EDITORIAL

Reductive cell death: the other side of the coin

The balance between oxidants and antioxidants within cells, known as redox homeostasis, is crucial for proper cellular function. Disruption of redox homeostasis has been implicated in the development and treatment of various human diseases, including cancer. Regulated cell death, a fundamental biological process, occurs in various forms and functions, often caused by oxidative stress damaging macromolecules (such as DNA, lipids, and proteins). Surprisingly, recent studies have suggested that aberrant reductive stress can also trigger regulated cell death (such as disulfidptosis and pyroptosis) in cancer and immune cells, which we collectively refer to as reductive cell death. Disulfidptosis is caused by disulfide stress-induced actin cytoskeleton dysfunction, while pyroptosis is triggered by inflammasome activation. Further exploring the relationship between oxidative and reductive cell death could lead to new treatments for diseases.

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Mammalian cells utilize redox reactions to produce energy (adenosine triphosphate [ATP]) and construct essential components (nucleic acids) from nutrients. Electrons transfer from reducing agents to oxidizing agents in these reactions. The main redox couples in cells are nicotinamide adenine dinucleotide (NAD^+)/reduced NAD^+ (NADH), phosphorylated NAD^+ ($NADP^+$)/reduced $NADP^+$ (NADPH), reduced glutathione (GSH)/GSH disulfide (GSSG), and reduced thioredoxin (TrxSH2)/oxidized thioredoxin (TrxSS) which perform most of the electron transfer and neutralize reactive oxygen species (ROS) to maintain a reducing environment [1]. Impaired redox homeostasis is a key factor in cancer development, as disruptions can cause oxidative stress, damaging cellular components, and even leading to cell death [2–4]. Tumor therapy often involves inducing oxidative cell death, but the role of reductive stress in cell death has been unclear [5]. However, recent studies have shown that impaired or excessive reductive stress can trigger regulated cell death in both cancer and immune cells, providing a novel avenue for targeted therapy [6–9]. This is highlighted in the following description.

Solute carrier family 7 member 11 (SLC7A11) is a functional light chain subunit of system xc^-, known for its role in cystine uptake [10]. This is used to produce cellular cysteines and subsequent GSH, which serve as antioxidants to counteract cellular oxidative stress and prevent ferroptosis [11, 12]. Previous studies have shown that SLC7A11-mediated cystine transport leads to cell death in cancer cells under glucose starvation [13, 14], but the mechanism remains unknown. The laboratory of Boyi Gan recently made a breakthrough discovery - they found that the accumulation of intracellular disulfides in cancer cells with high expression of SLC7A11 ($SLC7A11^{high}$) during glucose starvation leads to a novel form of cell death called disulfidptosis [6]. This process is the result of abnormal disulfide bonds in actin cytoskeleton proteins [6] (Fig. 1A).

The authors conducted an initial investigation into the impact of inhibitors or the removal of key regulators of various types of cell death on the cell death induced by glucose starvation in $SLC7A11^{high}$ cancer cells (UMRC6, H460, and A549) and in 786-O cells that overexpress SLC7A11. They found that the cell death was not caused by ferroptosis, apoptosis, necroptosis, or autophagy. This form of cell death was not caused by either ATP depletion or the formation of cystine crystals. However, agents that reduce disulfide stress prevented cell death induced by glucose starvation in $SLC7A11^{high}$ cells, while thiol oxidants that increase disulfide stress led to cell death [14]. The researchers further found that NADPH depletion during glucose starvation in $SLC7A11^{high}$ cells resulted in increased disulfide stress and the formation of disulfide bonds in the sulfhydryl groups of redox-sensitive proteins with reactive cysteine residues. This caused decreased cell viability, as NADPH plays a crucial role in preventing disulfide stress.

The researchers then performed a bio-orthogonal chemical proteomic analysis using Stable Isotope Labeling with Amino Acids in Cell Culture (SILAC) to investigate the impact of glucose deprivation on disulfide bonds in actin cytoskeleton proteins. The results revealed that this led to F-actin contraction and separation from the plasma membrane, resulting in cell death. Surprisingly, the use of ROS scavengers was ineffective in preventing this process, indicating that disulfidptosis is not dependent on ROS. Subsequently, the team used whole-genome CRISPR-Cas9 screening and found that Nck-associated protein 1 (NCKAP1), a component of the WAVE regulatory complex, positively regulates disulfidptosis in $SLC7A11^{high}$ cancer cells under conditions of glucose deprivation.

