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Disulfidptosis: a novel cell death modality induced by actin cytoskeleton collapse and a promising target for cancer therapeutics

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Abstract

Disulfidptosis is a novel discovered form of programmed cell death (PCD) that diverges from apoptosis, necroptosis, ferroptosis, and cuproptosis, stemming from disulfide stress-induced cytoskeletal collapse. In cancer cells exhibiting heightened expression of the solute carrier family 7 member 11 (SLC7A11), excessive cystine importation and reduction will deplete nicotinamide adenine dinucleotide phosphate (NADPH) under glucose deprivation, followed by an increase in intracellular disulfide stress and aberrant disulfide bond formation within actin networks, ultimately culminating in cytoskeletal collapse and disulfidptosis. Disulfidptosis involves crucial physiological processes in eukaryotic cells, such as cystine and glucose uptake, NADPH metabolism, and actin dynamics. The Rac1-WRC pathway-mediated actin polymerization is also implicated in this cell death due to its contribution to disulfide bond formation. However, the precise mechanisms underlying disulfidptosis and its role in tumors are not well understood. This is probably due to the multifaceted functionalities of SLC7A11 within cells and the complexities of the downstream pathways driving disulfidptosis. This review describes the critical roles of SLC7A11 in cells and summarizes recent research advancements in the potential pathways of disulfidptosis. Moreover, the less-studied aspects of this newly discovered cell death process are highlighted to stimulate further investigations in this field.

Keywords Programmed cell death, Disulfidptosis, Disulfide stress, SLC7A11, Rac1, WAVE regulatory complex, Arp2/3 complex, Actin dynamics, Cystine, Cancer treatment

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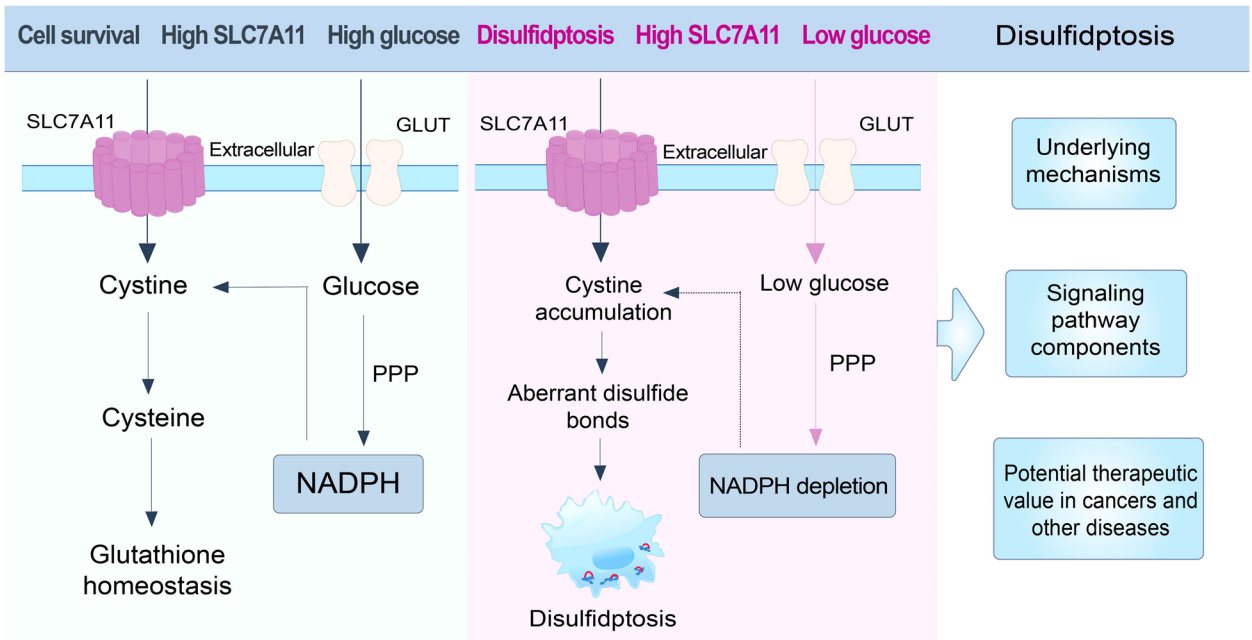
Graphical Abstract



Introduction

Rapid proliferation or metabolic abnormalities can result in excessive production of reactive oxygen species (ROS) within the organism, posing a threat to cell survival. To mitigate this damage, cells produce protective substances, such as glutathione (GSH), to scavenge excess ROS [1]. The Solute Carrier Family 7 Member 11 (SLC7A11), a crucial antiporter on the cell membrane, has been implicated in the transport of cystine into the cell in exchange for glutamate at a ratio of 1:1 [2]. Cystine can be reduced by nicotinamide adenine dinucleotide phosphate (NADPH) to cysteine, which serves as the raw material for synthesizing GSH, thus highlighting the critical role of SLC7A11 in the antioxidant defense system [3]. Studies have demonstrated that SLC7A11 expression is elevated in various tumors compared to normal tissues and maintains cellular redox balance and metabolic homeostasis [4, 5]. It is well established that the suppression of SLC7A11 leads to a decrease in GSH production, compromising the ability of glutathione peroxidase 4 (GPX4) to counteract iron-dependent ROS generation, ultimately resulting in ferroptosis [6]. Inhibition of SLC7A11 has also been repeatedly shown to induce apoptosis, necroptosis, and autophagy-dependent cell death [7–9]. Based on these facts, SLC7A11 helps tumors thrive in harsh environments, thereby promoting cell survival.

Recently, a novel form of programmed cell death (PCD) was found in SLC7A11-high cancer cells under glucose deprivation, characterized by an excessive accumulation of disulfide termed disulfidptosis. Gan et al. found that overexpression of SLC7A11 enhanced the glucose dependence of cancer cells and rendered them more susceptible to cell death induced by glucose starvation [10]. Glucose deprivation triggered a significant accumulation of intracellular cystine, disrupting the redox system and leading to rapid cell death in SLC7A11-high cancer cells, a process that can be rescued by preventing the accumulation of disulfides [11]. Subsequently, Liu et al. applied genetic or pharmacological inhibition of ferroptosis, apoptosis, necroptosis, and autophagic cell death on tumor cell lines [12]. Surprisingly, the treatment could prevent previously known cell death pathways but still failed to rescue glucose deprivation-triggered cell death [12]. Therefore, this mode of PCD differs from the known cell death pathways in that it is caused by an excessive accumulation of disulfides in SLC7A11-high cells under glucose starvation, hence the term disulfidptosis. Unrestrained intracellular cystine reduction will deplete NADPH generated from glucose, consequently inciting an increase in intracellular disulfide stress and aberrant disulfide bond formation in actin networks, ultimately culminating in cytoskeleton collapse and disulfidptosis (Fig. 1) [12]. Rac1- WAVE regulatory complex

