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REVIEW ARTICLE

NADPH debt drives redox bankruptcy: SLC7A11/xCT-mediated cystine uptake as a double-edged sword in cellular redox regulation

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Abstract Cystine/glutamate antiporter solute carrier family 7 member 11 (SLC7A11; also known as xCT) plays a key role in antioxidant defense by mediating cystine uptake, promoting glutathione synthesis, and maintaining cell survival under oxidative stress conditions. Recent studies showed that, to prevent toxic buildup of highly insoluble cystine inside cells, cancer cells with high expression of SLC7A11 (SLC7A11^{high}) are forced to quickly reduce cystine to more soluble cysteine, which requires substantial NADPH supply from the glucose-pentose phosphate pathway (PPP) route, thereby inducing glucose- and PPP-dependency in SLC7A11^{high} cancer cells. Limiting glucose supply to SLC7A11^{high} cancer cells results in significant NADPH "debt", redox "bankruptcy", and subsequent cell death. This review summarizes our current understanding of NADPH-generating and -consuming pathways, discusses the opposing role of SLC7A11 in protecting cells from oxidative stress—induced cell death such as ferroptosis but promoting glucose starvation—induced cell death, and proposes the concept that SLC7A11-mediated cystine uptake acts as a double-edged sword in cellular redox regulation. A detailed understanding of SLC7A11 in redox biology may identify metabolic vulnerabilities in SLC7A11^{high} cancer for therapeutic targeting.

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Introduction

The redox couples of nicotinamide adenine dinucleotide (NAD$^+$), nicotinamide adenine dinucleotide phosphate (NADP$^+$), and their corresponding reduced forms NADH and NADPH, play essential roles in cellular metabolism and redox maintenance. The NAD$^+$ and NADH redox couple is chiefly used in biological oxidation reactions involved in energy catabolism such as glycolysis and mitochondrial oxidative phosphorylation.[1] In contrast, the NADP$^+$ and NADPH redox couple has a critical role in maintaining cellular redox homeostasis and supporting the reductive biosynthesis during anabolism, such as syntheses of fatty acids, steroids, and nucleic acids. Under physiological conditions, the ratio of intracellular (and particularly cytosolic) NAD$^+$ to NADH is kept high, so that much NAD$^+$ can be available as an oxidizing agent for catabolism; whereas the ratio of intracellular NADP$^+$ to NADPH is kept low, thereby favoring the utilization of NADPH as a reducing agent for anabolism and redox maintenance. In this review, we will focus on NADPH, the universal cellular reduction "currency" in eukaryotic cells.

Amino acid cysteine and its oxidized dimeric form cystine have key functions in maintaining redox homeostasis and mediating cellular antioxidant defenses mainly via contributing to the biosynthesis of reduced glutathione (GSH), for which cysteine is as a limiting precursor. Whereas the intracellular GSH concentration is in the millimolar range, the intracellular concentration of free cysteine is kept in the low micromolar range and is much lower than that of GSH. Although cysteine can be synthesized from the de novo biosynthesis pathway in some cells, most cells rely on the amino acid transporter solute carrier family 7 member 11 (SLC7A11, also called xCT) to replenish intracellular cysteine pool from the extracellular environment by taking up cystine, which is then quickly reduced to cysteine once imported into cells.[2] The rationale for taking up cystine rather than cysteine is that, due to the more oxidizing extracellular conditions, extracellular cystine is much more stable than extracellular cysteine and therefore cystine concentration is much higher than that of cysteine in the extracellular environment (of note, the half-life of cysteine in culture media has been measured at only around 30 min[3]). SLC7A11-mediated cystine uptake has a pivotal role in promoting GSH biosynthesis, detoxifying reactive oxygen species (ROS), and supporting cell survival and growth. Consistent with this, many cancers exhibit upregulated expression of SLC7A11.[2]

In light of this well-established pro-survival function of SLC7A11, it is surprising that several recent studies highlight an emerging pro-cell death function of SLC7A11 in the context of glucose limitation.[4–8] Very recently, the role of SLC7A11 in promoting cell death under glucose deprivation was further linked to pentose phosphate pathway (PPP) dependency, NADPH consumption, and disulfide stress.[8] In this review, we will first summarize our current understanding of NADPH-generating pathways and major antioxidant systems that utilize NADPH, followed by a discussion of SLC7A11-mediated cystine transport and its known role in promoting GSH biosynthesis and suppressing oxidative stress-induced cell death such as ferroptotic cell death. We will then describe recent discoveries on the role of NADPH consumption in SLC7A11's function in promoting glucose—PPP dependency and discuss the double-edged sword role of SLC7A11 in regulating cellular redox balance and cell death/survival. We will end our discussion by highlighting a few unknown questions for future investigation.

NADPH-generating pathways

NADP$^+$ or NADPH cannot be directly acquired from extracellular sources and need to be generated via intracellular metabolic pathways. Intracellular NADPH is predominantly generated from NADP$^+$, while NADP$^+$ is mainly derived from NAD$^+$ through NAD$^+$ phosphorylation catalyzed by either cytosolic NAD kinase (NADK) or mitochondrial NADK2, both of which use ATP as the phosphoryl donor to convert NAD$^+$ to NADP$^+$ (Fig. 1).[9,10] NADK is required for maintaining the cellular NADPH pool by providing NADP$^+$ and is essential for cell survival. Recently it was reported that the N-terminal domain of cytosolic NADK serves to suppress NADK activity and Akt-mediated phosphorylation of residues in this region relieves its inhibitory effect, thereby stimulating NADK activity and function to generate NADP$^+$ and to maintain cancer cell survival.[11] Compared to NADKs as the only known pathway to generate NADP$^+$, there are at least four main metabolic pathways that mediate NADP$^+$ reduction to produce NADPH in eukaryotic cells, namely the PPP, malic enzyme (ME)-mediated malate metabolism, folate metabolism, and isocitrate dehydrogenase (IDH)-mediated isocitrate/α-ketoglutarate (α-KG) metabolism (Fig. 1), which will be further discussed in this section. Once formed, NADPH is consumed and subsequently recycled back to oxidized NADP$^+$ through reductive biosynthetic processes (such as fatty acid biosynthesis) and multiple NADPH-dependent antioxidant systems such as glutathione reductase (GR) and thioredoxin (Trx) reductase (TrxR or TR), which will be further discussed in the next section.

