene), but not to the glutathione reductase inhibitor BCNU ((1,3-bis(2-chloroethyl)-1-nitrosourea) (Angelini et al. 2002). This suggests that mainly system x_c^- and the thioredoxin system contribute to T cell activation.

Later on, hallmarks of these findings were confirmed in vivo (Castellani et al. 2008). Antigen injection causes a massive increase of thiols in lymph nodes and in germinal centers along with an increase in thioredoxin expression. While system x_c^- is absent in resting cells, T cell receptor stimulation triggers a fast upregulation of system x_c^- activity, supporting the idea that extracellular redox regulation, mediated at least in part by system x_c^-, plays an important role in the immune system (Castellani et al. 2008). This, however, is not the entire story. Regulatory T cells, which are involved in the suppression of immune response, appear to modulate the reducing microenvironment of the immune synapse (Yan et al. 2009). Addition of regulatory T cells to co-cultures of DCs and effector T cells leads to a strong decline in extracellular cysteine and intracellular GSH levels in effector cells as well as a clearly impaired proliferation of effector T cells (Yan et al. 2009). This lends to support the hypothesis that autoreactive T cells are suppressed by extracellular cysteine levels in the DC-T cell niche (Rubartelli and Sitia 2009). The mechanism how regulatory T cells "eliminate" extracellular cysteine in the immune synapse, however, remains unclear.

Immunosuppression of T cell-mediated antitumor immunity was reported also for myeloid-derived suppressor cells, which are involved in the inhibition of the innate and adaptive immunity (Srivastava et al. 2010). These cells apparently sequester, store and thus limit extracellular cysteine, which would be otherwise available for T cell activation. Since myeloid-derived suppressor cells lack the ASC transporter, release of stored cysteine is hampered. Consequently, tumor-bearing mice were shown to have reduced levels of serum cystine possibly resulting from increased storage (Srivastava et al. 2010). On the other hand, increased cysteine and/or cystine consumption of the tumor itself may also lead to decreased levels of circulating cysteine and cystine.

Nonetheless, the recognition that various cytokines or stimulators of the immune response are activated or inactivated by oxidation in the extracellular space strongly favors the idea for an important role of the cystine/cysteine cycle in T cell activation. For instance, the oxidation status of high-mobility group box-1 protein (HMGB1), a protein released from dying cells, determines whether inflammation is elicited or suppressed (Kazama et al. 2008). Similarly, galectin-1, a key mediator of regulatory T cells (Garin et al. 2007), is inactivated by cysteine residue oxidation, whereas TGF-β is activated upon oxidation (Blakytny et al. 2006).

Besides T cell activation, IL-1β processing and secretion by monocytes in response to Toll-like receptor (TLR) or nucleotide oligomerization domain 2 (NOD2) stimulation also requires externalization of cysteine mediated by system x_c^- (Tassi et al. 2009). Apparently, an initial signal, mediated by reactive oxygen species (ROS) and which stimulates the cystine/cysteine redox cycle and thioredoxin upregulation, is required for IL-1β processing and secretion. B cell differentiation into antibody secreting plasma cells was also shown to be triggered by extensive redox processes in a biphasic manner (Vene et al. 2010) like that observed for IL-1β processing (Tassi et al. 2009). LPS stimulation of naive splenocytes triggers a first NADPH oxidase-derived ROS signal required to induce an antioxidant response (Vene et al. 2010). Inhibition of this priming signal leads to impairment of B cell activation and differentiation. The ROS-induced antioxidant response involves increased expression of thioredoxin and of xCT, leading to augmented extracellular nonprotein thiols like GSH and cysteine. This initial ROS signal is not only important to switch on the antioxidant response to protect differentiating B cells from oxidative stress due to higher ROS levels, but also to increase the secretion of antibodies, which appears to be dependent on a reducing milieu (Vene et al. 2010).

In conclusion, emerging evidence established that the extracellular redox milieu, determined mainly by nonprotein thiols, has profound impact on various aspects of immune cell regulation.

