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Ferredoxins: master regulators in mitochondrial redox homeostasis and programmed cell death

Yajuan Lu [a,1], Yunyi Wu [b,1], Chen Yang [a,1], Yi Zhou [a], Xueying Ren [a], Haoran Li [a], Hangqi Huang [a], Feifan Pan [a], Aoli Deng [a], Yi Lu [a], Jiayi Yang [a], Xiangmin Tong [b,c,*], Yanchun Li [c,**], Jing Du [a,***]

[a] Laboratory Medicine Center, Department of Clinical Laboratory, Zhejiang Provincial People's Hospital (Affiliated People's Hospital), Hangzhou Medical College, Hangzhou, Zhejiang, 310014, China
[b] The Fourth School of Clinical Medicine, Zhejiang Chinese Medical University, Hangzhou First People's Hospital, 310006, China
[c] Department of Clinical Laboratory, Affiliated Hangzhou First People's Hospital, School of Medicine, Westlake University, Hangzhou, Zhejiang, 310006, China

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ABSTRACT

Ferredoxins (FDXs) are evolutionarily conserved iron-sulfur (Fe–S) proteins that serve as master regulators of mitochondrial redox homeostasis, governing critical processes including electron transfer, energy metabolism, Fe–S cluster biogenesis, and steroidogenesis. In humans, the mitochondrial isoforms FDX1 and FDX2 exhibit specialized yet complementary functions: FDX1 directs steroidogenesis, protein lipoylation, and copper redox cycling, while FDX2 is a core factor in Fe–S cluster assembly. Crucially, dysregulation of these proteins disrupts mitochondrial integrity, impairs redox balance, and activates multiple programmed cell death (PCD) pathways such as cuproptosis, ferroptosis, apoptosis, and autophagic cell death. This review systematically analyzes their isoform-specific roles in mitochondrial electron transport, Fe–S cluster dynamics, metabolic regulation, and summarizes major advances in understanding how FDX1 and FDX2 orchestrate mitochondrial-PCD crosstalk. The work further examines their critical functions in PCD execution, including FDX1-mediated cuproptosis through Cu^{+}-dependent aggregation of lipoylated proteins and FDX2-deficiency-driven ferroptosis via Fe–S cluster collapse and iron overload. Disease mechanisms across multiple pathologies, including cancer, neurodegeneration, cardiovascular disease, endocrine disorders, and genetic syndromes, are explored, highlighting links to FDX dysfunction, with emerging therapeutic strategies targeting FDXs also addressed. By elucidating the synergistic roles of FDX1 and FDX2 as metabolic-death gatekeepers, this review establishes a foundation for developing isoform-targeted therapies against diverse pathologies.

1. Introduction

Ferredoxins (FDXs) are a large class of iron-sulfur proteins first discovered in the obligate anaerobic non-photosynthetic bacterium, *Clostridium pasteurianum* in 1962 [1]. They are named for their unique property of being able to transfer electrons while containing only iron, without heme or flavin cofactors [1]. Ferredoxins are widely found in various organisms such as plants, animals, bacteria, and archaea and play roles in many fundamental biological processes, including photosynthesis, lipid metabolism, cellular respiration, iron-sulfur (Fe–S) cluster synthesis, steroidogenesis, bile acid production, and vitamin metabolism [2–5]. In particular, their involvement in redox processes makes them essential proteins in organisms ranging from non-photosynthetic anaerobic bacteria to photosynthetic single-cell and multicellular life forms [5]. Moreover, FDXs have been well-characterized as multifunctional regulatory proteins, demonstrating diverse roles in cellular processes. These include: modulating the expression of photooxidative stress-responsive genes [6]; orchestrating the dynamic reorganization of key protein complexes, particularly in photosynthetic and respiratory electron transport chains [7–9];

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: tongxiangmin@163.com (X. Tong), lycmed@163.com (Y. Li), dujing1@hmc.edu.cn (J. Du).
[1] These authors contributed equally: Yajuan Lu, Yunyi Wu, Chen Yang.