PPP

In most mammalian cells, cytosolic NADPH is mainly generated through the oxidative branch of the PPP. Once taken up into cells through glucose transporters (GLUTs),
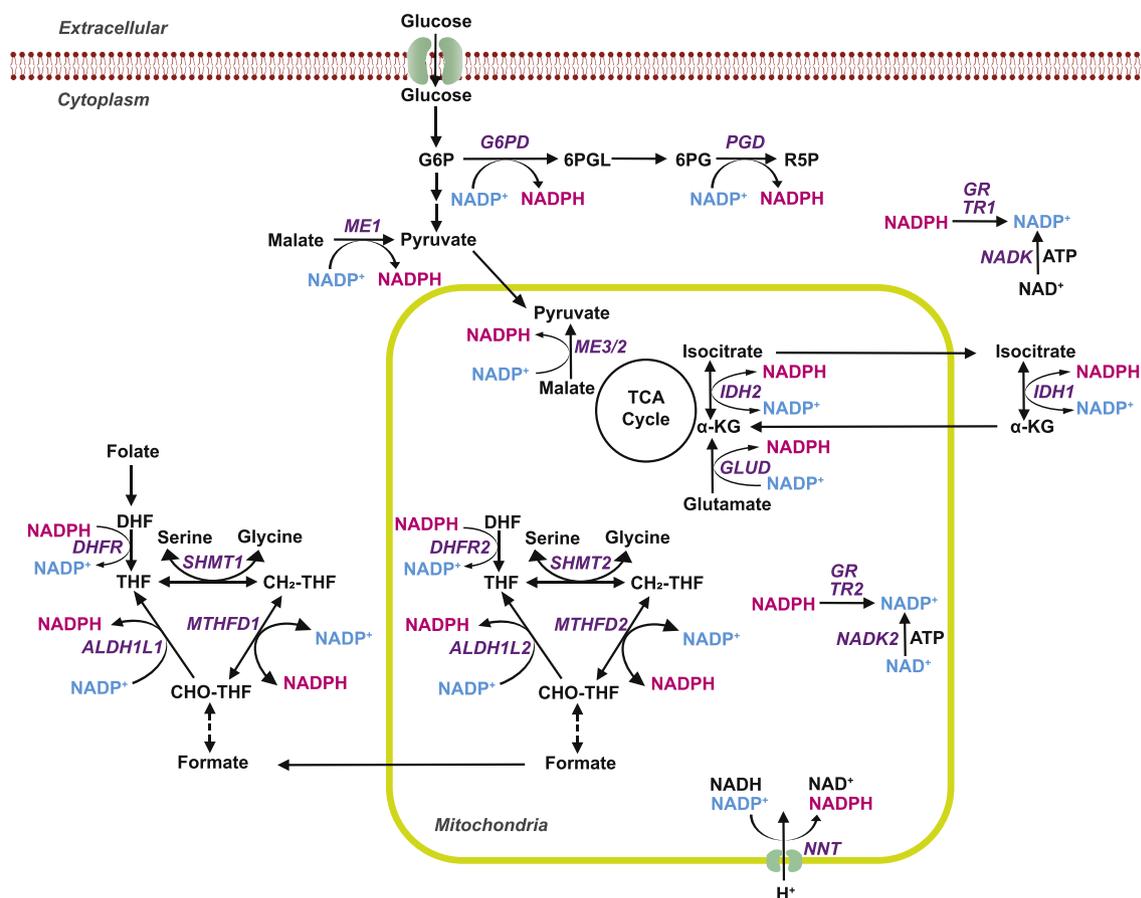
Figure 1 Intracellular sources of NADP$^+$ and NADPH in eukaryotic cells. NADP$^+$ is generated from NAD$^+$ by cytosolic NADK or mitochondrial NADK2. NADPH is produced from NADP$^+$ in cytosol or mitochondria. In cytosol, NADPH can be generated from the PPP via G6PD and PGD. In addition, ME1 converts malate to pyruvate generating NADPH. Folate is firstly reduced to DHF and then to the activated form THF that is catalyzed by cytosolic DHFR or mitochondrial DHFR2 which consumes NADPH. The later steps in folate metabolism then generate NADPH via MTHFD1/2 and ALDH1L1/2 in cytosol or mitochondria. Other enzymes also contribute to NADPH production, including ME2/3, IDH1/2, NNT, and GLUD. NADPH is used as a reducing power in GR- or TR1/2-mediated reactions and oxidized to NADP$^+$. Proteins are indicated in purple. Arrows indicate irreversible enzymatic steps, and bi-directional arrows indicate reversible reactions. G6PD: glucose-6-phosphate dehydrogenase; PGD: 6-phosphogluconate dehydrogenase; ME1/2/3: malic enzyme 1/2/3; GR: glutathione reductase; TR1/2: thioredoxin reductase 1/2; NADK/NADK2: NAD kinase/NAD kinase 2; IDH1/2: isocitrate dehydrogenase 1/2; GLUD: glutamate dehydrogenase; SHMT1/2: serine hydroxymethyltransferase 1/2; MTHFD1/2: methylenetetrahydrofolate dehydrogenase 1/2; ALDH1L1/2: aldehyde dehydrogenase family 1 member L1/2; NNT: nicotinamide nucleotide transhydrogenase; G6P: glucose-6-phosphate; 6PGL: 6-phosphogluconolactone; 6PG: 6-phosphogluconate; R5P: ribose-5-phosphate; α-KG: α-ketoglutarate; DHF: 7, 8-dihydrofolate; DHFR: dihydrofolate reductase; CH2-THF: 5,10-Methylene-THF; CHO-THF: 10-Formyl-THF.

glucose is converted to glucose-6-phosphate (G6P) through hexokinases. Subsequently, G6P can either continue its catabolism through the glycolysis or be shunted into the PPP. In the oxidative phase of the PPP, G6P is first dehydrogenated by glucose-6-phosphate dehydrogenase (G6PD) to yield 6-phosphogluconolactone (6PGL), which is coupled to the reduction of one molecule of NADP$^+$ to one molecule of NADPH. 6PGL is subsequently hydrolyzed spontaneously or catalyzed by 6-phosphogluconolactonase (PGL) into 6-phosphogluconate. This product is then catalyzed by 6-phosphogluconate dehydrogenase (PGD or 6PGD) to generate ribose-5-phosphate (R5P) and a second molecule of NADPH (Fig. 1). PPP also contributes to anabolic biosynthesis through its non-oxidative phase (which will not be discussed in detail here). The oxidative PPP directly

couples NADPH regeneration to the production of R5P, which is an obligate precursor for all nucleic acids. When the cellular demand for NADPH outstrips the demand for nucleic acid biosynthesis, the excessive R5P is funneled through a series of reactions in the non-oxidative PPP that convert it into intermediates of lower glycolysis. It is estimated that the oxidative branch of the PPP contributes to up to 60% of total cytosolic NADPH in some cells.[12,13]

G6PD is the rate-limiting enzyme of the PPP and its activity is increased in several types of cancer.[14] G6PD is essential for cell survival and growth in many cancer cells, and generation of NADPH and R5P has been proposed to be critical for G6PD's function in maintaining cancer cell survival and growth.[15] Conversely, it was reported that tumor suppressor p53 can suppress NADPH production through

direct inactivation of G6PD, resulting in inhibition of cancer cell growth and proliferation.[16] It was also shown that PGD plays important roles in promoting radioresistance in glioma cells and chemoresistance in hepatocellular carcinoma (HCC) cells partly through affecting NADPH generation.[17–19] The upregulation of these PPP genes in cancer cells makes them potential targets for cancer therapy. Notably, PPP was recently reported to regulate circadian oscillation through NADPH metabolism that modulates redox and circadian gene expression in flies, mice and human cells.[20] Together, these studies reveal that PPP-mediated NADPH generation plays a critical role in promoting cancer cell survival, growth and drug resistance.

MEs

Besides the PPP, MEs also contribute to NADPH generation (Fig. 1). MEs convert malate to pyruvate and CO_2 with the reduction of $NADP^+$ (or NAD^+) to NADPH (or NADH). There are three ME isoforms, including cytoplasmic $NADP^+$-dependent ME1, mitochondrial $NADP^+/NAD^+$-dependent ME2, and mitochondrial $NADP^+$-dependent ME3[21] (among which ME2 is the major isoform in mitochondria in most cancer cells[22]); consequently, ME1 produces NADPH in cytoplasm, whereas ME2 and ME3 generate NADPH in mitochondria. It should be noted that, while mitochondrial ME2 can use $NADP^+$ to generate NADPH, it binds NAD^+ with higher affinity and thus can more efficiently produce NADH. While it is generally believe that ME1 serves as a backup mechanism of the PPP to supply cytosolic NADPH, a recent study using deuterium (^2H)-labeled tracer revealed that most (~60%) NADPH is made by ME1, rather than by the PPP, in mouse adipocytes, and ME1 significantly contributes to fatty acid biosynthesis (which occurs in cytoplasm) through producing NADPH in liver and adipose tissues[23]; interestingly, hypoxia stress could switch the main NADPH source from ME1 to the PPP in adipocytes.[23] Another study showed that there exists cross-talks between ME1 and the PPP, and ME1 was reported to promote the PPP flux by forming a complex with PGD.[24] ME-mediated NADPH production has also been implicated in cancer biology. For example, it was reported that ME1 and ME2 promote NADPH production, lipogenesis, and glutamine metabolism in cancer cells, resulting in increased tumor growth.[22] ME1 and ME2 are transcriptionally repressed by tumor suppressor p53 and consequently are overexpressed in various cancers.[22]