Cancer

Cysteine is the substrate-limiting factor for GSH biosynthesis and essential for polypeptide synthesis. Tumor cells generate high amounts of ROS and are therefore well equipped with antioxidant systems (Schumacker 2006; Trachootham et al. 2009). In addition, tumor cells have a high demand for nutrients including amino acids, and thus upregulation of various transport systems including system x_c^- in cancer cells may be a cellular adaptation to accommodate with tumor growth (Ganapathy et al. 2009). Despite the well established role of system x_c^- in human malignant brain tumors (as discussed above), only limited information regarding its expression in primary tumors of other origins and its role in tumor development and metastasis is available to date. Nonetheless, it might be a promising approach to target system x_c^- alone for two reasons: (1) to sensitize tumors for conventional drug treatment by dampening intracellular GSH levels and/or (2) to directly interfere with tumor growth by shutting off tumor cells from cystine supply (Lo et al. 2008b). Along this line, Okuno et al. (2003) showed that the difference between sensitivity and resistance of the human ovarian cancer cell line A2780 as well as the human colonic cancer cell line HCT8 towards cisplatin is based upon higher cystine uptake and higher intracellular GSH levels as a consequence of higher endogenous xCT and 4F2 expression in the resistant cell line derivatives. A highly positive correlation between xCT and 4F2 expression was also found in 60 other human cancer cell lines (Huang et al. 2005).

Similar to the human ovarian cancer and the human colonic cancer cell lines, the high resistance of the pancreatic cell line PANC-1 towards gemcitabine is due to higher xCT expression compared to the pancreatic cell lines MIAPaCa-2 and BxPC-3 (Lo et al. 2008a). Consequently, combined expression of xCT and 4F2 in the MIAPaCa-2 cell line increases its resistance towards gemcitabine, indicating that higher system x_c^- activity, as also observed in primary ductal adenocarcinoma specimens, may increase tumor resistance. Likewise, the anti-inflammatory drug sulfasalazine inhibits the growth of small-cell lung cancer lines and this inhibitory effect can be abolished by co-administration of β-ME (Guan et al. 2009).

Experimental evidence for an involvement of xCT in cancer cell metastasis was provided by a combination of genetic and pharmacological means (Chen et al. 2009). xCT-deficient melanocytes, derived from *sut*/*sut* mice (see below), display an epithelial-like phenotype characterized by increased cell–cell adhesion and concomitant reduced cell–matrix adhesion. E-cadherin and caveolin-1, proteins involved in cell adhesion and migration, are highly upregulated in the knockout melanocytes. All these phenotypes could be phenocopied in the esophagal cancer cell line KYSE150 when treated with sulfasalazine (Chen et al. 2009). Most importantly, the number and size of lung metastases upon intravenous injection of KYSE150 cells into the tails and simultaneous treatment of mice with sulfasalazine could be greatly reduced compared to saline-treated animals. Yet, it remains to be determined whether this is due to impaired extracellular matrix adhesion or just due to impaired proliferation of tumor cells. Despite these first encouraging results, more studies are required to unequivocally assign a vital role for system x_c^- in tumorigenesis and metastasis.

Lessons from xCT loss of function models

Until now, three different mouse models for xCT loss of function have been generated and initially characterized during the last few years (Fig. 4). Chintala et al. (2005) analyzed mice with a pigmentary mutation, the subtle gray (*sut*) mutant, characterized by reduced yellow pigmentation. By positional cloning, they identified a large deletion region (481,280 bp) on chromosome 3 mapping to the *Slc7a11* gene (the gene encoding xCT). Thereby, exon 12 of the *Slc7a11* gene is replaced by an alternative downstream exon, leading to putatively altered *Slc7a11* transcripts at the 3′ end. However, no *Slc7a11* transcripts could be detected in brain or melanocytes of *sut*/*sut* mice suggesting that no functional protein is expressed in these mice. Loss of system x_c^- activity was further substantiated by the fact that cells isolated from the *sut*/*sut* mice indeed display only very little cystine uptake activity. Moreover, the ex vivo growth and survival of melanocytes and fibroblasts from *sut*/*sut* mice requires the presence of the sulfhydryl reducing agent β-ME in the culture medium. The same requirement of β-ME for the ex vivo proliferation of xCT-deficient cells holds true also for meningeal cells and astrocytes isolated from *sut*/*sut* mice (Shih et al. 2006), which suggests that this is a rather general in vitro phenomenon of xCT null cells. At least in part, cell death of *sut*/*sut* melanocytes occurs in an apoptotic manner and entails the activation of c-Jun N-terminal kinase (JNK), caspases-3/-9 and components of the endoplasmic reticulum stress-mediated cell death pathway like eIF2 and CHOP (Qiao et al. 2008). Breeding *sut*/*sut* mice with mice expressing a BAC clone containing exclusively *Slc7a11* restored the defects in pheomelanin production, strongly suggesting that cystine uptake is essential for proper