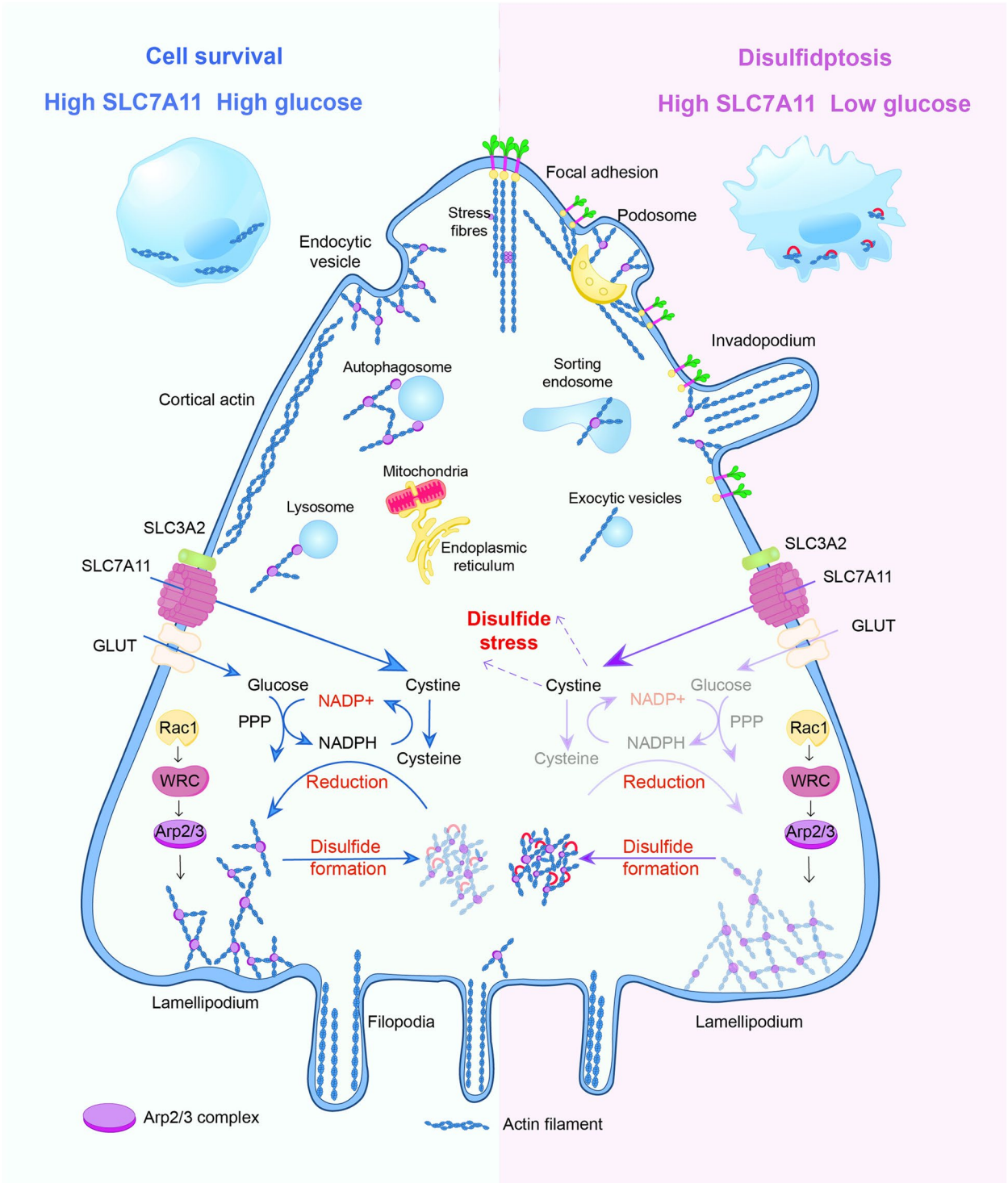


Fig. 1 Schematic of disulfidptosis mechanism. The upregulation of SLC7A11 facilitates the transportation of cystine into cells, which is essential for the maintenance of redox homeostasis. Sufficient glucose availability facilitates the production of NADPH through PPP, which enables the conversion of cystine to cysteine and inhibits the formation of disulfide bonds in cytoskeletal proteins (left side of the figure). Insufficient glucose availability hinders the generation of NADPH, leading to the accumulation of cystine and the abnormal formation of disulfide bonds between cytoskeletal proteins (right side of the figure). This process ultimately leads to disulfide bond-mediated cell death (i.e., disulfidptosis). Rac1-WRC pathway facilitates branched actin polymerization in lamellipodia, which may play a role in disulfidptosis by promoting the formation of disulfide bonds in a favorable cellular environment. In addition to lamellipodia, there are many cellular structures containing filament actin. The inquiry of whether disulfide stress has the potential to induce widespread disulfide bonding in other actin-based structures remains unresolved

(WRC)-mediated branched actin polymerization facilitates disulfidptosis, highlighting the critical role of Rac1 and WRC in this process. Nonetheless, knocking out WRC components only leads to partial resistance to this PCD, suggesting the involvement of additional actin formation pathways or actin-based structures in disulfidptosis. This review delves into the essential physiological roles of SLC7A11 and explores potential downstream effector molecules in disulfidptosis. Elucidating the precise mechanisms governing disulfidptosis and the components of its signaling pathways holds immense promise for broadening our understanding and therapeutic capabilities in a multitude of diseases, encompassing tumors, neurodegenerative disorders, and ischemia–reperfusion injuries.[13].

SLC7A11 regulates the cell fate by modulating PCD, redox homeostasis, and metabolic flexibility

Cysteine plays pivotal roles throughout the cell in redox maintenance, protein synthesis, catalysis, and trafficking [14]. To meet the increasing demand for antioxidant defense, most tumor cells enhance their uptake of cystine or cysteine from the extracellular environment via transporters [15]. Owing to the highly oxidative extracellular environment, cysteine readily oxidizes into its dimeric form, cystine [11]. A majority of cancer cells rely heavily on system Xc– to import cystine. Cystine is intracellularly reduced to cysteine, consuming NADPH in the process. System Xc– is composed of a heavy chain subunit, Solute Carrier Family 7 Member 11 (SLC3A2), and a light chain subunit, SLC7A11 (xCT). SLC7A11 is recognized as the functional unit, while SLC3A2 primarily facilitates the trafficking of the light chain and is not involved in amino acid transport [16]. Numerous studies have demonstrated that SLC7A11 contributes to various PCD, metabolic flexibility, and redox homeostasis in tumor cells [17].

SLC7A11's function in regulating cell death

Most recently, it was demonstrated that glucose deprivation can induce a novel form of PCD in SLC7A11-high cancer cells, characterized by actin cytoskeleton collapse induced by disulfide stress termed disulfidptosis (Fig. 1) [12]. The study observed a significant increase in disulfide bond formation in actin cytoskeleton proteins due to disulfide stress and NADPH depletion in glucose-starved SLC7A11-high renal cancer cells. NADPH depletion was the initial event under glucose starvation conditions, followed by excessive intermolecular disulfide bond formation and eventual cell death. Furthermore, the deletion of SLC7A11 or deprivation of cystine inhibited glucose starvation-induced disulfidptosis, suggesting that SLC7A11-mediated cystine uptake conditions disulfide bond formation in this form of PCD [12]. It was reported that 2-deoxyglucose (2DG) could produce NADPH via PPP, preventing the migration retardation of actin cytoskeleton proteins and rescuing SLC7A11-high cells from disulfidptosis under glucose deprivation [11, 12]. Additionally, β-mercaptoethanol (2ME), a disulfide-reducing agent, also delayed cell death by preventing disulfide bond formation. However, antioxidants designed to scavenge ROS failed to rescue cells from disulfidptosis [12]. Phalloidin staining indicated that glucose starvation induced actin filament (F-actin) contraction and detachment from the cell membrane, accompanied by cell shrinkage. Significantly, these morphological alterations occurred prior to massive cell death, and cystine deprivation, 2ME or 2DG treatment prevented these irregular changes from occurring, indicating that aberrant disulfur bond formation in the actin cytoskeleton triggers the disruption of actin networks during disulfidptosis. To delve deeper into the regulatory mechanism of disulfidptosis, Liu et al. performed a genome-wide CRISPR-Cas9 screen on cells overexpressing SLC7A11. They identified several key genes, including NCKAP1, which encodes Nck-associated protein 1 (NCKAP1), a subunit of the WRC. The WRC regulates actin dynamics and exists in the inactive state due to intrinsic inhibition in its basal state [18]. Ras-related C3 botulinum toxin substrate 1 (Rac1) is capable of binding to WRC, inducing its conformational changes that facilitate actin-related protein 2/3 (Arp2/3)-induced actin nucleation and lamellipodia formation [19]. Knockdown of NCKAP1 partially attenuated disulfidptosis in SLC7A11-high cells, while overexpression of NCKAP1 and RAC1 exacerbated cell death [12]. These results indicate that the Rac1-WRC-mediated actin polymerization and lamellipodia formation promote disulfidptosis, further underscoring that disulfidptosis differs from previously characterized forms of PCD. Finally, it was observed that glucose transporter inhibitors mirrored the effects of glucose starvation, causing extensive disulfide bond formation, F-actin collapse, and disulfide-induced cell death in SLC7A11-high tumor cells and mice models [12]. In summary, disulfidptosis represents a novel form of PCD, potentially offering a new therapeutic target for tumor treatment. Further studies are warranted to elucidate its detailed mechanism and interactions with other PCD pathways.

SLC7A11 also plays a vital role in other types of PCD, such as ferroptosis, autophagy, and apoptosis [8, 20–22]. Ferroptosis is an iron-dependent type of PCD marked by excessive accumulation of iron and lipid peroxidation in cells [23]. GPX4 reduces intracellular lipid peroxides, and its inactivation triggers lipid peroxidation in cell membranes, inducing ferroptosis [23, 24]. SLC7A11 regulates the synthesis of GSH by transporting cystine into

cells, and GSH serves as a crucial cofactor of GPX that facilitates the reduction of lipid peroxides [25]. Therefore, SLC7A11 protects cells from ferroptosis by facilitating GPX4 activation via cystine import and enhancing GSH synthesis [23]. On the contrary, targeted inhibition of SLC7A11 with erastin can diminish GSH synthesis, leading to GPX4 inactivation, thereby inducing ferroptosis [26]. Recent studies have identified Coenzyme A (CoA) as a regulator of ferroptosis, with SLC7A11 supplying cystine/cysteine for CoA synthesis, thereby safeguarding against this cellular demise [27]. Additionally, inhibiting SLC7A11 has been found to elevate intracellular glutamate levels, triggering ferroptosis via an intracellular metabolic pathway independent of cysteine [28, 29]. Notably, in the context of p53-mediated tumor suppression, transcriptional repression of SLC7A11 can directly activate the Arachidonate 12-lipoxygenase (ALOX12)-mediated lipid peroxidation directly and lead to ferroptosis [30]. Consequently, SLC7A11 modulates ferroptosis through diverse mechanisms, encompassing both cysteine-dependent and cysteine-independent pathways.

A recent study has unveiled another SLC7A11 inhibitor, HG106, which displayed selective cytotoxicity against KRAS-mutant lung adenocarcinoma by inducing apoptosis instead of ferroptosis [31]. Moreover, other studies have indicated that SLC7A11 possesses an anti-apoptotic function, suggesting that its inhibition can activate the apoptosis pathway in certain cell contexts [32–34]. It was reported that disruption of SLC7A11 led to the aggregation of punctate signals of the autophagy indicator, light chain 3 isoform B, in hepatocellular carcinoma cells, which was recovered upon treatment with the autophagy inhibitor 3-Methyladenine, suggesting a role for SLC7A11 in autophagy [20].