Folate metabolism

Similar to the PPP, folate-dependent one-carbon metabolism (also known as folate cycle) is critical for maintaining cell growth and survival by producing NADPH as well as nucleotide precursors. At the beginning of folate metabolism, NADPH is consumed to produce 5, 6, 7, 8-tetrahydrofolate (THF), followed by two oxidized reactions in later steps to produce NADPH, including methylenetetrahydrofolate dehydrogenase1/2 (MTHFD1/2)- and aldehyde dehydrogenase family 1 member L1/2 (ALDH1L1/2)-mediated reactions (Fig. 1). Notably, folate cycle can occur in both cytoplasm and mitochondria with compartmentalized enzymes. For example, while MTHFD1 and ALDH1L1 localize in cytoplasm, MTHFD2 and ALDH1L2 reside in mitochondria. Most folate intermediates are impermeable for mitochondrial membrane and cannot be transported between cytosol and mitochondria. Folate cycles in these two compartments are mainly linked via formate (Fig. 1), which is largely produced in mitochondria, exported to the cytosol, and then shunted into cytosolic folate cycle through its conversion to 10-Formyl-THF (CHO-THF).[25,26] Whereas most cells prefer to use the mitochondrial folate pathway to generate the one-carbon units required to produce CHO-THF, they can also switch to use the cytosolic folate pathway under some conditions.[27,28] These compartmentalized enzymes often exhibit differential expression in cancers, indicating differential importance of cytosolic vs. mitochondrial folate metabolism in cancer development. For example, while cytoplasm-localized ALDH1L1 is rarely expressed in cancer cells, mitochondria-localized ALDH1L2 is highly overexpressed in many cancers,[29–31] and its high expression correlates with poor prognosis in certain cancers,[32] indicating an important role of mitochondrial folate metabolism in cancer development. Importantly, while traditionally folate metabolism was not considered as a significant contributor to NADPH production, analyses using more recently developed tracing approaches revealed that folate metabolism can produce nearly comparable levels of NADPH to that produced by the PPP in some cells, therefore highlighting a previously underappreciated role of folate metabolism in NADPH generation.[33,34]

IDHs

IDHs are important metabolic enzymes catalyzing the oxidation of isocitrate to α-KG. There are at least three IDH isoforms, including cytoplasmic $NADP^+$-dependent IDH1, mitochondrial $NADP^+$-dependent IDH2, and mitochondrial NAD^+-dependent IDH3; therefore, IDH1 and IDH2 produce NADPH in cytoplasm and mitochondria, respectively (Fig. 1), whereas IDH3 generates NADH in mitochondria. Intriguingly, gain-of-function R132H mutation in *IDH1* or *IDH2* (which frequently occurs in glioblastomas and acute myelogenous leukaemias) generates IDH1 or IDH2 mutant proteins capable of converting α-KG to 2-hydroxyglutarate (2HG) through a reaction that consumes, rather than produces, NADPH,[35–37] resulting in decreased NADPH levels in glioblastomas with *IDH1* R132 mutation compared with those with wild-type *IDH1*.[38] Consequently, IDH1 mutant-mediated 2-HG production competes with other reactions that consume NADPH, such as reductive biosynthesis, resulting in PPP dependency and increased sensitivity to oxidative stress.[39] Notably, it was estimated that 2HG synthesis in IDH1 R132 mutant cells consumes NADPH at a similar rate to that of NADPH consumption by *de novo* lipogenesis (which generally is considered the largest NADPH-consuming process[40]).

Besides the afore-discussed NADPH-generating pathways, there are several other metabolic reactions producing NADPH in eukaryotic cells. In one reaction, NADPH can be generated in mitochondria by nicotinamide nucleotide transhydrogenase (NNT), which transfers hydride from NADH to $NADP^+$ coupled to H^+ translocation across the

inner mitochondrial membrane (Fig. 1).[41,42] As another example, mitochondrial glutamate dehydrogenase (GLUD)-mediated conversion of glutamate to α-KG can also produce NADPH (Fig. 1); however, since GLUD mainly uses NAD^+ as the coenzyme to generate NADH, it is believed that GLUD plays a limited role in replenishing intracellular NADPH pool.[43,44]

To summarize, among the four major NADPH-producing pathways we have discussed, the PPP is generally considered as the most important source to provide cytosolic NADPH; however, other three sources might also play equally important roles in producing NAPDH in certain cells/tissues or under certain conditions. It is important to note that NADPH is compartmentalized (i.e., NADPH produced in cytoplasm cannot directly enter into mitochondria, or vice versa for NADPH produced in mitochondria). For example, mitochondrial NADPH cannot be directly used for fatty acid biosynthesis, which occurs in cytoplasm (here we do not consider the scenario that mitochondrial NADPH might still indirectly contribute to cytoplasmic NADPH-consuming reactions through a series of metabolic pathways which shuttle from mitochondria to cytoplasm). Notably, MEs, folate metabolism, and IDHs all contain their respective isoforms to produce NADPH in either cytoplasm or mitochondria, such as ME1, IDH1, MTHFD1, and ALDH1L1 in cytoplasm vs ME2/3, IDH2, MTHFD2, and ALDH1L2 in mitochondria (Fig. 1). In contrast, the PPP only operates in cytoplasm and therefore mainly contributes to NADPH pool in cytosol (Fig. 1). This conceptual framework will be instrumental for our discussion of NADPH utilization in later sections. NADPH is mainly utilized in two broad biological processes, antioxidant systems and reductive biosynthesis (such as fatty acid synthesis). In the context of this review, we will only discuss antioxidant systems with their relevance to NADPH, which is the topic of the next section.

NADPH-utilizing antioxidant systems

Intracellular redox reactions involve the transfer of electrons between molecules and can take place in different subcellular compartments in eukaryotic cells. The differential redox environments in different compartments are critical for appropriate biological processes occurring in corresponding compartments. For example, under physiological conditions, the cytosolic environment is highly reducing whereas endoplasmic reticulum (ER) maintains an oxidative environment, therefore facilitating the oxidation of cysteine residues in nascent polypeptide chains in ER to form intramolecular disulfide bonds, which are critical for protein folding.[45,46] ROS are often produced by cellular metabolism as by-products. While low levels of ROS can act as second messengers to regulate various biological processes and therefore are critical for appropriate cell functioning, high levels of ROS cause damage in nucleic acids, proteins, and lipids, inducing cellular stress collectively called oxidative stress, which if unresolved can ultimately lead to cell cycle arrest and cell death. As discussed below, cells have evolved various antioxidant defense mechanisms to neutralize ROS, defend cellular oxidative stress, and therefore maintain redox balance in eukaryotic cells, which

include non-thiol-dependent and thiol-dependent antioxidant systems.[47−50] Non-thiol-based systems consist of superoxide dismutases (SODs) and catalase enzymes,[49,50] whereas thiol-dependent antioxidant systems mainly include GSH and Trx systems, which defend against oxidative stress through their disulfide reductase activities.[51] Of note, both GSH and Trx systems are dependent on NADPH to provide their reducing power (Fig. 2). In this section, we will discuss the mechanistic basis of these antioxidant systems as well as the role of NADPH in antioxidant defense.

GSH

GSH is a tripeptide-like thiol-containing molecule (γ-Glu-Cys-Gly), which is mainly synthesized and stored in the cytosol.[52] It is probably the most abundant low-molecular weight thiol compound synthesized in eukaryotic cells. Under physiological conditions, the intracellular GSH concentration is in the range of 1−10 mM and is higher than that of its oxidized form GSSG (the dimeric form of GSH linked by a disulfide bond) for 10−100 folds.[53,54] A steady GSH/GSSG ratio is essential for maintaining cellular redox homeostasis and for ensuring proper biological processes; consequently, abnormal accumulation of GSSG over GSH may cause oxidative stress-induced cell death.[47,55−57] GSH plays a versatile role in ROS detoxification and redox maintenance. As discussed below, the antioxidant function of GSH is mainly derived from its utilization by various GSH-dependent enzymes for antioxidant defenses. GSH can also directly react with free radical species via its thiol moiety and contributes to other non-enzymatic antioxidants such as vitamins E (α-tocopherol) and C (ascorbic acid).[58] Finally, GSH can undergo thiol-disulfide exchange reactions with glutaredoxins (Grxs) or serve as an electron carrier for enzymes involved in ROS reduction.[53,59,60] Throughout these reactions, two molecules of GSHs are oxidized to one molecule of GSSG. GSSG is then rapidly reduced back to GSH by GR, which is a NADPH-dependent flavoprotein.[61,62] In GR-mediated reaction to reduce GSSH to GSH, the electrons in NADPH are extracted and used to reduce the disulfide bond in GSSG, yielding GSH and $NADP^+$.[63] Therefore, NADPH serves as the critical reduction "currency" to maintain appropriate levels of intracellular GSH.