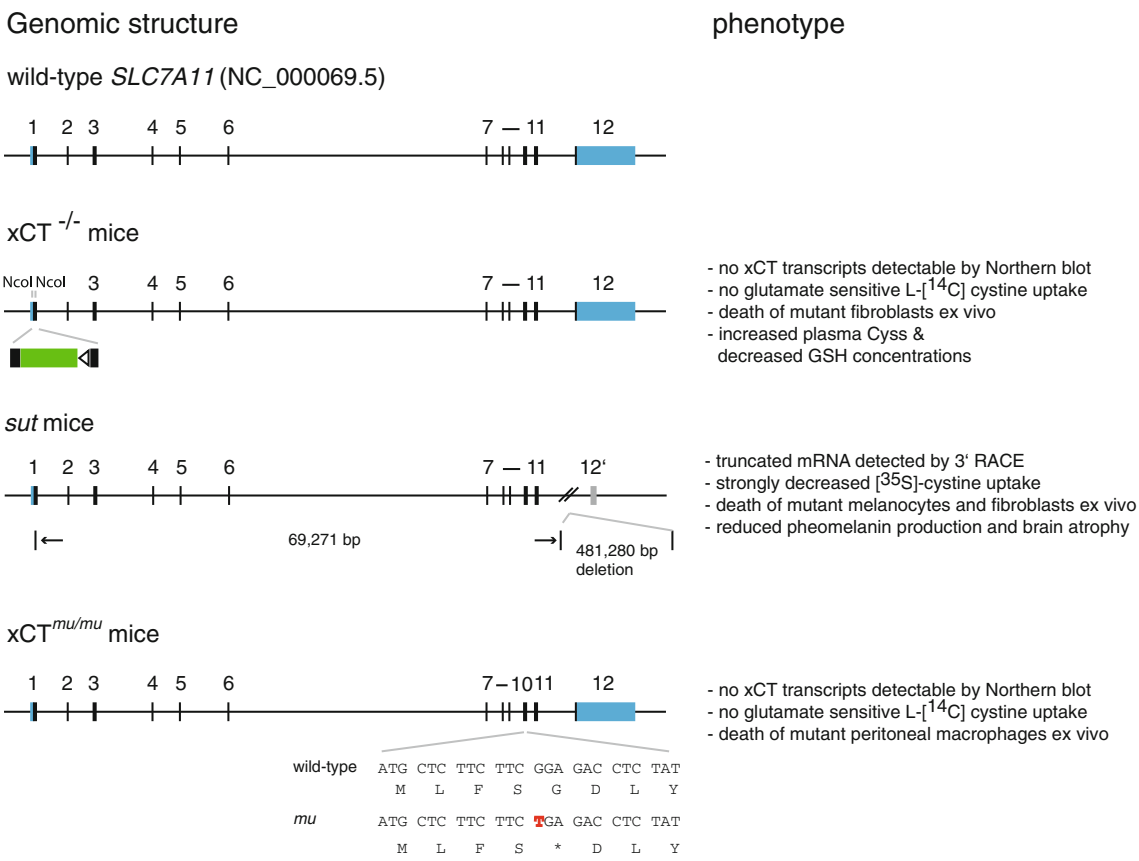

Fig. 4 Loss of function models for xCT in mice. The wild-type *xCT* (*Slc7a11*) gene consists of 12 exons (*filled boxes*), which localize on chromosome 3 (chromosome 4 in human) and span a region of 78,256 bases. In $xCT^{-/-}$ mice, the ATG start codon between the two *NcoI* sites is replaced by an eGFP expression cassette (*green box*) (Sato et al. 2005). In the subtle gray mutant (*sut/sut* mice), a large region containing exon 12 of the *Slc7a11* gene is deleted; thereby an alternative exon E12' (*gray box*) located just downstream of the deletion region leads to truncated xCT transcripts including E12' (Chintala et al. 2005). In $xCT^{mu/mu}$ mice ENU treatment caused a mutation in exon 10 (indicated in *red*) leading to an in-frame STOP codon in the xCT protein (Nabeyama et al. 2010). The major phenotypes of each mutant line reported in literature are given on the *right*. The 5′ and 3′ untranslated regions are represented in *blue*

pigmentation in vivo (Chintala et al. 2005). It is noteworthy that cysteine is the precursor of pheomelanin (Deibel and Chedekel 1984).

In the same year, we reported the phenotype of genetically engineered *xCT* knockout mice (Sato et al. 2005). The targeting strategy aimed at deleting the START codon in exon 1 of the *Slc7a11* gene. Removal of the ATG codon leads to undetectable levels of xCT expression in tissues like brain and thymus where xCT is constitutively expressed in wild-type mice. As a consequence, the rate of cystine uptake in LPS-stimulated $xCT^{-/-}$ macrophages drops to glutamate-insensitive background levels, confirming that the xCT knockout mice have no detectable