SLC7A11's function in regulating metabolic flexibility and nutrient dependency

To maintain redox homeostasis, the continuous overexpression of SLC7A11 facilitates the transport of cystine into the cancer cells, concomitant with the export of a large quantity of glutamate out of the cell, resulting in a partial depletion of intracellular glutamate [17, 35]. Glutamate plays critical roles in cell metabolism including redox homeostasis (participating in the synthesis of GSH) and energy formation (as the major source of tricarboxylic acid (TCA) cycle intermediate α-ketoglutarate(αKG)) [36]. Glutamine, the most abundant amino acid in the cytosol, can be converted to glutamate via glutaminase. The depletion of glutamate mediated by SLC7A11 overexpression enhances glutamine catabolism, driving SLC7A11-high cells to absorb more glutamine and resulting in their dependency on it [37]. Therefore, glutaminase inhibition or glutamine deprivation can suppress the proliferation of SLC7A11-high cancer cells [22, 38].

Glucose serves as the primary nutrient fueling most cellular metabolic processes and supports cell survival. Intracellular glucose is predominantly metabolized through two main pathways: the pentose phosphate pathway (PPP) and glycolysis [39]. The PPP plays a crucial role in generating NADPH, which provides reducing power to regulate cellular redox homeostasis and sustain reductive biosynthetic processes, while glycolysis produces pyruvate for the TCA cycle to generate ATP [40]. Recently, it has been reported that overexpression of SLC7A11 renders cancer cells highly dependent on glucose and promotes rapid cell death under glucose starvation conditions [10, 11, 41, 42]. Initially, the hypothesis was that the exportation of large amounts of glutamate out of SLC7A11-high cells was the mechanism leading to glucose dependency. Since both glutamate and glucose metabolites are involved in the TCA cycle (glucose is converted into pyruvate through glycolysis, and glutamate is the major source of αKG), the overexpression of SLC7A11 transports large amounts of glutamate out of the cells, making the cells more reliant on glucose to generate pyruvate for the TCA cycle [10, 41]. However, further research has shown that SLC7A11-high cells do not increase their glycolytic flux and are not sensitive to glycolysis inhibitors [10, 41]. Recent studies have suggested that it is cystine import rather than glutamate export that leads to glucose dependency and underlies glucose deprivation-induced cell death in SLC7A11-high cancer cells [11, 43]. Excessive cystine uptake through the overexpression of SLC7A11 needs to be reduced to cysteine, which depletes intracellular NADPH in the presence of glucose starvation, induces massive ROS generation, and promotes cell death [42]. Recently, Liu et al. attributed the glucose deprivation-promoted death of SLC7A11-high cells to cytoskeletal collapse due to highly increased cytosol cystine levels and extensive disulfide bonding of the actin cytoskeleton after NADPH depletion [12].

SLC7A11's function in maintaining redox homeostasis

ROS contribute to various physiological events and intracellular signaling pathways [44]. However, excessive ROS production can induce oxidative stress, leading to cellular dysfunction, and pathological changes, thereby initiating and exacerbating diseases such as inflammation, cancer, and neurodegenerative disorders [45]. This imbalance between antioxidant defense mechanisms and ROS production is often the underlying cause of diverse diseases [46]. GSH, a tripeptide comprising glycine, L-cysteine, and L-glutamate, acts as a major intracellular antioxidant, which protects cells from oxidative damage induced by ROS [47]. SLC7A11 enhances antioxidant activity

by translocating cystine into the cell, thereby providing cysteine, the rate-limiting precursor for the synthesis of GSH, through a NADPH-consuming reduction reaction [48]. Due to their unrestrained proliferation, cancer cells suffer significantly heightened oxidative stress due to elevated ROS levels compared to non-malignant cells. Therefore, various human cancer cells upregulate SLC7A11 expression to maintain sufficient levels of GSH to neutralize excessive ROS [49]. In addition to the synthesis of GSH, certain intracellular cysteine is exported out of the cell through amino acid transporters. Once outside the cell, cysteine undergoes oxidation to form cystine, thereby contributing to the establishment of a reducing microenvironment. Whereas cystine was then imported back into the cell through SLC7A11 and subsequently reduced to cysteine, thus establishing a cysteine/cystine cycle across the cell membrane [10, 48]. Banjac et al. demonstrated that elevated expression of SCL7A11 in B cells could result in high extracellular cysteine concentrations, thus creating a reducing extracellular microenvironment [50]. This environment played a more critical role in supporting tumor cell survival and proliferation than increasing intracellular GSH levels.

Intriguingly, other studies have found that in the presence of glucose deprivation, SLC7A11 overexpression promotes cell death by depleting NADPH and increasing ROS generation [11, 43, 51]. Liu et al. attributed the glucose deprivation-promoted death of SLC7A11-high cells to cytoskeletal collapse caused by excessively elevated cystine concentrations and extensive disulfide bonding of the actin cytoskeleton. They identified this cell death pathway as a novel form of PCD termed disulfidptosis [12]. These findings suggest that SLC7A11 may play contradictory roles in redox regulation and cell survival in different cell contexts.

The Rac1-WRC pathway is a crucial mechanism of disulfidptosis

Studies have shown that the Rac-WRC pathway participates in branched actin assembly [12, 13]. Moreover, Rac1–WRC-mediated branched actin network formation in lamellipodia was reported to promote disulfidptosis by enhancing disulfide bonding in SLC7A11-overexpressing cells[12]. Several stimuli, including ROS, inflammatory cytokines, shear stress, and mechanical stretch, can activate Rac1 to stimulate various downstream signaling [52, 53]. Rac1 binds to and activates WRC by releasing its WASP homology 2 (WH2)-Central-Acidic (WCA) motif, which drives Arp2/3 complex-mediated actin assembly in lamellipodia [54]. Functioning at the end of disulfidptosis, the Rac1-WRC pathway is recognized as an important modulator of this novel cell death.

Rac1 serves as a critical molecular switch in disulfidptosis

Rac1 is a core member of the Rho proteins, belonging to the Ras superfamily of small guanosine triphosphate phosphohydrolases (GTPases) [55]. This guanosine triphosphate (GTP)–binding protein is best known for its critical role in the regulation of cell adhesion, migration, and invasion owing to its role in cytoskeleton remodeling. Besides, Rac1 is implicated in various crucial cellular processes, including ROS production, inflammatory responses, vesicle transport, angiogenesis, cell proliferation, and apoptosis [56–58]. Recently, Liu et al. demonstrated that Rac1-WRC-mediated branched actin polymerization facilitated disulfidptosis, underscoring the pivotal role of Rac1 in this process [12]. Rac1 is extensively overexpressed in tumors, and its dysregulation contributes to a series of pathological conditions, including neurodegenerative disorders, cardiovascular diseases, kidney disorders, and infectious diseases [59–64]. Therefore, Rac1 is considered a promising target for the development of therapeutic interventions targeting cancer and other diseases.

Since its biosynthesis, Rac1 undergoes a series of modifications by particular signals meant to anchor it to the cell membrane and activate downstream effectors (Fig. 2)[65]. The initial step in Rac1 protein translocation involves the prenylation of the carboxy-terminal CAAX motif, wherein a geranylgeranyl isoprenoid is covalently attached to the cysteine residue. This process is catalyzed by geranylgeranyltransferase-I (GGTase-I) in the cytoplasm [66]. Following prenylation, Rac1 is sequestered in the cytosol by guanine nucleotide-dissociation inhibitors (GDIs), waiting for further instructions [67]. A polybasic amino acid sequence situated near the CAAX box regulates the docking of Rac1 into the lipid rafts of the plasma membrane and membrane ruffles [68]. Integrins enhance the affinity of Rac1 for lipid rafts, facilitating its translocation to target membranes and promoting subsequent dissociation of GDI from Rac1 [69]. Subsequently, under the action of guanine nucleotide exchange factor (GEF) and GTPase activating protein (GAP), Rac1 becomes activated and acts on downstream effectors [67]. In addition, Rac1 can be regulated by post-translational modifications, including phosphorylation, palmitoylation, ubiquitination, sumoylation, and adenylation, which also affect its effector functions, stability, and subcellular localization [70]. Altogether, the regulation of Rac1 involves a complex system with multiple activating and suppressing factors.

Among the above regulators, GEFs, GAPs, and GDIs play particularly important roles in the activation of Rac1. GEFs accelerate the binding of GTP to Rac1 and promote the interaction of Rac1 with various downstream effectors. GAPs enhance the intrinsic GTPase