Glutathione peroxidases

Glutathione peroxidases (GPXs) are important intracellular enzymes that directly break down hydrogen peroxides (H_2O_2) to water or alkyl hydroperoxides (ROOH) to their corresponding alcohols (ROH) (Fig. 2).[60,64] GPX protein family consists of at least eight enzymes in human, GPX1−8, among which GPX1 is the most abundant GPX member and is present virtually in all cell types.[65] While GPX5, GPX7, and GPX8 possess catalytic cysteine residues in their active sites, GPX1−4 and 6 instead use selenocysteine, an analog of cysteine in which the sulfur is replaced by selenium;[64,66] the distinct chemical properties of selenols present several advantages for biochemical reduction reactions in these GPX proteins.[67] The major function of GPX1 is to protect cells against H_2O_2 and a wide range of organic hydroperoxides,[60,64] whereas
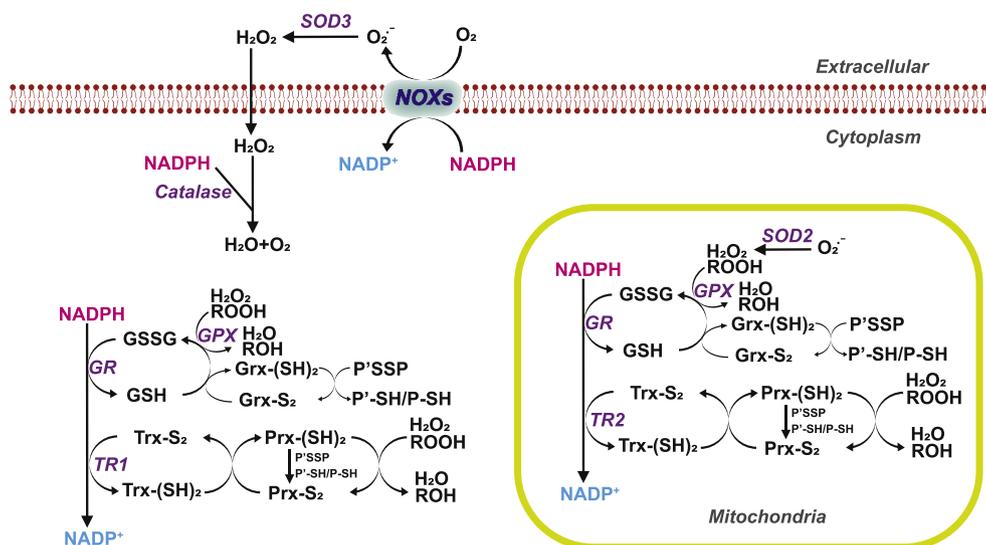
Figure 2 NADPH in regulating cellular redox homeostasis. NADPH is used as a reducing power in GR- or TR1/2-mediated anti-oxidant reactions. GR reduces GSSG to GSH, which is then used as a co-factor by GPXs, and GSH is re-oxidized to GSSG. In addition, the oxidation of GSH to GSSG is coupled with the reduction of glutaredoxin disulfide Grx-S$_2$ to glutaredoxin thiol Grx-(SH$_2$), which can conduct thiol–disulfide exchange reactions with protein disulfides (P'SSP), generating reduced protein thiols (P'-SH/P-SH). Cytosolic TR1 or mitochondrial TR2 catalyzes the reduction of thioredoxin disulfide Trx-S2 to thioredoxin thiols Trx-(SH)$_2$, which in turn promote the reduction of peroxiredoxin disulfide Prx-S2 to peroxiredoxin thiols Prx-(SH)$_2$. Prx-(SH)$_2$ may directly detoxify ROS or reduce protein disulfide by thiol–disulfide exchange. Transmembrane enzymes NOXs primarily catalyze the formation of superoxide anion O$_2^-$, which is rapidly converted to H$_2$O$_2$ by SOD3. In addition, NADPH binds to catalase converting H$_2$O$_2$ to H$_2$O. Proteins are indicated in purple. GR: glutathione reductase; TR1/2: thioredoxin reductase 1/2; SOD2/3: superoxide dismutase 2/3; NOXs: NADPH oxidases; ROOH: alkyl hydroperoxide; ROH: alkyl alcohol; P'SSP: protein disulfide; P'-SH/P-SH: protein thiol.

GPX4 is the only GPX enzyme that breaks down phospholipid hydroperoxides and recently has been shown to protect cells against ferroptosis, a form of iron-dependent regulated cell death caused by lipid peroxidation.[56,60] Recent studies further showed that selenium utilization by GPX4 is essential for blocking hydroperoxide-induced ferroptosis.[68]

Grx system

Grxs are a class of GSH-dependent oxidoreductase enzymes that specifically catalyze reversible thiol-disulfide exchange reactions between protein thiols and the intracellular thiol pool of GSH and GSSG (Fig. 2).[69,70] Grxs protect protein thiols (P-SH) from irreversible oxidation and regulate the cellular redox state and redox-dependent signaling pathways, for instance, by catalyzing reversible protein S-glutathionylation.[69,71] Moreover, Grxs are known to play a role in cellular iron homeostasis associated with GSH.[59]

Trx system

The Trx system comprises the redox-active protein Trx, the reductase enzyme TrxR, and NADPH (Fig. 2). This antioxidant system is essential for normal cellular and organismal functions, as evident by the observations that either *Trx* knockout or *TrxR* knockout mice are embryonic lethal.[72–74] The Trx system exerts its antioxidant activity mainly by transferring electrons to thioredoxin peroxidases (Prxs), which use a pair of SH groups as reducing equivalents to reduce peroxides, such as H$_2$O$_2$, ONOO$^-$ and ROOH.[51,75] These reactions generate the oxidized form of Prxs, which can be recycled back to its reduced form by the reduced Trx, while the reduced Trx is converted to the oxidized Trx, which then is converted back to its reduced form by TrxR at the expense of NADPH (Fig. 2).[75] Trx can also perform its antioxidant function through either directly quenching singlet oxygen and scavenging hydroxyl radicals, or reducing oxidized proteins.[76] TrxRs are the only known enzymes to mediate the reduction of Trx. There exist two TrxR members, including cytosolic TrxR1 and mitochondrial TrxR2, both of which are NADPH-dependent enzymes. Therefore, similar to its role in GSH system, NADPH plays a key role in Trx system to maintain cellular redox homeostasis.