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and governing posttranslational protein lipoylation, a crucial modification for cellular metabolism [10].

Humans possess two mitochondrially localized FDXs, namely ferredoxin 1 (FDX1) and ferredoxin 2 (FDX2, previously known as ferredoxin1L), encoded by adjacent genes on chromosome 11q22 [11]. These Fe–S proteins serve as crucial electron carriers in mitochondrial metabolic pathways, forming an essential redox chain with ferredoxin reductase (FDXR) to support fundamental processes including oxidative phosphorylation (OXPHOS), the tricarboxylic acid (TCA) cycle, and Fe–S cluster biogenesis. Notably, FDX1 and FDX2 exhibit distinct functional specialization: FDX1 primarily mediates steroid hormone biosynthesis and protein lipoylation, while FDX2 specifically drives [4Fe–4S] cluster assembly and maturation [11]. This functional dichotomy establishes FDXs as master regulators of mitochondrial metabolic homeostasis. Emerging research has further revealed their pivotal role as molecular nodes integrating metabolic signaling with cell fate determination. FDXs dynamically interface with multiple programmed cell death pathways, including apoptosis [12], autophagy [13], ferroptosis [12,14], and cuproptosis [15]. This paradigm shift highlights FDXs as central players in the crosstalk between cellular metabolism and death signaling networks.

This review synthesizes mechanistic insights into how FDX1 and FDX2 govern mitochondrial bioenergetics, Fe–S cluster dynamics, and redox balance, while their dysregulation propagates death signals through cuproptosis, ferroptosis, autophagy, and apoptosis. We further highlight how isoform-specific targeting offers promising therapeutic avenues for oncogenesis and cancer progression, particularly in chemoresistant malignancies, neurodegenerative disorders, cardiovascular pathologies, metabolic disorders, and genetic diseases. By systematically dissecting the co-evolution of FDXs from electron carriers to death regulators, we illuminate their emerging translational potential in precision medicine.

2. The role of FDXs in mitochondrial homeostasis

Mitochondria, the cellular powerhouses, orchestrate two pivotal functions: energy transduction via TCA cycle coupled with OXPHOS, and biosynthesis of Fe–S clusters – indispensable cofactors for enzymes governing redox homeostasis. These processes fundamentally rely on finely-tuned electron transfer networks where mitochondrial FDXs emerge as central mediators. As evolutionarily conserved Fe–S proteins, FDXs operate at the nexus of mitochondrial redox biochemistry. In animals, FDX1 and FDX2 exhibit compartmentalized specialization: FDX1 primarily fuels steroidogenesis and protein lipoylation, while FDX2 dominates Fe–S cluster biogenesis, a process vital for electron transport chain (ETC) integrity and metabolic enzyme activation. This functional dichotomy positions FDXs as molecular rheostats coordinating three cornerstone aspects of mitochondrial physiology: (i) energy metabolism through substrate channeling to the ETC, (ii) dynamic maintenance of Fe–S cluster pools, and (iii) redox equilibrium via electron partitioning. The structural framework demonstrates the functional specialization of FDX1 and FDX2 in mitochondrial redox biology (Fig. 1). Mechanistic analyses of these specific pathways will be presented in subsequent sections.

2.1. The role of FDXs in electron transfer

FDX1 and FDX2 both interact with the NADPH-dependent reductase FDXR, acquiring electrons from NADPH via FDXR and subsequently transferring these electrons to target systems involved in various biological processes. Although they exhibit a high degree of homology in both primary and three-dimensional structures, critical differences exist between them. First, their electrostatic surface potentials around the [2Fe–2S] clusters differ significantly - FDX1 displays a stronger negatively charged region, while FDX2 has a more neutral surface potential [10]. Additionally, their redox potentials exhibit distinct values (FDX1: −267 mV; FDX2: −342 mV) [11]. More importantly, they demonstrate