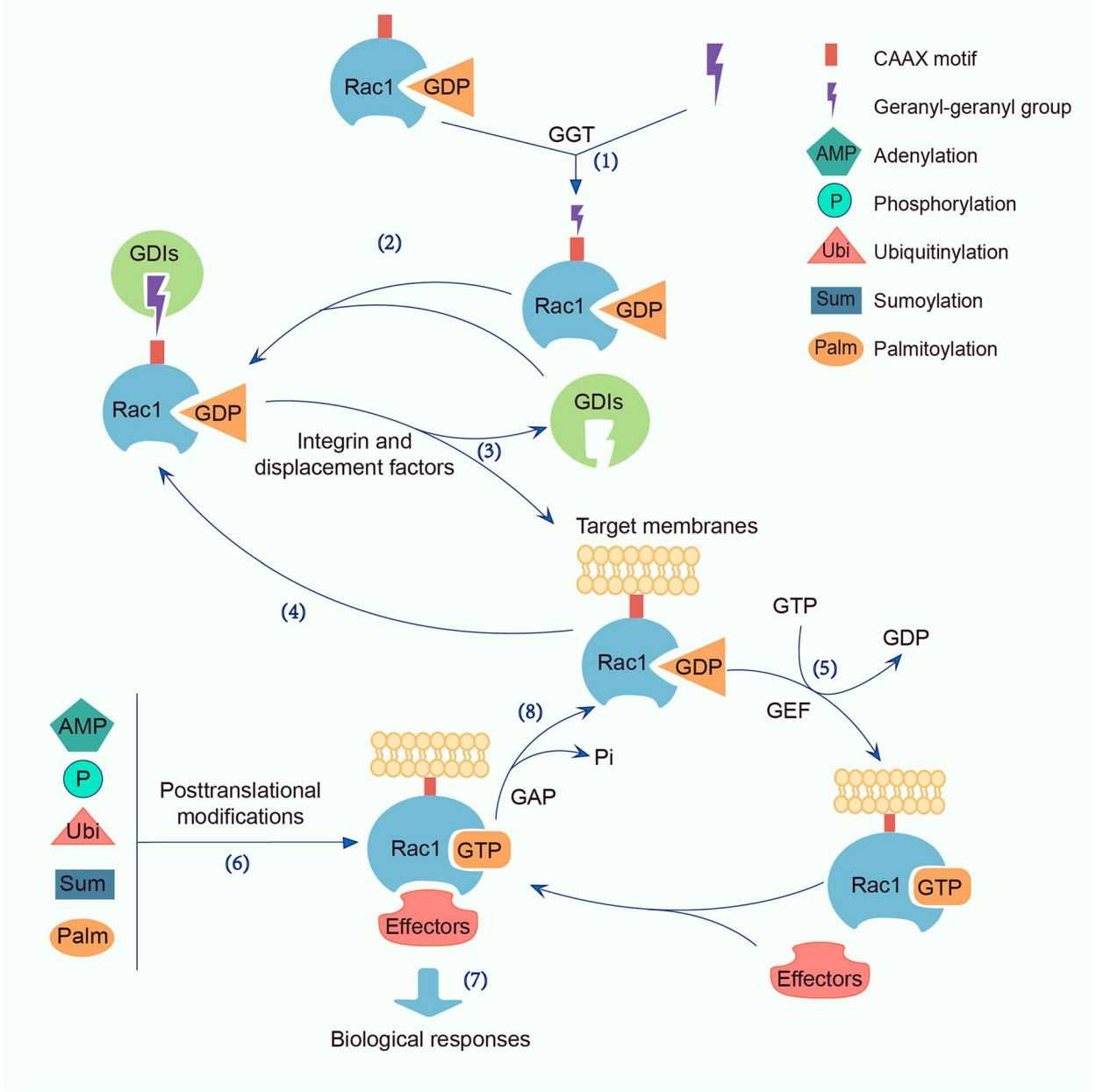


Fig. 2 Schematic of Rac1 regulation. The prenylation of the carboxy-terminal CAAX motif of Rac1 is a crucial step in its translocation and activation processes. This involves the covalent attachment of a geranylgeranyl group to the cysteine residue (1). Subsequently, GDIs sequester Rac1 in the cytoplasm or organelles by shielding its isoprenyl groups, awaiting further instructions (2) and (4). Integrins and other regulatory factors facilitate the dissociation of the geranyl–geranyl motif of Rac1 from GDIs and its subsequent integration into the target membranes (3). GEFs facilitate the activation of Rac1 by promoting its transition from the inactive GDP-bound state to the active GTP-bound state (5). Furthermore, post-translational modifications such as phosphorylation, palmitoylation, ubiquitination, sumoylation, and adenylation have been identified to influence the effector functions, stability, and subcellular localization of Rac1 (6). Activated Rac1 elicits specific biological responses by modulating diverse effectors (7). GAPs augment the inherent activity of Rac1 and facilitate the hydrolysis of GTP, leading to the reversion of Rac1 to its inactive GDP-bound state (8)

activity and promote GTP hydrolysis, leading to the inactive guanosine diphosphate (GDP)-bound state. While the GDIs stabilize Rac1 in its GDP-bound, inactive state, effectively pausing its activity until further instructions are received in certain subcellular locations (Fig. 2) [56–58]. More than 20 Rac1-related GEFs have been identified, acting on distinct downstream effectors of Rac1 [52]. These Rac1-targeted GEFs play pivotal roles in

modulating cytoskeletal dynamics by activating Rac1 and diverse downstream signaling pathways [71–78]. Furthermore, at least 35 GAPs have been identified to enhance the slow intrinsic GTPase activity, thereby inducing its inactivation across eukaryotes, ranging from yeast to humans [52]. Functioning as a small GTPase, Rac1 serves as a molecular switch, transforming between its inactive GDP-bound form and active GTP-bound form under the regulation of these factors. Consequently, it would be valuable to investigate whether these Rac1 regulators are implicated in disulfidptosis in future studies.

The WAVE regulatory complex is a crucial mediator in disulfidptosis

It has been reported that deletion of NCKAP1 or other components in the WRC suppresses disulfidptosis, confirming the vital role of this complex in the PCD [79]. Activation of the WRC by upstream Rho-family GTPases, such as Rac1, drives the Arp2/3 complex-mediated actin assembly in diverse cellular processes, ranging from intracellular vesicle trafficking to cell motility [80, 81]. The WRC is a 400-kDa, hetero-pentameric complex, which comprises two elongated subcomplexes: a dimer formed by cytoplasmic FMR1 interacting protein1/2 (CYFIP1/2) and NCKAP1, and a trimer formed by WAVE1/2/3 (Scar1/2/3), hematopoietic stem/progenitor cell protein 300 (HSPC300), and Abelson interactor1/2/3 (Abi1/2/3) (Fig. 3) [82].

The WCA module of WAVE is known to activate the Arp2/3 complex and initiate actin polymerization. The conserved concave surface of CYFIP1, in conjunction with the conserved meander region of WAVE, keeps WRC in the autoinhibited state by sequestering the WCA element [83]. It has been documented that Rac1 molecules bind to CYFIP, activating WRC by releasing the WCA motif. In addition, Arf GTPases can interact with NCKAP, which together with Rac1, augments the activation of WRC [80, 84]. Furthermore, the Abi polyproline-rich domain and Src-homology-3 (SH3) sequence facilitates Abl tyrosine kinase phosphorylation of the meander region in WAVE, triggering the direct release of the WCA motif and leading to the activation of actin nucleation by the Arp2/3 complex (Fig. 3) [83].

WAVE

WAVE 1–3 proteins are members of the Wiskott-Aldrich syndrome family proteins (WASP), pivotal in responding to a broad spectrum of upstream signals originating from small GTPases to activate Arp2/3-mediated actin polymerization [85]. These proteins harbor four conserved sequences: a WAVE homology domain (WHD, also known as the Scar homology sequence) at the amino terminus, a basic region, a polyproline-rich domain, and a C-terminal WCA (also called Verprolin-homology-Central-Acidic (VCA)) motif [86]. The N-terminal WHD of WAVE interacts with the other four component proteins to form the WRC [83, 87]. The proline-rich region contains SH3 binding sites, mediating interactions with SH3 domains in other regulatory proteins [88]. The WASP family proteins share the conserved WCA sequence, which binds to and activates the Arp2/3 complex, thereby regulating actin assembly [85]. The WH2 motifs bind to actin monomers and recruit them to the Arp2/3 complex, which is activated by the WCA domains to initiate actin polymerization [89, 90]. Typically, the conserved concave surface of CYFIP1, along with the conserved meander region of WAVE, inhibit the WCA element by sequestering its WH2 and Central regions (Fig. 3) [83].

CYFIP1

Previous studies have revealed two binding sites of Rac1 on the subunit of WRC, located on the opposite ends of CYFIP1. The one positioned adjacent to the WCA-binding site is named the "A Site", and the other one toward the C terminus of CYFIP1 is named the "D Site" (Fig. 3) [84, 91]. Chen et al. demonstrated that two molecules of Rac1 can bind to these two sites simultaneously through distinct mechanisms, with the D site exhibiting 40-fold to 100-fold higher binding affinity than the A site [80, 84]. Although their affinity is lower, only the binding of Rac1 to the A site can lead to conformational changes of the meander region of WAVE1, abolishing autoinhibition of the WRC complex and making the WCA sequence accessible to the Arp2/3 complex [84, 91]. Earlier research has elucidated the pivotal role of the A site in initiating the formation of lamellipodia in vivo, while the D site, though not necessary for activating WRC, can enhance the functionality and structural integrity of lamellipodia [80, 84, 91].

NCKAP1

NCKAP1 also referred to as hematopoietic protein-2 (Hem-2), is expressed in all tissues except hematopoietic cells. Its counterpart, Nck-associated protein 1-like (NCKAP1L, Hem-1), is predominantly expressed in urogenital and hematopoietic tissues [92]. NCKAP1 plays a critical role in regulating neuronal cytoskeletal dynamics and facilitating final neuronal differentiation in the cerebral cortex [93]. NCKAP1L is involved in actin immunoregulatory mechanisms, and mutations in its coding gene may lead to autoimmune disorders and recurrent viral and bacterial infections [94–96]. A recent study discovered a binding site on NCKAP1/NCPAK1L for Arf GTPases, which collaborates with Rac1-binding sites on CYFIP1/2 to enhance the recruitment and activation of WRC (Fig. 3) [97].