Catalase and NADPH

NADPH also participates in cellular antioxidant defense through promoting the enzyme activity of catalase (Fig. 2).[49,77] The catalase is a major enzyme that directly detoxifies H$_2$O$_2$ by cleaving two molecules of H$_2$O$_2$ into two molecules of H$_2$O and one molecule of O$_2$. While NADPH does not directly participate in the catalase reaction, mammalian catalases nonetheless possess a high-affinity NADPH-binding domain. NADPH is believed to donate a reducing equivalent to protect the catalytic heme from inactivation.[49,78]

Oxidative role of NADPH oxidase

As discussed above, NADPH has a well-established protective role in cellular antioxidant systems. Paradoxically, excess accumulation of NADPH can lead to reductive stress and act as the substrate for NADPH oxidase (NOX) to generate ROS such as superoxide anion O_2^- (Fig. 2).[79,80] NOX family is composed of seven members, including NOX1–5 and dual oxidase (DUOX) enzymes DUOX1/2.[81,82] NOXs are membrane-associated hetero-oligomeric complexes that transfer electros across biological membranes to oxygen.[83] NOXs can produce either O_2^- (by NOX1–3, 5) or H_2O_2 (by NOX4 and DUOX1/2) in an NADPH-dependent manner.[84–86] Superoxide is then rapidly converted to H_2O_2 either spontaneously (such as under low pH environment) or catalyzed by SOD.[87,88] Unlike many other biological processes or reactions that generate ROS as a by-product, NOXs generate ROS as their primary function. NOXs are expressed in a cell- or tissue-specific manner and have functions under a wide range of physiological and pathophysiological conditions.[79] Except for NOX4 (which is constitutively active), other NOX enzymes are normally inactivated and their activities are heavily regulated by their interacting subunits or under diverse stimuli.[80,89] NADPH is the substrate of NOXs as well as likely a regulator of their enzyme activity. Recent structural analysis of *Cylindrospermum stagnale* NOX5 revealed its NADPH-binding site on C-terminal region, which also corresponds to the regulatory site of enzymatic activity for human NOX5 and NOX2.[83]

In summary, NADPH can act as a universal reduction "currency" in cellular antioxidant systems or the substrate for NOXs to drive ROS production; therefore, NADPH can have double-edged functions in redox maintenance. With our introduction of major NADPH-producing reactions and NADPH-utilizing antioxidant systems, in following sections we will shift our focus to SLC7A11-mediated cystine uptake and its role in antioxidant defense, which is ultimately linked to NADPH utilization.

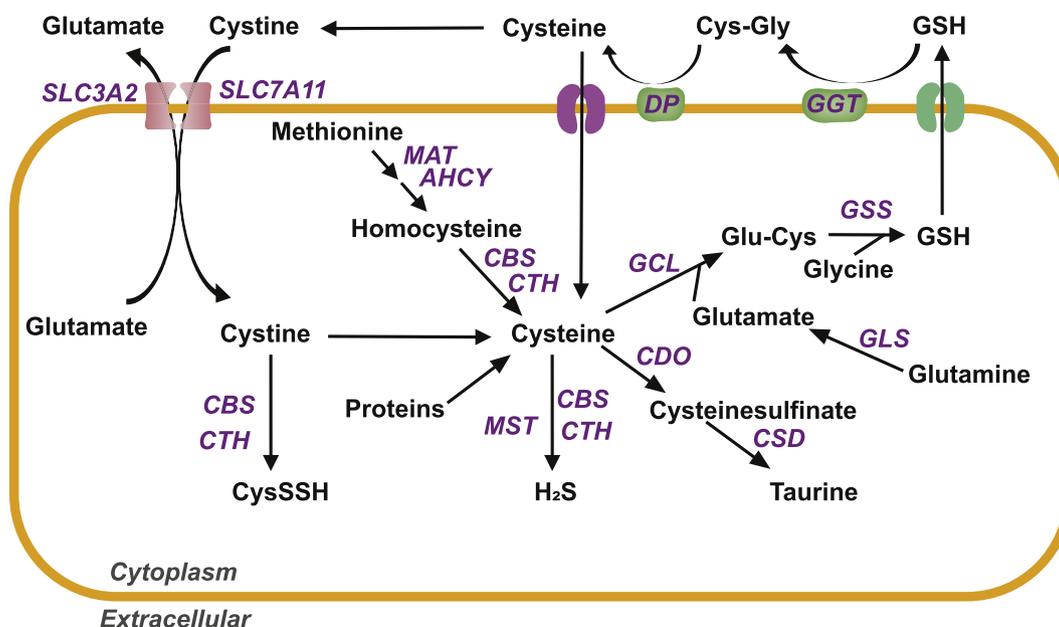## SLC7A11-mediated cystine uptake in antioxidant defense

Despite being one of the least abundant proteinogenic amino acids, cysteine plays critical roles in diverse cellular processes besides contributing to protein synthesis. Similar to lysine and serine, cysteine is a key residue on proteins that is subjected to multiple posttranslational modifications to control appropriate protein functions.[90] Moreover, cysteine has a vital role in maintaining cellular redox homeostasis, by serving as an essential component in major antioxidants such as GSH as well as functioning as a potent antioxidant itself. This versatile role of cysteine in redox maintenance stems from its reactive thiol group.[2]

Cells have evolved multiple mechanisms to maintain a constant supply of cysteine, including (1) *de novo* synthesis of intracellular cysteine (in which homocysteine can be converted to cysteine through the transsulfuration pathway) in some cells and tissues,[27,90] (2) protein degradation to recycle intracellular cysteine, and (3) uptake from extracellular environment mainly in the form of cystine, followed by the reduction of intracellular cystine to cysteine (Fig. 3). It should be noted that cysteine can be directly taken up from extracellular environment through cysteine transporters such as excitatory amino acid transporter 3 (EAAT3, also known as SLC1A1) and the alanine, serine, cysteine transporters (ASCT1/2) in some cell types or under certain conditions.[91,92] However, due to oxidizing extracellular environment, cysteine is unstable and easily oxidized to cystine, and the concentration of extracellular cysteine is lower than that of extracellular cystine typically by an order of magnitude. Consequently, most cells largely rely on cystine transporters to acquire extracellular cystine in order to supply intracellular cysteine. Cystine transport is mainly mediated by the system X_c^-, a sodium-independent antiporter that imports extracellular cystine and exports intracellular glutamate at a 1:1 ratio.[93] This amino acid transporter is a heterodimer comprising disulfide-linked light chain subunit SLC7A11 and heavy chain subunit solute carrier family 3 member 2 (SLC3A2; also known as 4F2hc or CD98).[2,93] SLC7A11 is responsible for the primary cystine transporting activity and substrate specificity,[94] whereas SLC3A2 mainly functions as a chaperone protein for SLC7A11 (as well as other amino acid transporters).[2,95] To keep consistency, in our following discussion we will refer to this cystine transporter primarily as SLC7A11.

Extracellular cystine is transported into cells primarily through SLC7A11 and then is quickly reduced to cysteine in the highly reducing environment of cytosol. Subsequently, cysteine is used as a precursor for synthesis of protein and other biomolecules, most notably GSH biosynthesis, which occurs in cytosol and for which cysteine is the limiting precursor.[53,96,97] The heterodimeric enzyme γ-glutamate-cysteine ligase (GCL, consisting of GCLC and GCLM), catalyzes the conjugation of cysteine with glutamate to form γ-glutamylcysteine (γ-Glu-Cys), which is the first and rate-limiting step in GSH biosynthesis. Glutathione synthetase (GSS) then mediates the second step in GSH biosynthesis by adding glycine to the C-terminus of γ-Glu-Cys, forming GSH (Fig. 3). The embryonic lethality of *GCLC* knockout mice at the gastrulation stage underlines the importance of GSH in mammalian development.[55] Interestingly, GSH can also be recycled back to cysteine in a process which occurs extracellularly, wherein enzymes located on the extracellular surface of cells, including ectoenzyme γ-glutamyl-transpeptidase (GGT) and membrane-bound dipeptidase (DP), catalyze the degradation of GSH to cysteine (which is then quickly oxidized to cystine due to oxidizing extracellular environment) (Fig. 3).[97–99] The reason that this process occurs extracellularly is probably related to the fact that cells excrete GSH conjugates (and GSSG) to the extracellular space as a way of detoxifying waste products.