cystine uptake activity mediated by system x_c^- (Sato et al. 2005). Like that of cells isolated from *sut/sut* mice, the proliferation of $xCT^{-/-}$ mouse embryonic fibroblasts (MEFs) is strictly dependent on thiol containing antioxidants, such as β-ME and NAC, while the lipophilic antioxidant vitamin E only prevents rapid cell death, but does not allow cells to proliferate. Deprivation of a thiol-containing source from the cell culture medium causes rapid drops of intracellular GSH in $xCT^{-/-}$ cells and thereby triggers cell death ex vivo. Interestingly, xCT knockout mice display higher plasma cystine levels while the levels of amino acids other than cysteine are comparable between knockout and wild-type mice (Sato et al. 2005). The redox potential of the plasma cystine/cysteine couple of knockout mice (−89 mV) was shown to be clearly more oxidized than wild-type mice (−100 mV) (Sato et al. 2005), indicating that system x_c^- contributes to maintaining the plasma cystine/cysteine redox balance at least in part. These results may also indicate that the xCT knockout mice age faster than wild-type counterparts do; yet, experimental proof is still lacking for a possible accelerated ageing of xCT null mice. The brain atrophy of relatively young *sut/sut* mice (Shih et al. 2006), however, has not yet been observed in $xCT^{-/-}$ mice (unpublished observation). Whether these differences are due to the different backgrounds of

the mouse strains used in the studies remains unclear ($xCT^{-/-}$ were crossed for more than 10 generations on the C57BL/6j background, whereas *sut/sut* mice were on the C3H/HeSnJ background) (Chintala et al. 2005; Shih et al. 2006).