Finally, the authors revealed that glucose transporter solute carrier family 2 member 1 (SLC2A1, also known as GLUT) inhibitors (BAY-876 or KL-11743) increased $NADP^+$/NADPH ratio and induced disulfide bonding in actin cytoskeleton proteins through glucose uptake inhibition, leading to F-actin network collapse and disulfidptosis in UMRC6 cells. Animal studies showed that BAY-876 treatment resulted in more frequent cell death and disulfide bonding in actin compared to control group. BAY-876 was tested in two lung cancer patient-derived xenograft models with high or low expression of SLC7A11 and found to suppress growth and induce disulfide bonding in $SLC7A11^{high}$ tumors without significant side effects. These results highlight the role of glucose starvation and cystine uptake in disulfidptosis and suggest SLC2A1 inhibitors as a therapeutic target for $SLC7A11^{high}$ tumors.

Protein disulfide bonds are formed by thiol-disulfide exchange reactions between free thiols and substances that have formed disulfide bonds, wherein the free thiol form is in the reduced state and the disulfide form is in the oxidized state [15, 16]. The source

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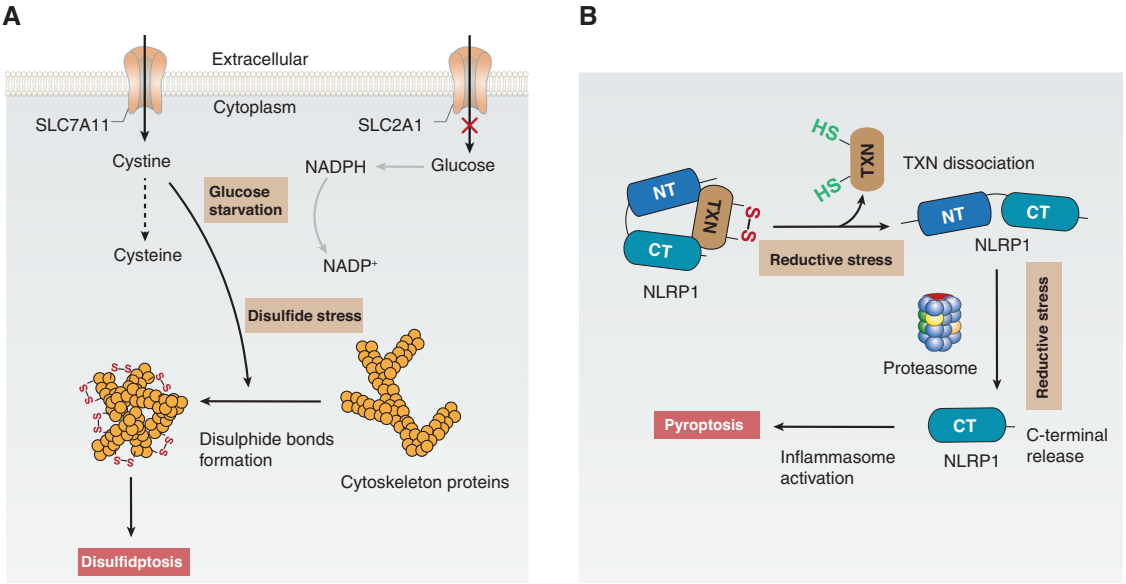

Fig. 1 Signaling and mechanism of reductive cell death. A Glucose starvation leads to NADPH depletion and the accumulation of intracellular disulfide and cystine, thereby increasing disulfide stress in SLC7A11-overexpressing cancer cells. Disulfide stress further induces excessive disulfide bonds formation in actin cytoskeletal proteins, resulting in disulfidptosis. **B** On the one hand, reductive stress eliminates the oxidized form of TXN in the NLRP1 protein complex and removes TXN; on the other hand, reductive stress accelerates proteasome-mediated degradation of the repressive NT fragments of NLRP1 and releases the inflammasome-forming CT fragments from autoinhibition, thereby promoting inflammasome activation and subsequent pyroptosis in immune cells.

of free reduced thiols is sulfhydryl-containing compounds like cysteine and the antioxidant GSH, generated from cystine [17, 18]. Overall, the study conducted by the laboratory of Boyi Gan concluded that an excess of cystine results in abnormal disulfide bonds in cytoskeletal proteins, leading to disulfidptosis [6]. The next step is to investigate whether endoplasmic reticulum (ER) stress is involved in the formation of these disulfide bonds, as they occur in the ER [19]. Despite glucose being the primary source of NADPH production through the pentose phosphate pathway, some solid tumors exhibit glucose deficiency and therefore alternative metabolic pathways may need to be utilized to preserve disulfidptosis sensitivity in certain cancer cells [20].