Although intracellular cysteine can be synthesized through the transsulfuration pathway, the *de novo* synthesis is insufficient to provide adequate cysteine supply for GSH biosynthesis and antioxidant defense in many cells; therefore, cells, particularly cancer cells, are largely dependent on taking up extracellular cystine to maintain redox homeostasis and cell survival. Consistent with this, it was observed many years ago that cystine deprivation in cell culture medium results in significant decrease of intracellular GSH and subsequently cell death,[100–102] although the

Figure 3 SLC7A11-mediated cystine transport in cellular metabolism. SLC7A11 mediates the uptake of extracellular cystine, which is reduced to cysteine. Cysteine is subsequently used for GSH biosynthesis, wherein cysteine is first conjugated with glutamate to form the dipeptide Glu—Cys by GCL; the second enzyme GSS then adds one glycine to the C-terminus of Glu—Cys to produce GSH. GSH can be secreted to extracellular space and degraded to cysteine by transmembrane enzymes GGT and DP. Extracellular cysteine is quickly oxidized to cystine, which is re-transported into cell via SLC7A11. Alternatively, extracellular cysteine may be imported into cell by cysteine transporters. The intracellular trans-sulfuration pathway can synthesize cysteine from methionine through several enzymes as indicated. Besides contributing to GSH biosynthesis, cystine and cysteine can also indirectly act as antioxidants through generating CysSSH, H$_2$S, and taurine, all of which have significant antioxidant properties. Proteins are indicated in purple. MAT: S-adenosylmethionine synthase; AHCY: adenosylhomocysteinase; CBS: cystathionine beta-synthase; CGL/CTH: cystathionine gamma-lyase; MST: 3-mercaptopyruvate sulfurtransferase; CDO: cysteine dioxygenase; CSD: cysteinesulfinate decarboxylase; GLS: glutaminase; Glu—Cys: γ-glutamylcysteine; Cys—Gly: γ-cysteinylglycine; GCL or named GCS: γ-glutamate-cysteine ligase; GSS: glutathione synthetase; GGT: γ-glutamyltranspeptidase; DP: dipeptidase; CysSSH: cysteine persulfide.

nature of cystine starvation-induced cell death had remained elusive until recently. Studies in the past few years revealed that cystine deprivation or blockade of SLC7A11-mediated cystine uptake induces a form of regulated cell death termed ferroptosis.[2,56] Specifically, cystine starvation or SLC7A11 inactivation results in GSH depletion and then cripples the detoxification of lipid hydroperoxides catalyzed by GPX4, which is dependent on GSH, ultimately resulting in excessive lipid peroxidation and ferroptotic cell death.[56] Recently ferroptosis has emerged as a tumor suppression mechanism, and tumor suppressors such as p53 and BRCA1-associated protein 1 (BAP1) were reported to suppress tumor formation at least partly by promoting ferroptosis via suppressing *SLC7A11* expression.[103,104] Furthermore, recent studies revealed that common cancer therapies such as radiotherapy and immunotherapy induce ferroptosis and that SLC7A11 overexpression in certain cancers promotes radioresistance largely through inhibiting ferroptosis.[105—107] Other studies also reported that SLC7A11 promotes drug- or chemo-resistance in cancer cells, presumably through its role in promoting GSH biosynthesis.[108,109]

In most of these studies, the protective role of SLC7A11 under stress conditions has been attributed to its functions to import cystine and to promote GSH biosynthesis.

However, emerging evidence also suggests that SLC7A11 can exert its roles in regulating redox balance in GSH-independent manners (Fig. 3). It was shown that ectopic expression of SLC7A11 promotes cell proliferation and survival in GSH-depleted cells upon either genetic knockout of *GCLC* or pharmacological inhibition of GCLC with buthionine sulfoximine (BSO).[57] Recently it was demonstrated that cystine can be converted to cysteine persulfide (CysSSH) by cystathionine beta-synthase (CBS) and cystathionine gamma-lyase (CGL or CTH) (Fig. 3).[110] Because reactive persulfide species have higher nucleophilicity than do cysteine and GSH, it was suggested that CysSSH also exhibits strong antioxidant activity.[110] Besides its role in acting as a precursor for GSH, cysteine can also serve as a precursor of hydrogen sulfide (H$_2$S) and taurine, both of which have important antioxidant properties.[90,91,111,112] H$_2$S production from cysteine is catalyzed by aforementioned CBS and CTH, as well as 3-mercaptopyruvate sulfurtransferase (MST) (Fig. 3).[113—116] Endogenous H$_2$S is an important second messenger and can act as an antioxidant, anti-inflammatory, and neuroprotective agent.[112] In addition, cysteine dioxygenase (CDO) catalyzes the oxidation of cysteine to form cysteinesulfinate, which is subsequently oxidized to form taurine by cysteinesulfinate decarboxylase (CSD) (Fig. 3). Taurine acts as an antioxidant

through inhibiting ROS generation in mitochondria and may also indirectly prevent the loss of antioxidant enzymes from oxidative damage.[117]

Previous studies largely performed in cancer cell lines established the antioxidant and pro-survival roles of SLC7A11. However, mice with genetic ablation of *Slc7a11* are grossly healthy and fertile, indicating that SLC7A11 is dispensable for mammalian development.[118] In contrast, pharmacological or genetic inhibition of SLC7A11 substantially suppresses tumor growth *in vivo*.[119-121] It is possible that *de novo* cysteine synthesis and other transporters that take up cystine (or cysteine) can compensate for obtaining intracellular cysteine in normal cells or tissues with *SLC7A11* deficiency. However, due to the increased oxidative stress, cancer cells have more dependency on SLC7A11-mediated cystine uptake to maintain redox homeostasis and cell survival, resulting in a more essential role for SLC7A11 in tumors than in normal tissues. Consistent with this hypothesis, many cancers exhibit upregulated expression of SLC7A11.[2]

In summary, SLC7A11-mediated cystine uptake plays a critical role in antioxidant defense through promoting GSH biosynthesis and inhibiting ferroptosis, as well as other GSH-independent antioxidant functions. SLC7A11's antioxidant and pro-survival functions are particularly important in cancer cells. With this as a backdrop, below we will discuss a counterintuitive role of SLC7A11 in promoting cancer cell death under glucose limiting conditions.

SLC7A11 induces glucose-PPP dependency and promotes cell death under glucose starvation

As discussed above, it is well established that, by promoting cystine uptake and GSH synthesis, SLC7A11 protects cells from oxidative stress-induced cell death such as ferroptosis. In 2017, several groups independently identified a counterintuitive function of SLC7A11 wherein SLC7A11 promotes cell death under glucose deprivation in a wide variety of cancer cell lines.[4-6] In one study, SLC7A11 expression was identified to be upregulated under glucose starvation.[5] Considering that SLC7A11 expression is often induced by stress conditions to promote GSH biosynthesis and to protect cells from stress-induced cellular insults, it was initially postulated that glucose starvation-induced SLC7A11 expression likely represents a typical adaptive response to detoxify glucose starvation-induced ROS and to promote cell survival under glucose starvation.[5] However, subsequent experimental evidence, including SLC7A11 overexpression and its inactivation by knockdown or pharmacological inhibition, revealed that SLC7A11 promotes cancer cell death under glucose starvation.[5] In another study, an unbiased loss-of-function screening identified both SLC7A11 and SLC3A2 as top ranking genes whose inactivation suppresses cancer cell death induced by glucose starvation; conversely, it was demonstrated that SLC7A11 overexpression promotes glucose starvation-induced cell death.[4] These findings were subsequently confirmed by another study in glioblastoma cells.[6] Considering that glucose starvation induces oxidative stress and that SLC7A11 exerts a well-documented protective effect under oxidative stress, it was initially surprising and

puzzling that SLC7A11 would execute a pro-cell death, rather than a pro-cell survival, function under glucose starvation.