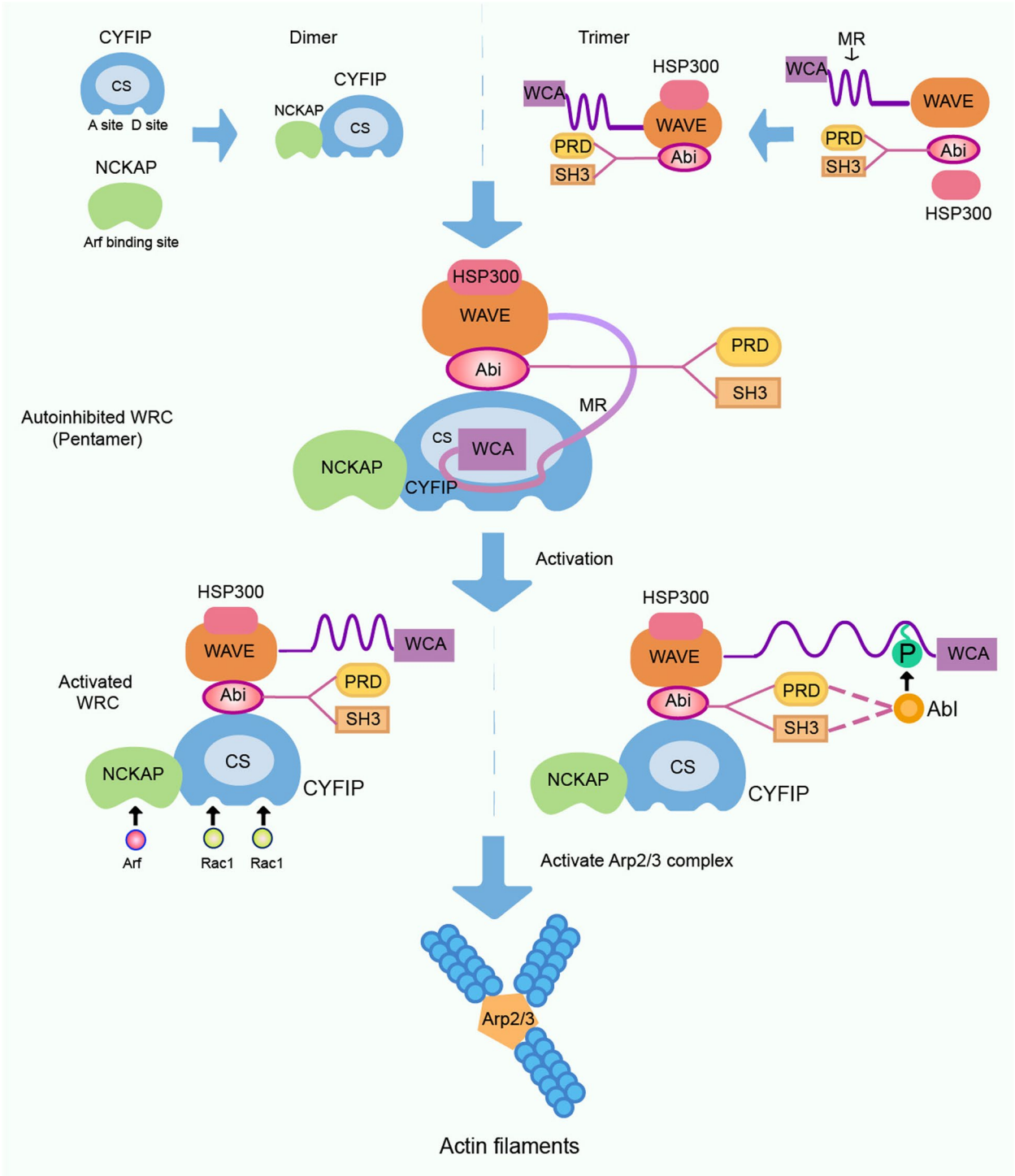


Fig. 3 Composition, assembly, and activation of WRC. WRC is a hetero-pentameric complex consisting of two subcomplexes: a dimer composed of CYFIP and NCKAP, and a trimer composed of WAVE, HSPC300, and Abi. The conserved concave surface (CS) of CYFIP1, in conjunction with the conserved meander region (MR) of WAVE, sequesters the WCA element by interacting with its WH2 and Central regions. This interaction keeps WRC autoinhibited in its resting state. Two Rac1 molecules can concurrently bind to A and D sites on CYFIP, thereby activating WRC through the release of the WCA motif. In addition to Rac1, Arf GTPases have the capability to interact with NCKAP, thereby cooperating with Rac1 to augment the recruitment and activation of WRC. Furthermore, the Abi polyproline-rich domain (PRD) and Src-homology-3 (SH3) sequence facilitate the phosphorylation of WAVE by Abl kinase in the conserved meander region. This phosphorylation event directly releases the WCA motif, leading to the activation of actin nucleation by the Arp2/3 complex

HSPC300

HSPC300, the smallest subunit of the WRC (approximately 8 kDa), is not only the least characterized, but also the most highly conserved [82]. Unlike other components that exist solely within the complex, HSPC300 can exist as a free form, comprising homotrimers, which serve as precursors for assembling the functional WRC [98]. Extensive research has revealed its role in activating the Arp2/3 complex and its importance in cytoskeletal remodeling in Arabidopsis and Drosophila cells [99, 100]. However, some studies have suggested that HSPC300 may not be essential for WRC formation or Arp2/3-mediated actin filament nucleation in human cervical carcinoma HeLa cells [87, 101].

Abi

The Abi protein family consists of three members: Abi1, Abi2, and Abi3, with Abi1 and Abi2 initially demonstrated to interact with the Abl tyrosine kinase through a proline-rich sequence and SH3 domain [102, 103]. Abl, in turn, can phosphorylate WAVE on the conserved Tyr150 or Tyr151, disrupting the CYFIP1-WAVE interaction in the meander region and freeing the WCA region to activate the Arp2/3 complex (Fig. 3) [83]. The Abi Proline-rich sequence and SH3 domain are necessary for Abl phosphorylation of WAVE2 on the conserved Tyr150 [104, 105]. Abi proteins play a role in Rac1-activated actin dynamics by forming complexes with other related proteins such as WAVE2, Eps8, or Sos1 [106, 107]. Moreover, Abi has been found to associate with enabled/vasodilator-stimulated phosphoprotein (Ena/VASP), contributing to its accumulation in the leading cell edges of cells. Strikingly, Abi/VASP double-KO might be fatal to cells, highlighting the significance of their interaction for intracellular homeostasis, which is not entirely dependent on the WRC-Arp2/3 pathway [108].

Consistent with prior research findings in eukaryotic organisms, Liu et al. demonstrated that knocking out any component would generally lead to the degradation of other WRC components. Moreover, they observed that elimination of any of the WRC subunits inhibited disulfidptosis [12]. Therefore, understanding the structural composition and regulatory mechanisms of WRC will reveal valuable insights into its potential roles in modulating disulfidptosis.

Actin polymerization factors might be potential downstream components of disulfidptosis

Liu et al. found that the Rac1-WRC-mediated actin polymerization and lamellipodia formation contributed to disulfidptosis, possibly because the actin network in the lamellipodia served as the target for disulfide bonds [12]. Knockout of the WRC components resulted in partial resistance to this PCD, suggesting the involvement of other actin formation pathways or actin-based structures in disulfidptosis. Actin polymerization, comprising actin nucleation and elongation, drives numerous essential cellular processes, including cell motility, endocytosis, and cytokinesis [109, 110]. The initial stage in de novo actin filament formation is nucleation, by which a stable seed composed of two or more actin monomers is formed [111]. Although there is a plentiful presence of globular (G-) actin monomers in the cytoplasm and nucleoplasm, the spontaneous nucleation of new filaments is suppressed by proteins that bind to and sequester G-actin (e.g. β-thymosin and profilin), along with the instability of actin multimer intermediates [112, 113]. Cells initiate nucleation by regulating three major classes of actin nucleators via diverse mechanisms: (1) the Arp2/3 complex, which combines with nucleation promoting factors (NPF) to act as a nucleus for the new actin filament based on preformed F-actin [114, 115]; (2) the formins, comprising 15 family members, which not only elongate linear actin filaments but also serve as nucleators that associate with stabilizing actin dimers or trimers [114, 116]; (3) the tandem-monomer-binding (TMB) nucleators recruit actin monomers together to form a polymerization nucleus by tandem G-actin-binding motifs [117, 118].