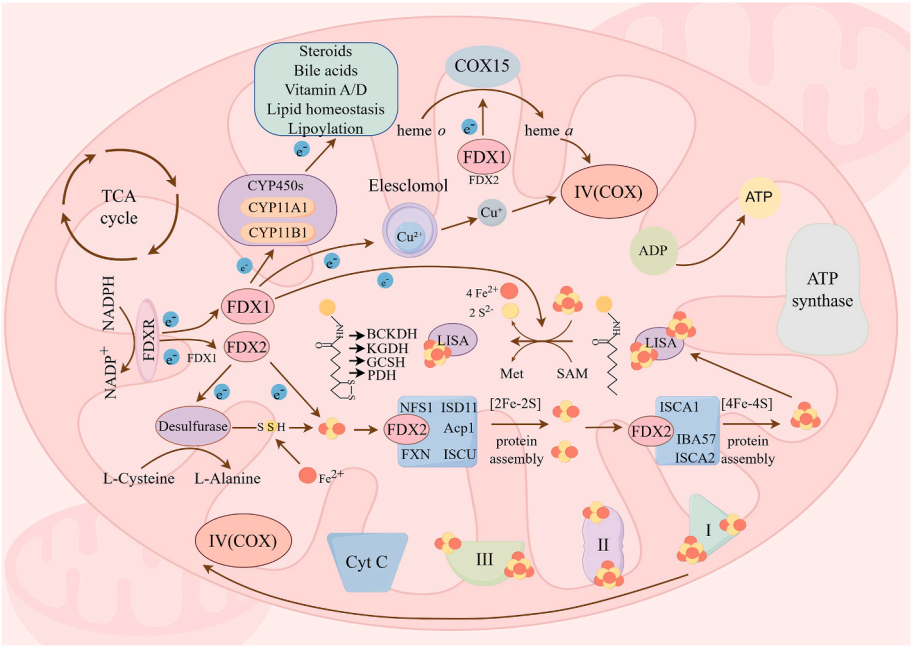

Fig. 1. The role of FDXs in mitochondrial homeostasis

FDX1 serves as a dedicated electron donor through FDXR and NADPH, facilitating steroidogenesis via CYP450 enzymes, protein lipoylation through LIAS (modifying PDH, GCSH, KGDH, and BCKDH), copper reduction from elesclomol to Cu^{+}, and heme *a* biosynthesis via COX15. It directly interacts with LIAS to activate its catalytic function in lipoic acid synthesis, enabling lipoylation of TCA cycle enzymes and supporting mitochondrial respiration. In contrast, FDX2 drives Fe–S cluster biogenesis as an electron donor to NFS1 complex, facilitating [2Fe–2S] cluster formation via ISCU and [4Fe–4S] maturation via ISCA1-ISCA2 for ETC complexes I-III and aconitase. Structurally, FDX1 and FDX2 exhibit distinct redox potentials (−267 mV vs. −342 mV) and electrostatic surfaces, ensuring non-redundant roles, as illustrated by their size differences in the figure. Together, they maintain OXPHOS, TCA cycle flux, and redox homeostasis.

systematic differences in redox properties and substrate specificity.

2.1.1. The electron transfer function of FDX1

FDX1 specifically donates electrons to mitochondrial cytochrome P450 enzymes (e.g., CYP11A1, CYP11B1) in steroidogenic tissues (adrenals, testes, kidneys). Most mitochondrial P450 enzymes require cofactors such as NAD(P)H. In the human body, CYPs are not self-sufficient and cannot directly interact with their primary electron donor, NAD(P)H, to receive electrons. Instead, they rely on redox partners to transfer electrons. Mitochondrial P450 enzymes (e.g., CYP11A1) depend on the ETC composed of adrenodoxin (Adx, namely FDX1) and adrenodoxin reductase (AdR, namely FDXR). The electron transfer chain NADPH-FDXR-FDX1-P450 converts cholesterol to pregnenolone, cortisol, and aldosterone [11,16]. Besides, FDX1 is also involved in the metabolism of vitamin D and bile acids [5].