Studies conducted by the laboratory of Daniel Bachovchin have linked increased reductive stress to cell death in three separate investigations [7–9]. The research showed that excessive reductive stress in immune cells activated the NLR family pyrin domain containing 1 (NLRP1) inflammasome, leading to pyroptosis as a result of proteasome-mediated degradation of the repressive N-terminal (NT) fragments of NLRP1 and caspase recruitment domain family member 8 (CARD8) (Fig. 1B). Both NLRP1 and CARD8 undergo autoproteolysis in their function-to-find (FIIND) domain, creating NT autoinhibitory and C-terminal (CT) inflammatory fragments [21, 22]. Dipeptidyl peptidase 8 (DPP8) and dipeptidyl peptidase 9 (DPP9) complex suppresses inflammasome activation by binding to the C-terminus of NLRP1. However, inhibitors like valboroPro (VbP) can accelerate proteasome-mediated degradation of NT fragments, freeing CT fragments, and inducing pyroptosis [23, 24].

The first study found that the oxidized form of the oxidoreductase thioredoxin (TXN, also known as TRX1) binds to the NACHT-leucine-rich repeat (LRR) domains of NLRP1 and suppresses inflammasome activation [7]. However, reductive stress, which removes the oxidized form of TXN, leads to NLRP1 inflammasome activation and pyroptosis. The researchers identified mutations in Cys^{32} and Cys^{35} that impacted the reaction with disulfide bonds in oxidized TXN protein. The interaction between NLRP1 and TXN was found to be weakened by reducing agents dithiothreitol (DTT) and reduced GSH, but strengthened by the oxidizing agent hydrogen peroxide. Additionally, they found that binding of oxidized TXN stabilized the self-inhibitory formation of NLRP1 and ATP hydrolysis was necessary to form the inactive complex. No antioxidants were found to decrease the TXN redox state, but the data suggests that reductive stress may enhance NLRP1 activation and pyroptosis.

The second study found that reductive stress induced by free radical-scavenging antioxidants like JSH-23 accelerated the proteasome-mediated degradation of the repressive NT fragments of both NLRP1 and CARD8 in immune cells, releasing the inflammasome-forming CT fragments from autoinhibition [8]. JSH-23 induced the degradation of NLRP1 and CARD8 in DPP8/9-deficient cells, which was blocked by the proteasome inhibitor bortezomib. However, JSH-23 did not weaken the repressive DPP8/9 complex and the released CT fragments were kept in check by DPP8/9. JSH-23 triggered a danger signal that quickened the degradation of NT fragments, but the DPP8/9 complex prevented the free CT fragments from causing pyroptosis. Radical-trapping antioxidants like ferrostatin-1, which inhibit RSL3-induced ferroptosis, were found to enhance macrophages' sensitivity to pyroptosis, suggesting that blocking ferroptosis may raise pyroptosis sensitivity.

The third study demonstrated that DPP9-binding ligands are required to disrupt the complex and allow oligomerization of CT fragments into inflammasomes in immune cells, leading to NLRP1- and CARD8-dependent pyroptosis [9]. The researchers found that aminopeptidase inhibitors, such as bestatin methyl esters, induced peptide accumulation and protein folding stress to accelerate NT degradation, and induced the accumulation of XP-containing peptides, which may disrupt the inhibitory DPP8/9 complexes in monocytes and macrophages.

Of interest, glucose deprivation can cause an increase of intracellular cysteine, leading to stress on proteins and the formation of abnormal disulfide bonds [6]. This also decreases the levels of intracellular ATP and activates the NLRP1 inflammasome [25]. The disruptions caused by glucose deprivation can result in significant reductive stress, triggering inflammasome activation or protein stress. This can then cause dysfunctions in

cytoskeleton proteins or further activation of the inflammasome, ultimately leading to cell death. The inflammasome plays a vital role in innate immunity, inflammation, and cell death, and has significant implications in cancer development and control [26]. It would be fascinating to study whether the NLRP1 inflammasome regulates disulfidptosis in cancer cells. Antioxidants work by eliminating free radicals, binding metal ions, breaking down hydrogen peroxide or hydroperoxides, neutralizing co-oxidants, and repairing cellular damage. Further research is necessary to fully understand the role of antioxidants in cell death and the effect of reductive stress on cell metabolism. Additionally, the connection between reductive cell death and tumor immunity requires further investigation. Developing ways to counteract the negative impact of reductive stress is crucial.

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ADDITIONAL INFORMATION

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