The Arp2/3 complex

The Arp2/3 complex catalyzes the formation of new branched actin networks, which are essential for assembling actin structures such as lamellipodia and invadosomes [119, 120]. Hence, the Arp2/3 complex potentially plays a vital role in disulfidptosis [12]. This multiprotein complex comprises seven highly conserved subunits in eukaryotic evolution, including Arp2, Arp3, and ArpC1-ArpC5. Among them, ArpC1-ArpC5 proteins bind to the maternal actin filament, anchoring the complex, while Arp2 and Arp3, as actin-related proteins, act as a nucleus to initiate the formation of a nascent branch from the preformed filament [121, 122]. Under equilibrium conditions, the complex remains in an inactive conformational state, with Arp2 and Arp3 spatially separated by the five additional subunits. Two classes of NPFs can activate the Arp2/3 complex, initiating the formation of a new filament [111, 123]. In mammals, class I NPFs mainly include WASP, neural WASP (N-WASP), WASP and Scar homologue (WASH), WAVE1-3, WASP homolog associated with actin, membranes and microtubules families (WHAMM), and junction mediating and regulatory protein (JMY). Besides, some bacterial-derived activators, such as RickA from Rickettsia, ActA from Listeria monocytogenes, and BimA from Burkholderia thailandensis, also belong to type I NPFs [124,

125]. They share a conserved C-terminal WCA module responsible for promoting the binding of Arp2/3 to the existing filament and recruiting 1–2 actin monomers as the initial step in forming a new filament branch [126, 127]. Two molecules of type I NPFs, each carrying an actin monomer, can more efficiently activate actin nucleation by simultaneously binding to two distinct sites on Arp2/3 complex (one site on Arp2 and ArpC1, and the other on Arp3) [128]. A representative of Class II NPFs is cortactin with an N-terminal acidic (NTA) motif, which functions to activate the Arp2/3 complex. Cortactin's activation effect on the complex is weaker than that of type I NPFs due to its lack of actin recruiting function [129, 130]. Moreover, cortactin can synergize with class I NPFs to stabilize Arp2/3 at branch points and elongate actin filaments [131, 132]. Recently, a novel type of NPFs, the WISH/DIP/SPIN90 family, was found to activate the Arp2/3 complex, promoting the polymerization of unbranched filaments without directly binding to actin monomers or preformed F-actin [133–135]. These NPFs localize to various cellular membranes, anchoring themselves and promoting the generation of branching actin networks to execute various cellular functions. Correspondingly, factors that inhibit and debranch the Arp2/3 complex are also localized at these cellular membranes to counteract specific NPFs and maintain the dynamic balance of branching actin network formation [123]. The Arp2/3 inhibitors, such as Arpin and Gadkin, contain a canonical C-terminal acidic A motif that binds to the complex but lacks the required WH2 sequence for recruiting actin monomers. Thus, they act as competitive inhibitors of corresponding NPFs, and their inhibitory ability is regulated by specific small GTPases (e.g. Rac regulates with Arpin) [136–138]. In addition, coronin, cofilin/actin-depolymerizing factor (ADF), and glia maturation factor (GMF) can promote the debranching of the Arp2/3 complex through distinct mechanisms. Coronins bind to both actin filaments and the Arp2/3 complex to enhance the disassembly of branched actin structures [139, 140]. Cofilin/ADF competes with Arp2/3 for binding sites in F-actin and induces structural changes that disfavor the combination of Arp2/3 with the filament, thereby exerting a debranching function [141]. Although GMF belongs to the cofilin superfamily, it binds to Arp2, instead of F-actin, dissociating new filaments from branched networks by inserting its binding sites between actin subunits and Arp2 [142–144]. In fact, these debranching proteins play critical roles in promoting the circulation of intracellular actin monomers and Arp2/3 complexes to sustain normal cellular functions [123]. In summary, the Arp2/3 complex participates in numerous actin-dependent physiological processes and is regulated by several factors. Further research is needed to determine whether these regulators are implicated in disulfidptosis.

Formin

The mammalian formin family comprises 15 members, all of which promote the polymerization of linear filaments by both de novo nucleating actin and progressively elongating F-actin at the barbed end [145]. All formins are characterized by two conserved domains: the formin homology (FH)2 domain, which stabilizes actin dimers to catalyze actin nucleation, and the FH1 domain, which delivers profilin-actin to the FH2 domain by profilin-binding motifs to modulate F-actin elongation [146, 147]. Members of the Diaphanous-related formin (Drf) subfamily exist in an autoinhibited state due to the intramolecular interaction between the Dia autoregulatory domain (DAD) and Dia inhibitory domain (DID) [148]. A subset of Rho GTPases (e.g. Rac, Rho, or Cdc42) can directly bind to the DID motifs, disrupting their interaction with the DAD sequences and activating the Drfs [146, 147]. Formins are implicated in de novo nucleation and subsequent elongation of actin filaments at the membrane surface during lamellipodia assembly [145]. Therefore, it is essential to study whether the Rho GTPase-Drf signaling pathway is involved in the mechanism of disulfidptosis.

TMB

The third class of actin nucleators, known as TMB nucleators, includes JMY, Spire, Vibrio parahaemolyticus and Vibrio cholerae (VopL/VopF) factors, leiomodin (Lmod), adenomatous polyposis coli (APC), and cordon-bleu (Cobl) [149, 150]. These nucleators possess multiple tandem WH2 domains, which enhance the assembly of polymerization seeds by binding to and bringing together G-actin monomers. Studies have demonstrated that JMY can nucleate both linear and branched actin filaments, while the others promote the polymerization of unbranched filaments [120]. However, recent studies have indicated that the tandem WH2 sequences are inadequate for efficient nucleation. Additional cooperative factors, such as Fmn-family formins, are necessary to enhance optimal nucleation by inducing TMB dimerization [151–153].

JMY, which contains a CA domain and three WH2 domains, binds to the Arp2/3 complex through the CA motif and serves as an NPF, catalyzing the polymerization of branched actin networks. In the absence of Arp2/3, JMY can directly initiate the nucleation of unbranched actin filaments through a G-actin binding linker and the third WH2 sequence [154]. JMY is located at the leading edge of the cell, which likely facilitates the migration via its dual actin polymerization function.

Initially, it nucleates linear filaments and activates the Arp2/3 complex, promoting the formation of branched actin networks based on the linear filament generated in the initial step [154].

Spire, in conjunction with formins and profilin, has been demonstrated to play an essential role during oocyte maturation in both flies and mammals [155, 156]. Human Spire comprises four WH2 domains capable of sequestering four actin monomers to form a complex. This complex synergizes with formin and profilin, exhibiting multifunctional properties such as nucleating, severing, and capping of actin filaments at the barbed ends [157].

VopL and VopF are actin assembly factors derived from gram-negative bacteria. They both possess a C-terminal dimerization domain and three WH2 domains, which collectively confer nucleation activity to VopL and VopF. The dimerization of VopF or VopL enhances their nucleation activity by stabilizing lateral connections between actin subunits in the nucleus [151, 158, 159]. When expressed in transfected or infected cells, VopL/F promotes the formation of stress fibers and filopodia [158, 160, 161]. Currently, whether VopL/F is associated with the pointed or barbed end of the filament during filament assembly is a subject of intense scientific debate. However, a recent study found that VopL/F primarily nucleated actin filaments from the pointed end [158, 162, 163].

Cobl is predominantly expressed in the brain, where it regulates neuronal morphology through its actin nucleation activity. The actin polymerization activity of Cobl has been traced to its C-terminal three WH2 motifs separated by linkers. Initially, a long linker (linker-2) between the second and third WH2 domains was proposed to be crucial for the nucleation process [118]. Nevertheless, a subsequent study suggested that a lysine-rich sequence (K-region) adjacent to the WH2 domains, together with the WH2 motifs, is primarily responsible for Cobl's actin polymerization function [164]. Besides its nucleation function at low Cobl: actin ratios, Cobl also demonstrates the ability to sequester actin monomers at high Cobl: actin ratios [164].

Lmod exists in three isoforms: smooth-muscle Lmod, cardiac Lmod, and fetal Lmod, all primarily expressed in the myocardial and skeletal muscles [165–167]. It contains an actin-binding site and a WH2 domain, rendering it a potent nucleator. Deficiency in Lmod severely compromises sarcomere assembly in muscle cells [168, 169]. Tropomyosin enhances Lomd's nucleation activity and mediates its localization to the middle of muscle sarcomeres, where both Lmod and tropomodulin-1 regulate F-actin polymerization dynamics [169–171].

APC is an important regulator of actin filament dynamics and is known to improve microtubule stability in epithelial tissues. Mutations in APC are particularly harmful to the intestinal epithelium and are strongly associated with most gastrointestinal cancers [172]. The C-terminal "basic" domain of APC (APC-B) constitutes a homodimer and combines four actin subunits to form a polymerization seed. Additionally, APC-B synergizes with the formin mDia1 to overcome the inhibitory effects of profilin and capping protein, facilitating the assembly of actin filaments [173].

In summary, JMY can potentially contribute to disulfidptosis by acting as an NPF for the Arp2/3 complex, thereby catalyzing the polymerization of branched actin networks during lamellipodia formation. Other TMB nucleators primarily promote the polymerization of unbranched actin filaments and more research is urgently needed to prove whether unbranched filamentous actin can also provide a permissive condition for facilitating disulfide crosslinking.

After filament nucleation, actin elongation proteins shield the barbed ends of filaments from capping proteins and regulate the elongation rate. Apart from formins, another well-known actin elongation factor is Ena/VASP, which is ubiquitously expressed in human tissues [174, 175]. The Ena/VASP family proteins comprise three highly conserved parts: a central, proline-rich domain that recruits actin monomers and profilin-actin complexes to promote actin assembly, an N-terminal EVH1 segment that has been proposed to bind to key ligands in cytoskeletal proteins and regulate the subcellular localization of Eva/VASP proteins, and a common C-terminal EVH2 region that are essential for the interactions with F-actin and actin monomers, as well as for tetramerization of the molecules [176, 177]. Unlike formins, the elongation activity of Ena/VASP proteins relies on the tethering or clustering of Ena/VASP to actin-rich zones such as lamellipodia, filopodial tips, stress fibers, and focal adhesions [109, 175]. Further investigations are warranted to identify the specific actin polymerization factors involved in disulfidptosis.