Protein lipoylation is a key modification in cellular metabolism. FDX1 functions as a dedicated electron donor in mitochondrial lipoylation, exhibiting specific binding affinity for lipoic acid synthase (LIAS) with a dissociation constant (Kd ≈ 6 μM) [17–19]. This interaction facilitates the direct transfer of electrons to LIAS's auxiliary [4Fe–4S] cluster, maintaining its catalytically essential reduced state. The electron shuttle mechanism enables LIAS to activate sulfur atoms derived from cysteine and other sulfur donors for insertion into lipoyl-CoA. Critically, FDX1 deficiency does not degrade the Fe–S cluster structurally but abolishes its redox activity, demonstrating that FDX1 regulates lipoylation through electron transfer-dependent activation rather than cluster stability [19]. This electron donation system operates as a glucose-sensitive checkpoint, with FDX1 becoming indispensable under low-energy conditions to sustain LIAS activity.

Copper is an essential micronutrient required for balanced growth and normal physiological functions in humans. FDX1 plays a pivotal role in copper ion metabolism and holds therapeutic potential for treating copper deficiency disorders, such as Menkes disease, by regulating copper ion distribution [20]. Specifically, FDX1 promotes the release of copper from the copper ionophore elesclomol (ES) into mitochondria by catalyzing the reduction of ES-Cu^{2+} to bioavailable Cu^{+} [21]. The generated Cu^{+} is directly utilized for the metallation of mitochondrial copper enzymes, particularly cytochrome *c* oxidase (COX)- a key component of the ETC that relies on copper cofactors for its function [20,22,23]. Recent electron paramagnetic resonance (EPR) spectroscopy studies have shown that FDX1 can directly transfer electrons to ES-Cu^{2+} complexes both in vitro and in vivo. Notably, ES-Cu^{2+} exhibits a higher electron affinity than free Cu^{2+}, demonstrating its high efficiency as the primary electron scavenger for FDX1 in cells [24].

This specific interaction with ES-Cu^{2+}, distinct from FDX2's substrate preferences, underscores the functional specialization between the two FDXs and establishes FDX1 as the primary gateway for mitochondrial copper redox chemistry. However, this essential copper metabolism can become pathological when dysregulated: copper overload induces FDX1-mediated reduction of Cu^{2+} to Cu^{+}, triggering cuproptosis, which is characterized by proteotoxic stress from Cu^{+}-dependent aggregation of lipoylated TCA cycle proteins and Fe–S cluster depletion [25,26]. The hepatic toxicity mechanism of $Cu(OH)_2$ nanopesticide involves targeting FDX1 and its associated lipoic acid and iron-sulfur cluster pathways. It increases FDX1 expression, activates lipoylated TCA cycle proteins to trigger cuproptosis, and disrupts bile acid synthesis and energy metabolism, ultimately leading to liver dysfunction [27]. Importantly, FDX1's dual role in this process stems not only from its Cu^{2+}-to-Cu^{+} reduction activity but also from its specific electron transfer mechanism to ES-Cu^{2+}, which directly links copper overload to lethal protein aggregation. In the following sections, we will further elaborate on the relationship between FDX1 and cuproptosis.