Since SLC7A11-mediated metabolic function is not directly linked to glucose metabolism, the metabolic underpinning of the exquisite sensitivity of cancer cells with high SLC7A11 expression (SLC7A11^high) to glucose starvation has remained elusive. Glucose has various vital metabolic functions in cells, including providing energy "currency" ATP via glycolysis, the tricarboxylic acid (TCA) cycle, and oxidative phosphorylation, contributing to redox maintenance through generating NADPH via the PPP, and donating carbon intermediates for the biosynthesis of diverse macromolecules. It was initially proposed that SLC7A11-mediated glutamate export partially depletes intracellular glutamate and glutamate-derived TCA cycle intermediates such as α-KG; consequently, combining SLC7A11 overexpression with glucose starvation would further exacerbate the depletion of TCA cycle intermediates and impairs oxidative phosphorylation in mitochondria, resulting in enhanced cell death.[4,5] (Below we will discuss the evidence from more recent studies that argues against this model.) Instead, a recent study revealed a surprising finding that SLC7A11-associated cystine metabolism promotes cancer cell dependency on the glucose-PPP route to supply NADPH in order to reduce intracellular cystine to cysteine; consequently, glucose starvation and subsequent NADPH depletion leads to toxic accumulation of intracellular cystine in SLC7A11^high cancer cells under glucose starvation, resulting in rapid cell death.[8]

In an effort to characterize metabolic alterations associated with SLC7A11 overexpression in cancer cells, global metabolomic analyses uncovered that the levels of PPP intermediates, most notably 6PG, are increased in cancer cells with SLC7A11 overexpression. Isotope tracing experiments further revealed that SLC7A11 overexpression promotes oxidative PPP flux and increases the contribution of the PPP to cytosolic NADPH generation, but does not affect the expression levels of PPP enzymes such as G6PD and PGD.[8] It is known that increased NADPH consumption (as indicated by an increased $NADP^+/NADPH$ ratio) can promote the PPP flux through feedback activation of the PPP rating-limiting enzyme G6PD.[122] Consistent with this, it was shown that SLC7A11 overexpression moderately increases $NADP^+/NADPH$ ratio under glucose replete conditions but markedly increases $NADP^+/NADPH$ ratio under glucose starvation; conversely, *SLC7A11* deletion in SLC7A11^high cancer cells largely abolishes NADPH depletion under glucose starvation.[8] Therefore, the increased PPP flux is likely caused by the increased NADPH consumption in SLC7A11-overexpressing cancer cells. As a key piece of evidence to causally link NADPH depletion to SLC7A11 overexpression-induced cell death under glucose starvation, it was further shown that treatment with 2-deoxyglucose (2DG, a glucose analog which blocks glycolysis but in fact supplies NADPH as a PPP substrate) restores NADPH levels and rescues glucose starvation-induced cell death in SLC7A11-overexpressing cancer cells.[8] As noted above, earlier studies suggested that SLC7A11-mediated glutamate export partially depletes TCA cycle intermediates, thereby rendering SLC7A11-overexpressing cancer cells to be more dependent on glucose to supply the TCA cycle through

glycolysis.[4,5] This model would predict that SLC7A11-overexpressing cells should be sensitive to glycolysis inhibitors, in much the same way that they are sensitive to glucose starvation. The observation that 2DG, which efficiently blocks glycolytic ATP production while sparing NADPH generation, in fact rescues the cell death phenotype in SLC7A11-overexpressing cancer cells seems to argue against this model.

These observations raised the question of how SLC7A11 promotes NADPH depletion in the context of glucose starvation. Since SLC7A11's transporter activity relates to importing cystine, it was further tested whether removing cystine from the medium would affect NADPH depletion and cell death in SLC7A11-overexpressing cancer cells under glucose starvation. These analyses revealed that cystine removal abolishes NADPH depletion and cell death in SLC7A11-overexpressing cells under glucose deprivation,[6–8] thereby suggesting that SLC7A11-mediated cystine transport underlies these phenotypes. At first glance, it seems difficult to understand the observation that cystine starvation blocks glucose starvation-induced cell death, because cystine in culture medium is critical for maintaining cell survival and it is expected that cystine starvation would induce ferroptotic cell death. It should be pointed out that SLC7A11^{high} cancer cells have high intracellular cysteine reserves and therefore are generally resistant to ferroptosis and that glucose starvation-induced cell death in SLC7A11^{high} cancer cells is not ferroptosis.[8] Therefore, in the context of SLC7A11^{high} cancer cells, glucose starvation exerts much more rapid cell killing effects than does cystine starvation; at early time points when cystine starvation has not induced obvious ferroptosis, it actually protects SLC7A11^{high} cancer cells from glucose starvation-induced cell death. Taken together, these data reveal that both SLC7A11 and cystine starvation have dual roles in regulating cell death and survival: SLC7A11 protects cells from ferroptosis yet promotes glucose starvation-induced cell death, whereas cystine starvation induces ferroptosis yet inhibits glucose starvation-induced cell death.

Cystine reduction to cysteine presents a significant drain on intracellular NADPH pool in SLC7A11^{high} cancer cells

These observations raised additional question of how high flux of cystine uptake would lead to NADPH depletion and cell death under glucose starvation. SLC7A11-mediated cystine uptake *per se* is not known to consume any NADPH. Once transported into cells, cystine is then converted to cysteine through a reduction reaction that consumes NADPH. Interestingly, even though SLC7A11 overexpression was confirmed to significantly increase cystine uptake, metabolite measurement revealed that intracellular cystine concentration is not significantly increased upon SLC7A11 overexpression under normal culture conditions; instead, SLC7A11 overexpression results in a dramatic increase of intracellular cysteine levels.[8] These data suggest that, once transported into cells via SLC7A11, cystine is quickly reduced to cysteine. Because cystine reduction occurs in the cytosol, this requires supply of cytosolic NADPH, which, as discussed in a previous section,

is mainly generated by the PPP. Therefore, it seems to make sense that SLC7A11 and the PPP are coupled as revealed from the metabolomic analyses. In further support of this model, it was shown that glucose starvation in SLC7A11-overexpressing cancer cells leads to a marked accumulation of intracellular cystine and other disulfide molecules such as GSSG, which is accompanied with ROS induction, GSH and NADPH depletion, and rapid cell death.[7,8]

In previous sections, we mentioned and discussed several known NADPH-consumption pathways, including reductive biosynthesis and various NADPH-utilizing antioxidant systems. However, to the best of our knowledge, cystine reduction has not been proposed in the literature as a significant NADPH consumption pathway in human cells or tissues. This further raises the question of why cystine reduction causes such a substantial drain on intracellular NADPH in SLC7A11^{high} cancer cells. We reason that this might at least partly relate to the constitutive nature of SLC7A11-mediated cystine uptake and the high insolubility of cystine. Cystine is the least soluble among all common amino acids; indeed, there are at least two inborn errors of metabolism involving defective cystine transport that result in the toxic buildup and subsequent crystallization of insoluble cystine either in the bladder (cystinuria) or intracellular lysosomes (cystinosis).[123,124] Therefore, significant buildup of intracellular cystine (as well as other disulfide molecules) likely is toxic to cells. In many metabolic pathways, downstream metabolites can inhibit upstream enzymatic reactions through negative feedback regulation, which serves to prevent aberrant buildup of downstream metabolites and to maintain appropriate homeostasis in cellular metabolism. However, as far as we know, SLC7A11's transporter activity is not controlled by intracellular cyst(e)ine levels through negative feedback regulation (such that a buildup of intracellular cyst(e)ine can prevent further cystine uptake by SLC7A11). Instead, cystine import by SLC7A11 is primarily driven by intracellular glutamate. Because intracellular glutamate is very abundant, its constant export by SLC7A11 is coupled with constitutive, active import of a large amount of extracellular cystine into SLC7A11^{high} cells. Subsequently, these cells are forced to maintain intracellular cystine at nontoxic levels by rapidly reducing it to cysteine (through which the solubility can be improved by more than 1000 fold), which causes a substantial drain of intracellular NADPH and the dependency on glucose and the PPP in SLC7A11^{high} cancer cells. In support of this model, treatments that prevent cystine accumulation, such as N-acetyl cysteine (NAC) and penicillamine (which can convert cystine back to cysteine via disulfide exchange), restores NADPH levels and rescues all redox defects and cell death in SLC7A11-overexpressing cells under glucose starvation.[8] These data therefore indicate that glucose starvation-induced cell death in SLC7A11^{high} cancer cells is likely caused by disulfide stress resulting from NADPH depletion and toxic buildup of cystine and other disulfide molecules.

Together, these studies suggest a model that, while SLC7A11-mediated cystine transport is critical for GSH synthesis and therefore beneficial for cancer cells by strengthening their antioxidant defenses and suppressing ferroptosis, this benefit does not come for free but instead

requires a substantial NADPH "investment" to maintain intracellular cystine at non-toxic levels. In our view, this cost is mainly derived from the differential redox natures between extracellular and intracellular environments (i.e., oxidizing extracellular environment vs reducing intracellular environment). Consequently, in order to obtain cysteine from extracellular environment, cells have to mainly rely on cystine uptake that is fundamentally coupled to the cost of reducing cystine to cysteine. Under normal conditions with ample glucose supply and intact PPP, SLC7A11^{high} cancer cells can meet this demand by supplying sufficient amount of NADPH through the PPP (Fig. 4, left and middle panels). However, if somehow NADPH demand cannot meet its supply (such as in SLC7A11^{high} cancer cells under glucose limiting conditions), this can lead to significant NADPH "debt" and ultimately redox "bankruptcy" and cell demise (Fig. 4, right panel).