A third loss of function model for xCT was published very recently by using *N*-ethyl-*N*-nitrosourea (ENU)-induced mutagenesis (Nabeyama et al. 2010). Hematopoietic progenitor cells from ENU mutants ($xCT^{mu/mu}$) were screened according to their inability to form macrophages and DCs in response to cytokine stimulation. As revealed by mapping and sequencing, ENU mutagenesis induced an in-frame STOP in exon 10. Thus putative xCT products would lack the last 3 out of 12 transmembrane spanning domains, in case they are expressed. Like already observed for the two other models, proliferation of MEFs and DCs requires the presence of β-ME in vitro, whereas the in vivo homeostasis of DCs and macrophages is not altered. In addition, LPS-activated macrophages die very rapidly in cell culture possibly in a necrotic manner, although the lack of xCT does not impinge on TNFα secretion 24 h after LPS stimulation (Nabeyama et al. 2010). Previously, it was shown that LPS profoundly stimulates xCT expression in macrophages (Sato et al. 2001). Since chronic inflammation is considered to be closely related to tumor initiation, chemically induced fibrosarcoma development using 3-methylcholanthrene (3-MCA) was studied in the $xCT^{mu/mu}$ mice (Nabeyama et al. 2010). Already 3 days after 3-MCA injection, IL-1β and TNF-α levels were found to be markedly upregulated in skin at the site of injection, and one week later *xCT* mRNA levels were highly upregulated due to the presence of infiltrated macrophages. Coinciding with a possible increased unremitted inflammation in xCT mutant mice, tumor incidence in $xCT^{mu/mu}$ mice is slightly accelerated, although tumor growth progresses with similar pace as in wild-type mice (Nabeyama et al. 2010).

Despite these rather specific phenotypes, two intriguing lessons could be learned from these models: first, it was indeed surprising that the absence of xCT has hardly any impact on the viability and survival of mice, particularly in view of the early embryonic lethal phenotype of γ-GCS knockout mice (Shi et al. 2000). Second, all three studies independently demonstrated the stark discrepancy between the in vivo and in vitro functions of xCT. While xCT appears to be essential for cell growth in vitro in various cellular systems, including fibroblasts, melanocytes, astrocytes and hematopoietic (stem) cells, in vivo xCT is dispensable for viability and survival of mice. Even the combined deficiency of xCT and superoxide dismutase 1 (SOD1) does not aggravate the phenotypic consequences of SOD1-deficiency alone (Iuchi et al. 2008), which again is unexpected in light of the vital role of xCT for in vitro survival of cells. It seems likely that the function of xCT in vivo is compensated by the renal cystine transport system $b^{0,+}$ expressed in kidney.

First in vivo proof that xCT plays indeed an important role in protecting tissues from oxidative stress was obtained by using a model of ischemia–reperfusion-induced acute renal failure (ARF) (Shibasaki et al. 2009). Here, xCT expression is induced in kidney 24 h after transient ischemia. Consequently, mice lacking xCT show more signs of ARF and higher concentrations of blood urea nitrogen and creatinine upon transient ischemia–reperfusion (Shibasaki et al. 2009). Despite these initial studies, more stress models are certainly required to corroborate the long-suspected protective role of system x_c^- in stress protection in vivo.

Conclusion and outlook

The cystine/glutamate antiporter system, system x_c^-, has emerged as an additional and GSH-independent redox system whose impact on many redox and thiol dependent cellular processes we only begin to understand. While its vital role in the provision of cystine for GSH biosynthesis for the in vitro survival and proliferation of many cells is well established, evidence for its widely debated protective functions in vivo is still scarce. So far, system x_c^- mediated glutamate release is mainly associated with detrimental effects in brain disease including glioma growth. Attempts to pharmacologically target system x_c^- in glioma patients failed and had to be sadly terminated due to severe toxic side effects of sulfasalazine, an FDA-approved drug for the treatment of inflammatory bowel disease. It remains to be shown whether a protective (antioxidant) role exists for system x_c^- in the maintenance of cellular redox balance in the brain, being well aware of the fact that this will be always hard to show in vivo due to the inevitably intertwined functions, cystine uptake and glutamate release. Another, yet unanswered question is whether loss of xCT causes premature ageing as oxidative stress and perturbed cellular redox balance have been frequently linked with ageing, albeit this is still a matter of intense discussions. Moreover, it will be equally important to use the xCT loss of function models in pharmacological or experimental models of human disease and to crossbreed these models with models of genetic disease. Finally, one should keep an eye on a putative role of extracellular glutamate in cell–cell communication in tissues other than in brain similar to that what has been shown for extracellular cysteine in the immune synapse.

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