2.1.2. FDX2 as the central electron donor in Fe–S cluster biogenesis

FDXs can bind to cysteine desulfurase and donate electrons for the biosynthesis of Fe–S clusters, with FDX2 playing the principal role. Notably, FDX2 can directly interact with the protein complex responsible for iron-sulfur cluster assembly, whether in its oxidized or reduced state. This complex comprises cysteine desulfurase (NFS1), ISD11 (also known as LYRM4), and acyl-carrier protein (Acp1). FDX2 exhibits a higher binding affinity to this complex compared to FDX1 (with a Kd value of 14.9 ± 1.2 μM, in contrast to 41.7 ± 2.6 μM for FDX1). Nevertheless, FDX2-dependent Fe–S cluster assembly is tightly regulated by divalent ions. Recent studies reveal that zinc ions (Zn^{2+}) competitively bind to the metal coordination site of ISCU via residues C35, D37, C61, H103, which block iron (Fe^{2+}) insertion and thereby inhibit FDX2-mediated persulfide reduction. This results in inefficient [2Fe–2S] cluster formation and forces cells to rely on non-physiological assembly pathways such as excess L-cysteine. In contrast, removing zinc enables Fe^{2+} binding and restores FDX2's electron transfer function, thereby highlighting zinc as a critical modulator of FDX2 activity in Fe–S cluster biogenesis [28]. Moreover, in vitro experiments have demonstrated that the reduced state of FDX2 can support the assembly of Fe–S clusters on ISCU, a scaffold protein for iron-sulfur cluster assembly, with significantly higher efficiency than FDX1. Beyond its direct role, FDX2 may also cooperate with other proteins (such as FXN and ISCU) during iron-sulfur cluster biosynthesis, further enhancing the efficiency of iron-sulfur cluster assembly [29,30]. FDX2 not only supplies electrons for the synthesis of [2Fe–2S] clusters but also plays a highly specific role in the maturation of [4Fe–4S] proteins. Indeed, neither FDX1 nor other cellular reduction systems, such as the glutaredoxin/GSH and thioredoxin systems, can substitute for FDX2. Specifically, FDX2, via its NADPH-coupled reductase FDXR, FDX2 transfers electrons to the ISCA1-ISCA2 complex and IBA57. Through specific interactions with these partners, it accelerates the reductive assembly of [2Fe–2S] clusters, a process that leads to the formation of [4Fe–4S] clusters, which are then transferred to the target proteins, thereby completing the maturation of [4Fe–4S] proteins [31]. Therefore, knocking down the expression of FDX2 using RNAi technology leads to decreased activities of multiple iron-sulfur proteins. However, knocking down FDX1 did not produce such an effect, demonstrating the irreplaceability of FDX2 in iron-sulfur cluster biosynthesis [11]. The absolute reliance of [4Fe–4S] protein maturation on FDX2 underscores its role as the keystone of Fe–S cluster biogenesis. Consequently, any impairment of FDX2 function, whether by genetic deficiency, zinc dysregulation, or oxidative stress, has a domino effect, destabilizing a wide range of Fe–S-dependent processes, from energy metabolism to iron homeostasis, and thereby creating a cellular context primed for metabolic catastrophe and specific forms of cell death, such as ferroptosis.

A recent nuclear magnetic resonance (NMR) experiment targeting hyperfine shifts and fast relaxation resonance near the cluster revealed the pathway of electron spin density transfer in FDX2 through N–H—S–Fe^{3+} and C–H—S–Fe^{3+} interactions. Notably, the C–H—S–Fe^{3+} interaction may be weak or absent in FDX1. Combined with quantum chemical calculations, the study showed an asymmetry in the electron spin density distribution of the two Fe^{3+} ions in FDX2, which may affect its electron transfer function. This finding not only provides a new perspective for understanding the unique role of FDX2 in iron-sulfur cluster biogenesis but also offers fresh insights into the structural similarity yet functional diversity between the FDX1 and FDX2 [32].

2.2. The role of FDXs in mitochondrial energy metabolism

As elaborated in the previous section, FDX family proteins regulate the function of mitochondrial metabolic enzymes by driving the biosynthesis of Fe–S clusters and protein lipoylation through electron transfer. Building on this, the present section will further explore their specific yet interconnected contributions to energy metabolism from two perspectives: on one hand, analyzing the role of FDX1 in maintaining lipoylation, and on the other hand, investigating how FDX2-mediated Fe–S cluster defects disrupt mitochondrial metabolism.