Conclusions and future perspectives

During tumor development, in order to adapt to a harsh microenvironment with increased oxidative stress, cancer cells reprogram their metabolic networks to enhance antioxidant systems,[125] prominent among which is the upregulation of SLC7A11 expression to increase cystine uptake and GSH biosynthesis. While SLC7A11^{high} cancer cells are equipped with stronger antioxidant systems and can survive and grow better under oxidative stress conditions, they also have to endure the significant cost that is associated with increased cystine uptake. Significant amount of NADPH as reducing power has to be derived from glucose through the PPP to prevent toxic buildup of intracellular cystine, leading to glucose dependency in SLC7A11^{high} cancer cells. Therefore, SLC7A11 appears to act as a double-edged sword in regulating cellular redox balance. These recent findings

raise additional intriguing questions that merit further studies in the future. Below we will further discuss these questions from the perspective of both fundamental redox biology and translational implication.

Currently, the reductase(s) that mediates the reduction of cystine to cysteine remains to be further studied. GR and cytosolic TrxR1 are the presumed cystine reductases. In addition, thioredoxin-related protein of 14 kDa (TRP14) was also identified to be able to catalyze cystine reduction at the biochemical level.[126] However, the definitive biological evidence that any of these proteins acts as a cystine reductase is still lacking (i.e., to show that genetic ablation of any of these genes results in accumulation of intracellular cystine). Considering that some of these enzymes (such as TrxR1) are essential in many cell types and that these enzymes might share redundant functions in cystine reduction, it might be challenging to address their exact role as potential cystine reductase.

Exactly how disulfide stress caused by aberrant cystine accumulation and NADPH depletion induces cell death remains elusive. It is known that disulfide stress leads to inter- or intra-molecular disulfide bond formation in proteins, which is capable of regulating protein functions such as enzyme activity, protein—protein interaction, protein stability or subcellular localization.[127—129] Interestingly, a recent study showed that glucose starvation in SLC7A11^{high} cancer cells induces the phosphorylation of BMI1, a polycomb repressive complex 1 (PRC1) component, and histone 2A (H2A) deubiquitination, resulting in transcriptional alteration of genes involved in ER stress response and subsequent cell death.[46] Notably, NAC or 2DG treatment rescues BMI1 phosphorylation and H2A deubiquitination, much like its rescuing effect on cystine accumulation, in SLC7A11^{high} cancer cells under glucose starvation,[46] suggesting a possibility that glucose starvation might induce BMI1 phosphorylation through disulfide stress-mediated
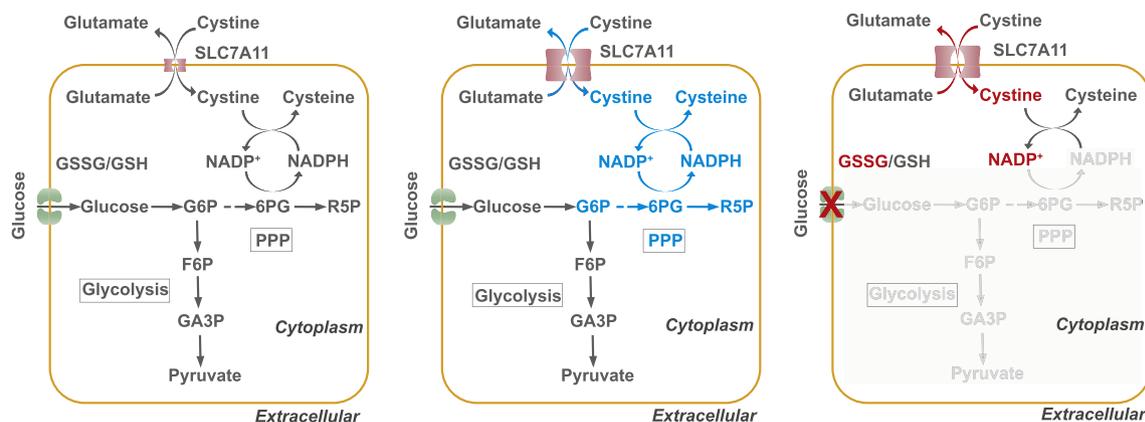
Figure 4 SLC7A11 regulates glucose dependency through promoting NADPH consumption. Compared to normal cells with relatively low expression of SLC7A11 (left panel), cancer cells with high expression of SLC7A11 import considerate amount of cystine. To maintain cystine at non-toxic levels, cystine is quickly reduced to cysteine through a NDPH-consuming reduction reaction, therefore promoting the PPP flux from glucose to supply more NADPH (middle panel). When NADPH production is limited from the PPP by glucose starvation (or GLUT inhibition) in the context of high SLC7A11 expression, abundant intracellular cystine imported via SLC7A11 would drain cellular NADPH, which leads to a massive accumulation of cystine and other disulfide molecules (such as GSSG), resulting in a series of redox defect and rapid cell death (right panel). F6P, fructose-6-phosphate; GA3P, glyceraldehyde-3-phosphate.

modification of an upstream kinase or phosphatase. These data suggest a hypothesis that in SLC7A11^{high} cancer cells under glucose starvation, disulfide stress can initiate downstream cellular signaling through modifying disulfide bond formation in key signaling proteins. Future studies will be directed to test this intriguing hypothesis.

It is known that other stress conditions, such as oxidative stress, nutrient deprivation, genotoxic stress, and radiation, can induce SLC7A11 expression,[130] and the induction of some of these stresses (such as oxidative stress) is also accompanied with NADPH depletion. While SLC7A11 generally plays a protective role under stress conditions, whether and how the NADPH dependency imposed by SLC7A11-mediated cystine uptake can affect cellular adaptation to these other stress conditions remain to be further studied.

Finally, the exquisite sensitivity of SLC7A11^{high} cancer cells to glucose starvation also reveals a metabolic liability for therapeutic targeting in SLC7A11[high] tumors, and suggests that SLC7A11^{high} cancer cells or tumors may be sensitive to pharmacologic inhibition of GLUT-mediated glucose uptake, the first step in glucose metabolism. Of the four main GLUTs (GLUT1−4), GLUT1 is the main GLUT member that is widely overexpressed in a number of cancers.[131] Considerable interest therefore has been directed toward developing GLUT1 inhibitors in cancer therapy.[132] In addition, whereas GLUT3 mRNA is ubiquitously expressed in human tissues, its protein is primarily detected in the brain and testis.[133] Similar to GLUT1, GLUT3 is also overexpressed in various cancers, thereby justifying the development of GLUT1 and GLUT3 dual inhibitors in cancer treatment. Indeed, it was recently shown that the GLUT1 inhibitor BAY-876 or GLUT1/3 dual inhibitor KL-11743 exerts much more potent cell killing effect in SLC7A11^{high} cancer cells than in SLC7A11^{low} cancer cells. Further preclinical studies using cell line- and patient-derived xenografts showed that KL-11743 selectively suppresses SLC7A11[high] tumor growth.[8] Aberrant expression of SLC7A11 is often caused by specific mutations in cancer. For example, BAP1 mutation leads to de-repression of SLC7A11 in renal and other cancers.[103,134,135] In addition, KEAP1 mutation stabilizes NRF2, leading to aberrant expression of NRF2 transcriptional targets including SLC7A11 in lung cancer.[136,137] Therefore, BAP1 or KEAP1 mutations might be used as potential biomarkers to select SLC7A11[high] cancers for GLUT inhibition. These preclinical studies suggest that SLC7A11[high] tumors may also be sensitive to PPP inhibitors, which await further investigation in future studies.

Conflict of Interests

K.O. is a full-time employee of Kadmon Corporation, LLC. No potential conflict of interest was reported by other authors.

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