Actin-based cellular structures might serve as targets for excessive disulfide bond formation, facilitating disulfidptosis

Inhibition of lamellipodia formation via the Rac1-WRC pathway only results in partial resistance to disulfidptosis, suggesting that other proteins, including F-actin, may play a role in the establishment of conditions conducive to disulfide bond formation [12]. Therefore, it is imperative to clarify the potential implication of other actin filament structures in disulfidptosis (Fig. 1). Below, we offer a concise overview of these actin-containing subcellular structures, along with their respective functions, and regulatory factors (Table 1).

Table 1 Actin-based cellular structures

Structures	Main functional roles	Actin Regulators	References
Lamellipodia	Cell migration, adhesion, and signal transduction	Rac, Cdc42, WAVE complex, Arp2/3 complex, FMNL2/3, Ena/VASP, ADF/cofilin, coronin, capping protein, cortactin, Arpin	[87, 110, 138, 183, 185–190] [191]
Filopodia	Cell exploration, invasion, and axon growth	Cdc42, Rif, DIAPH3(mDia2), FMNL2/3, Ena/VASP, Arp2/3 complex, Daam1, cofilin, fascin	[110, 186, 192–197]
Podosomes	Cell adhesion, migration, and invasion	Cdc42, RhoA, WASP, Arp2/3 complex, FMNL1, INF2, FHOD1, cortactin	[110, 191, 198–202]
Invadopodia	Cell adhesion, migration, and invasion	Cdc42, RhoA, N-WASP, Arp2/3 complex, DIAPH1-DIAPH3, FHOD1	[198, 203–208]
Cell cortexes	Cell migration, division, morphology maintenance, and plasma membrane dynamics	Rho, Arp2/3 complex, DIAPH1(mDia1)	[110, 209, 210]
Stress fibers and focal adhesions	Cell adhesion and contraction	Rho, vinculin–Arp2/3 hybrid complexes, FHOD1, DIAPH1(mDia1)	[110, 211–213]
Endocytic vesicles	Endocytosis	Cdc42, Cip4/Toca, N-WASP, WIP/WIRE, Arp2/3 complex	[214–219]
Endosomes	Intracellular transport, sorting, and turnover of substances	WASH complex, Arp2/3 complex	[110, 220, 221]
Lysosomes	Protein degradation	WASH complex, Arp2/3 complex	[110, 222]
Autophagosomes	Autophagy	WHAMM/JMY, Arp2/3 complex	[223, 224]
Exocytic vesicles	Exocytosis	Spire1, Spire2, FMN2	[225, 226]
ER-mitochondrial intersections	Mitochondria dynamics	Spire1C, INF2	[110, 227]

Lamellipodia

Lamellipodia are dynamic wave-like structures found at the leading edge of cells, composed of densely packed branched actin filaments assembled by the Arp2/3 complex (Fig. 1). Studies have demonstrated that they have important functions in processes such as cell migration, adhesion, and signal transduction [18]. The branched actin networks in lamellipodia protrusion are mainly generated through the Rac1-WAVE-Arp2/3 signaling pathway [178, 179]. Capping protein (CP) is abundant in lamellipodia and serves as the major barbed-end terminator due to its high affinity for actin filament barbed ends. Elevated CP activity hinders actin filament elongation, thus facilitating nucleation by the Arp2/3 complex by augmenting the concentration of actin monomers in the cytoplasm [180]. The ADF/cofilin family facilitates actin treadmilling by severing preformed cortical actin filaments, generating new barbed ends and filaments to which the Arp2/3 complex can bind, thereby promoting the formation of branched networks in lamellipodia [181]. Cortactin stimulates the Arp2/3 complex to produce branched actin filaments and enhances their stability, thereby affecting lamellipodial persistence [182]. Coronin family proteins counteract cortactin by destabilizing branches, replacing the Arp2/3 complexes in mature dendritic networks in lamellipodia [183]. Besides, Arp2/3 complex inhibitors, such as Arpin, collaborate with the Rac1-WAVE-Arp2/3 activatory circuit to regulate Arp2/3-dependent actin polymerization and the directionality of cell migration [138]. Furthermore, studies have shown that under the regulation of Cdc42, formin-like protein 2 (FMNL2) and FMNL3, facilitate actin filament nucleation and elongation at the tips of lamellipodia and filopodia, independently of the Arp2/3 complex [184, 185]

Filopodia

Filopodia, dynamic and finger-like exploratory protrusions of the plasma membrane, contain parallel bundles of actin filaments (Fig. 1). Functioning as a sensory extension for the cell to explore its surroundings, filopodia play a vital role in multiple biological processes, including cell migration and invasion, wound healing, immune cell function, neurite outgrowth, and epithelial sheet fusion during development [228–230]. Two alternative models have been proposed to explain the initiation of filopodial actin structures: the "convergent elongation" theory suggests that the filaments originate from the dendritic network nucleated by the Arp2/3 complex, whereas the "de novo nucleation" model proposes that the filaments are directly nucleated by mammalian Diaphanous-related formin-like proteins FMNL2, FMNL3, and mDia2 [11, 193, 194]. Filopodial parallel actin filaments are elongated by formins and Ena/VASP proteins, then spatially organized into a bundle by formin Daam1 and fascin [195, 197]. Additionally, filopodia formation also necessitates inverse Bin-amphiphysin-Rvs (IBAR) proteins and myosin-X [231, 232]. During the filopodium disassembly process,

cofilin mediates actin filament severing, a process that is potentiated by the collaboration of fascin [196].

Invadosomes

Invadosomes, including podosomes and invadopodia, are actin-rich protrusions involved in adhesion and invasion, capable of degrading the extracellular matrix (ECM) through the secretion of matrix metalloproteinases (MMPs) (Fig. 1) [233, 234]. Podosomes are observed in various physiological contexts and cell types such as endothelial, monocytic, and smooth muscle cells, while invadopodia are predominantly found in cancer cells [234]. Actin polymerization, facilitated by the Arp2/3 complex, forms the core of podosomes, surrounded by a ring of adhesion plaque proteins (e.g. talin, vinculin) and integrins [200]. The formins FMNL1 and INF2 localize at the cap structure of the podosome adjacent to the core, polymerizing unbranched filaments and regulating contractile events by linking the core with the ring structure. Formin homology 2 domain containing 1 (FHOD1), on the other hand, is present around the core and likely facilitates the bundling of a second set of unbranched filaments, thereby mediating inter-structural contractility between podosomes [191, 201]. Invadopodia also originate from an Arp2/3-nucleated actin network, which is subsequently anchored to the cell membrane by a ring structure composed of adhesion plaque proteins and integrins. Furthermore, formins such as Diaphanous-related formins DIAPH1-DIAPH3, FHOD1, and the Arp2/3 complex collaborate in the further protrusive growth of invadopodia [207, 208].

Cell cortex

The cell cortex, a thin layer closely underlying the cell membrane and attaching to it, contains both unbranched and branched actin filaments along with myosins (Fig. 1), which generate tension crucial for numerous biological processes such as cell division, migration, and tissue development [235, 236]. Proteins like mDia1 (DIAPH1), mDia1-like formin ForA, and the Arp2/3 complex play significant roles in nucleating the cortical actin [210, 237].

Stress fibers

Stress fibers, consisting of crosslinked bundles of actin filaments (Fig. 1), are categorized into three types: dorsal stress fibers, transverse arcs, and ventral stress fibers. Among these, the ventral stress fiber is particularly prominent in mature cells and plays a crucial role in essential cellular functions such as mechanosensing, migration, and adhesion. At the ventral membrane of cells, the thick actomyosin bundles are anchored at both ends to focal adhesions. Focal adhesions are multi-protein complexes containing vinculin and talin, which serve to connect the intracellular actin cytoskeleton with ECM [238, 239]. Ventral stress fibers and focal adhesions are highly interdependent, with disruption of one structure resulting in the downregulation of the other [238]. Formins like mDia1 and FHOD contribute to the generation of actin filaments in stress fibers and focal adhesions [211, 213]. Although the canonical Arp2/3 complex is not typically observed in stress fibers and adhesions, a vinculin-Arp2/3 hybrid complex has been identified, regulating the formation of focal adhesion [212].

Intracellular organelles and vesicles

In addition to its role in controlling cell edge protrusion and morphology, actin polymerization is also crucial for maintaining the normal function of intracellular organelles and vesicles. Proteins like Cdc42 and F-BAR protein Cip4/Toca1 recruit N-WASP-WIP complex to endocytosis sites and activate Arp2/3 complex-dependent actin polymerization. This process promotes the formation of endocytic vesicles and its actin comet tail [216, 217]. Besides, WASH-mediated actin nucleation on the surface of endosomes contributes to efficient endosomal fission and its cargo trafficking function [216, 220]. On the surface of the lysosome, actin polymerization driven by WASH leads to the removal of vacuolar adenosine triphosphatase, which helps maintain lysosomal neutralization and enables efficient autophagic and phagocytic digestion [222, 240]. WHAMM and JMY are involved in autophagy by directing the number and size of the autophagosome, as well as the formation of branched actin comet tail [224]. Formin-2, Spire1, and Spire2 orchestrate the assembly of a robust actin network on vesicle surfaces, facilitating their attachment and enabling long-range intracellular transport along these actin tracks [226, 241]. In addition, Spire and inverted formin 2 (INF2) regulate actin polymerization at endoplasmic reticulum–mitochondria intersections, generating the necessary force for mitochondrial constriction and division [227].