2.2.1. FDX1 in lipoylation maintenance and mitochondrial energy regulation

Lipoylation involves the covalent attachment of lipoyl groups to proteins. As a specific form of acylation, it occurs exclusively on the conserved lysine residues of four key mitochondrial metabolic complexes. These include the glycine decarboxylase complex (GDC, also known as the glycine cleavage system) and crucial metabolic complexes in the TCA cycle, such as pyruvate dehydrogenase (PDH) complex, α-ketoglutarate dehydrogenase (KGDH) complex, and branched-chain α-ketoacid dehydrogenase (BCKDH) complex. Lipoylation is crucial for the enzymatic functions of these acylated complexes. FDX1 ensures the efficient operation of the TCA cycle and normal mitochondrial respiratory chain function by maintaining the lipoylation of key metabolic enzyme complexes. Knocking down FDX1 leads to lipoylation defects, impairs TCA cycle flux, and forces cells to rely on glycolysis [10,19]. Meanwhile, cellular metabolic disorder caused by lipoylation deficiency induced by FDX1 knockout activates an integrated stress response (ISR). As a key marker of ISR, GDF15 shows significantly upregulated expression, and FDX1 indirectly regulates GDF15 expression by maintaining lipoylation to ensure metabolic homeostasis [33–35]. Moreover, the metabolic dysregulation caused by FDX1 deficiency is not limited to lipoylation defects. The collapse of mitochondrial metabolism can create an environment permissive to the accumulation of inhibitory metabolites such as phosphoethanolamine, which is known to directly impair mitochondrial OXPHOS [36,37]. Therefore, FDX1 not only directly regulates TCA cycle flux and OXPHOS, but also prevents the accumulation of respiratory inhibitors, thereby safeguarding mitochondrial function. This coordinates mitochondrial homeostasis and cellular metabolic adaptation at multiple levels.

Early functional studies of human ferredoxins relied on bacterial/yeast reconstitution models or in vitro assays, which often failed to recapitulate their native roles in human cells. This led to incomplete and sometimes conflicting conclusions about FDX1's contributions, particularly regarding heme synthesis [11,38,39]. Heme *a* is a crucial cofactor of COX, which serves as the terminal enzyme (namely Complex IV) of the ETC. COX is responsible for transferring electrons to oxygen, thereby generating water. Its biosynthesis requires multiple assembly factors, including COX15, which mediates the conversion of heme *o* to heme *a*. Early yeast complementation studies (e.g., the Gal - YAH1 model) suggested that human FDX1 could not functionally substitute for yeast Yah1—a finding that initially obscured FDX1's conserved role in heme metabolism [11].

However, recent studies have clarified that FDX1 is indispensable for heme *a* maturation and COX integrity [10,40]. CRISPR-Cas9 knockout studies in human embryonic kidney (HEK) 293 cells demonstrated that FDX1 deficiency significantly reduces COX enzymatic activity, which correlates with impaired COX15-dependent heme *a* synthesis [10]. Yeast humanization models further confirmed that FDX1 serves as the primary human ferredoxin supporting COX15 activity, establishing its conserved function across species [10]. Meanwhile, studies in rat H9c2 cardiomyocytes showed that FDX1 knockout leads to reduced protein levels of COX subunits COX1 and COX4, disrupts heme a/a_3 absorption spectra [40], and that COX15 overexpression can partially rescue COX1 levels, indicating FDX1 facilitates heme biosynthesis for COX assembly [40]. These findings collectively reveal that FDX1 plays a critical role in mitochondrial respiratory chain function through dual mechanisms: it directly donates electrons to COX15 to support heme *a* biosynthesis, while also coordinating with COX15 to maintain the structural integrity of the COX complex. FDX1 deficiency impairs the function of ETC complex IV by simultaneously disrupting heme *a* synthesis and COX complex assembly, ultimately leading to disorders in cellular energy metabolism.

In fact, the impacts of FDX1 gene defects go far beyond this. In hepatocellular carcinoma (HCC) cells, overexpression of FDX1 increases the mitochondrial membrane potential and reduces the levels of reactive oxygen species (ROS), whereas FDX1 knockdown produces the opposite effects [41]. This demonstrates the important role of FDX1 in maintaining the intracellular redox balance.

2.2.2. FDX2 deficiency-induced Fe–S cluster defects and mitochondrial metabolic dysfunction

In mitochondria, Fe–S proteins are essential components of the ETC, where Fe–S clusters facilitate electron transfer through Complex I [42], Complex II [43] and Complex III [44] to cytochrome *c*. Given the critical role of FDX2 in Fe–S cluster biogenesis, its dysfunction disrupts mitochondrial function. Knockdown of FDX2 in HEK 293 cells results in a significant reduction in mitochondrial Fe–S protein levels, including NDUFS1 and SDHB, impaired activities of respiratory chain complexes I, II, and III, decreased COX activity, and inhibition of OXPHOS. Consequently, cells compensate by shifting toward glycolysis for energy production [10].