In summary, actin-based structures are widely distributed in cells and regulate diverse cellular physiological processes (Fig. 1). Apart from lamellipodia and the Rac-WRC-mediated branched actin network, whether other types of actin filaments (e.g. unbranched actin filaments), actin-based structures, and corresponding nucleation factors are associated with disulfidptosis needs to be verified in the future.

Disulfidptosis might provide new treatment strategies for cancers and other diseases

In recent years, as the mechanism of PCD has been studied in depth, its potential application in the treatment of tumors and other diseases has gained increasing attention and demonstrated significant efficacy. In

cancer treatment, PCD can effectively inhibit cancer cell growth and spread, offering high specificity and selectivity. This reduces damage to normal cells and reduces dependence on traditional chemotherapy and radiotherapy [242–244]. Beyond tumor treatment, PCD also holds promise in the treatment of other diseases. For instance, in neurodegenerative diseases, regulating the processes of PCD can slow neuronal damage and death, thus delaying disease progression [245]. Moreover, PCD has demonstrated potential therapeutic value in fields such as cardiovascular disease and autoimmune diseases [246, 247]. Modulating disulfidptosis may also hold promise for treating the aforementioned diseases. Glucose transporter 1 (GLUT1) inhibitors like BAY-876 or GLUT 1 and GLUT 3 inhibitor KL-11743 can inhibit glucose uptake, deplete intracellular NADPH, and induce extensive disulfide bond formation in actin cytoskeleton proteins, ultimately leading to the collapse of F-actin and disulfidptosis in SLC7A11-high tumor cells. Consistent with cell experiment findings, GLUT inhibitors also induce disulfidptosis and demonstrate anti-tumor effects in SLC7A11-high cancers in mouse models, without causing significant pathological changes in major organs or affecting weight [11, 12]. Hence, disulfidptosis serves as a mediator for the therapeutic efficacy of GLUT inhibitors in the treatment of SLC7A11-high cancers. Further investigation is warranted to explore whether GLUT inhibitors can be synergistically combined with other drugs to enhance disulfidptosis induction and anticancer effects. Liu et al. revealed that 2DG could generate NADPH via PPP, thereby preventing the migration retardation of actin cytoskeleton proteins and rescuing SLC7A11-high cells from disulfidptosis under glucose deprivation. Furthermore, 2ME also delays this mode of cell demise by impeding disulfide bond formation [11, 12]. As a recently identified form of PCD, whether disulfidptosis might occur in normal cells and contribute to associated diseases (e.g., neurodegenerative diseases, cardiovascular disease, and autoimmune diseases) remains unclear, with only a handful of drugs demonstrated to modulate it. Further investigations are needed to elucidate the involvement of disulfidptosis in diverse diseases and to devise corresponding pharmaceuticals. With the in-depth study of the mechanism of disulfidptosis and the ongoing advancements in technology, it is anticipated that additional therapeutic strategies for this PCD and targeted medications can be developed in the future, providing novel approaches to disease treatment.

Conclusions and future perspectives

Disulfidptosis is a novel form of PCD involving crucial physiological processes in eukaryotic cells, such as cystine and glucose uptake, NADPH metabolism, and actin dynamics [12]. To counteract oxidative stress during rapid proliferation, tumor cells increase cystine absorption and GSH generation by upregulating the expression of SLC7A11, leading to substantial NADPH consumption [251–253]. Under glucose deprivation conditions, excessive intracellular cystine reduction can deplete NADPH, resulting in increased intracellular disulfide stress and aberrant disulfide bond formation in actin networks, ultimately causing cytoskeleton collapse and triggering disulfidptosis [12]. With remarkable advancements in this emerging field, several researchers have raised outstanding questions regarding the potential mechanisms of disulfidptosis (Table 2), outlining directions for future research

Table 2 Outstanding questions and potential research directions in disulfidptosis

Questions or future directions	References
Could other conditions or methods besides glucose deprivation promote disulfidptosis in SLC7A11-high cells?	[79, 248, 249]
Apart from the Rac1-WRC pathway, what other downstream factors are involved in disulfidptosis?	[79, 248]
What is the threshold level of disulfide required to trigger disulfidptosis?	[79, 250]
What are the specific biomarkers associated with disulfidptosis?	[79, 249]
How can disulfidptosis be leveraged in the treatment of tumors and other diseases?	[79, 249, 250]
Are there beneficial effects of disulfidptosis on dying cells and their surrounding tissues?	[3]
Does the collapse of the actin cytoskeleton impact glycolysis and energy metabolism?	[3]
Could the actin cytoskeleton buffer intracellular disulfide stress?	[3]
Is metastatic cancer more susceptible to disulfidptosis due to enhanced leading-edge protrusions?	[3, 250]
What are the roles of various organelles in disulfidptosis?	[79, 250]
Is actin cytoskeleton collapse the only mechanism causing disulfidptosis?	[249]
Can GLUT inhibitors be combined with other drugs to enhance disulfidptosis induction and anti-cancer effects?	[249]
Would disulfidptosis occur in normal cells and lead to corresponding diseases?	[248]
Is disulfidptosis implicated in the developmental process?	[79]
Does pore-forming protein participate in disulfidptosis?	[79]

[3, 79, 248–250]. In addition, there are numerous pressing issues concerning the crosstalk between disulfidptosis and other physiological processes, as well as the association between actin dynamics and excessive disulfide bond formation. (1) Due to the multifaceted functionalities of SLC7A11 within cells, more studies are needed to illustrate the interaction between disulfidpotosis and other physiological processes, including other types of PCD, nutrient metabolism, and redox homeostasis. (2) Apart from branched actin networks, it remains to be determined whether unbranched actin filaments also contribute to aberrant disulfide bonding. (3) While lamellipodia have been implicated in disulfidptosis, further investigation is needed to ascertain whether other actin-based cellular structures are also related to this form of PCD. Additionally, it is crucial to explore whether disulfide stress affects the function of related structures before reaching the threshold for disulfidptosis. (4) Numerous factors and signaling pathways are known to influence actin assembly and turnover. What additional pathways, beyond the Rac1-WRC axis, contribute to the induction of disulfidptosis? (5) The newly assembled actin filament and aged filament structures are not completely the same. For instance, the newly polymerized actin filaments (barbed ends) contain ATP-bound actin monomers, which gradually hydrolyze into ADP-actin as the filaments age, with the filaments far from the barbed ends characterized by ADP-bound actin subunits [254]. It's important to investigate whether both newly assembled and aged filaments equally contribute to the formation of disulfide bonds.

In summary, disulfidptosis presents a fresh therapeutic target for a range of conditions including tumors, neurodegenerative diseases, ischemia–reperfusion injuries, etc. This newly found PCD raises intriguing questions and identifies valuable avenues for future investigation. More studies are warranted to elucidate the precise mechanisms underlying disulfidptosis and its implications in the pathogenesis and treatment of diseases.

Abbreviations

PCD	Programmed cell death
ROS	Reactive oxygen species
GSH	Glutathione
SLC7A11	Solute carrier family 7 member 11
GPX4	Glutathione peroxidase 4
SLC3A2	Solute carrier family 3 member 2
GLUT	Glucose transporters
PPP	Pentose phosphate pathway
Rac1	Ras-related C3 botulinum toxin substrate 1
WRC	WAVE regulatory complex
Arp2/3	Actin-related protein 2/3
TCA	Tricarboxylic acid
αKG	α-Ketoglutarate
CoA	Coenzyme A
ALOX12	Arachidonate 12-lipoxygenase
2DG	2-Deoxyglucose
2ME	β-Mercaptoethanol
actin	Actin filament
NCKAP1	Nck-associated protein 1
NCKAP1L	Nck-associated protein 1-like
GTPases	Guanosine triphosphate phosphohydrolases
GEFs	Guanine nucleotide exchange factors
GAPs	GTPase activating proteins
GDIs	Guanine nucleotide-dissociation inhibitors
GGTase-I	Geranylgeranyltransferase-I
FTase	Farnesyltransferase
RCE1	Ras converting CAAX endopeptidase 1
ICMT	Isoprenylcysteine carboxyl methyltransferase
CYFIP	Cytoplasmic FMR1 interacting protein
HSPC300	Hematopoietic stem/progenitor cell protein 300
Abi	Abelson interactor
WASP	Wiskott-Aldrich syndrome family proteins
WHD	WAVE homology domain
PRD	Polyproline rich domain
WH2	WASP homology 2
WCA	WASP homology 2-Central-Acidic
VCA	Verprolin-homology-Central-Acidic
SH3	Src-homology-3
CS	Concave surface
MR	Meander region
Hem-2	Hematopoietic protein-2
Ena/VASP	Enabled/vasodilator-stimulated phosphoprotein
NPF	Nucleation promoting factors
TMB	Tandem-monomer-binding
WHAMM	WASP homolog associated with actin, membranes and microtubules families
JMY	Junction mediating and regulatory protein
NTA	N-terminal acidic
ADF	Actin-depolymerizing factor
GMF	Glia maturation factor
FH	Formin homology
Drf	Diaphanous-related formin
DAD	Dia autoregulatory domain
DID	Dia inhibitory domain
VopL/VopF	Vibrio parahaemolyticus and Vibrio cholera
Lmod	Leiomodin
APC	Adenomatous polyposis coli
Cobl	Cordon-bleu
CP	Capping protein
IBAR	Bin-amphiphysin-Rvs
ECM	Extracellular matrix
MMPs	Matrix metalloproteinases
INF2	Inverted formin 2
FHOD1	Formin homology 2 domain containing 1
FMNL	Formin-like protein

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