Abnormalities in FDX2 disrupt mitochondrial energy metabolism by impairing Fe–S cluster biosynthesis, a process essential for ETC function and other Fe–S proteins like aconitase and SDHB. This deficiency triggers dual consequences: (i) elevated ROS from compromised ETC activity, which drives compensatory superoxide dismutase 2 (SOD2) upregulation yet fails to prevent oxidative damage (ii) Dysregulated iron metabolism, causing mitochondrial iron accumulation, a hallmark of defective Fe–S biogenesis. Together, these interconnected defects in energy production, oxidative stress, and iron homeostasis underpin mitochondrial pathogenesis in FDX2-related disorders [45–48]. Moreover, the mitochondrial dysfunction resulting from FDX2 deficiency and subsequent Fe–S cluster instability may be further amplified by disrupting the balance of the reactive species interactome (RSI) [49,50]. The RSI represents a complex network of redox-active molecules, including ROS, reactive nitrogen species (RNS; e.g., nitric oxide, NO, and peroxynitrite, $ONOO^-$), and reactive sulfur species (RSS; e.g., hydrogen sulfide, H_2S, and polysulfides) [49–53]. Fe–S clusters are highly vulnerable to attack by various components of the RSI [28]. For instance, the superoxide anion ($O_2^{\bullet-}$), whose production is elevated due to ETC impairments from Fe–S cluster deficiency, can directly oxidize and disrupt [4Fe–4S] clusters [54]. Furthermore, it is well-established that RNS, like NO, can target the iron centers within Fe–S clusters, leading to their disassembly and the release of free iron [55]. This RSI-mediated damage creates a vicious cycle: initial Fe–S cluster loss increases the production of reactive species, which in turn attack the remaining clusters, exacerbating oxidative stress, iron overload, and ultimately contributing to the activation of cell death pathways such as ferroptosis. Therefore, although FDX2 is not a direct source of RSI components, its essential role in Fe–S cluster biogenesis suggests that it may indirectly regulate mitochondrial susceptibility to RSI-mediated damage, based on current indirect evidence from oxidative stress models and Fe–S cluster dysfunction studies. However, direct experimental validation is needed to confirm this regulatory relationship.

Given the distinct functional specializations of FDX1 and FDX2 in mitochondrial electron transfer and cluster biogenesis, we have systematically summarized these differential comparisons, highlighting their non-overlapping roles in maintaining core metabolic processes (Table 1). As delineated in this section, both FDX1 and FDX2 are indispensable for mitochondrial energy transduction and core metabolism, albeit through complementary mechanisms. FDX2 is the cornerstone of Fe–S cluster biogenesis, directly providing essential cofactors for the ETC and key metabolic enzymes. In parallel, FDX1 sustains mitochondrial respiration by regulating the lipoylation of TCA cycle dehydrogenases and supporting heme a biosynthesis. The profound functional specialization of FDX1 and FDX2 not only defines their distinct physiological roles but also their respective pathological potentials. We therefore posit that the FDX family functions as a central "metabolism-death axis" within the mitochondrion. The following sections will explore how their influence extends to systemic lipid homeostasis and, crucially, how the dysregulation of their distinct physiological activities becomes the principal determinant of cell fate,

Table 1
Core functional and structural comparison of FDX1 and FDX2 in mitochondria.

Characteristic	FDX1	FDX2	Reference
Core Functions	Electron transfer for steroidogenesis (CYP11A1/CYP11B1); Lipoylation regulation via LIAS; Copper metabolism (Cuproptosis).	Essential for mitochondrial Fe–S cluster biogenesis; Specialized in [4Fe–4S] cluster maturation.	[5,10,11, 16,19,20, 22,24–27]
Metabolic Regulation	Drives steroid hormone synthesis Activates LIAS (K_d ~ 6 μM); Maintains TCA cycle via PDH/KGDH lipoylation; Regulates TCA cycle (PDH/ KGDH).	Assembles [2Fe–2S], [4Fe–4S] clusters; Stabilizes ETC complexes I-III; Supports Fe–S enzyme activity.	[10,11,19, 29–31, 45–48]
Redox Properties	Redox potential: 267 mV; Strong negative surface charge near [2Fe–2S] cluster.	Redox potential: 342 mV; Neutral surface potential near [2Fe–2S] cluster.	[10,11]
Structural Specificity	Preferential binding to LIAS and ES-Cu^{2+}; Lower affinity for Fe–S complexes (K_d ~ 41.7 μM).	High-affinity binding to NFS1-ISD11-Acp1 complex (K_d ~ 14.9 μM); Irreplaceable for ISCA1-ISCA2/IBA57 interaction.	[19,29,30]
Consequences of Deficiency	Lipoylation defects-glycolytic shift; COX assembly failure; SREBP1/2 activation-lipid dysregulation; Cuproptosis resistance.	ETC complex I-III dysfunction; Global Fe–S enzyme deficiency; Mitochondrial iron overload; Increased ROS production.	[10,11,19, 40,45–47]
Unique Mechanisms	Glucose-sensitive LIAS regulation; Cu^{2+} – Cu^{+}reduction triggers cuproptosis.	Asymmetric electron spin density in [2Fe–2S] cluster; No functional redundancy in [4Fe–4S] maturation; Zn^{2+} competes with Fe^{2+} for ISCU binding, inhibiting FDX2-mediated persulfide reduction and [2Fe–2S] cluster formation.	[24,25,28, 32]

channeling stress into specific disease pathways and programmed death signals.

3. The role of FDXs in lipid regulation

Beyond their canonical roles in electron transfer and Fe–S cluster biogenesis, FDX1 and FDX2 also critically govern lipid metabolism. They exert this influence through both shared and distinct mechanisms, not only bridging mitochondrial redox biology with systemic lipid balance but also thereby playing an equally critical role in maintaining cellular homeostasis. This section analyzes the direct regulation of FDX1 on lipid homeostasis and developmental processes, as well as the auxiliary regulation of FDX2 (Fig. 2).

3.1. FDX1's role in lipid metabolism and embryonic development

Genetic studies demonstrate that FDX1 is indispensable for mammalian embryonic development, as knockout of both alleles results in embryonic lethality in mice. FDX1 heterozygous mice exhibit significantly shortened lifespans and are prone to developing steatohepatitis [5]. Mechanistically, FDX1 deficiency simultaneously activates sterol regulatory element-binding proteins SREBP1/2—master regulators of lipid metabolism—thereby triggering a cascade of reactions [56]. On one hand, it enhances the synthesis of cholesterol and fatty acids. On the other hand, it suppresses the expression of the cholesterol transporter ABCA1, impeding cholesterol efflux. These combined effects ultimately lead to significant accumulations of both cholesterol and triglycerides. Knockout of FDX1 can reduce the secretion of 3β-hydroxy-5-cholestenate (3BH5C) in hepatocytes and decrease cholesterol efflux from macrophages, while overexpression of FDX1 can rescue these phenotypes [57]. Specifically, FDX1 deficiency decreases ABCA1 protein expression while markedly elevating levels of the mature nuclear form of SREBP2 and its downstream target MVD; there is also a modest increase in the mature form of SREBP1 [5]. Of note, while SREBP2 activation is a well-established downstream consequence of FDX1 loss, it remains an open question whether SREBP2 directly binds to the FDX1 promoter to form a feedback regulatory loop. Current evidence does not address this transcriptional mechanism, representing a significant gap in understanding the full regulatory network. Notably, similar lipid abnormalities, encompassing disruptions in cholesterol, triglycerides, acylcarnitines, ceramides, phospholipids, and lysophospholipids, are detected in human colon cancer cells with FDX1 deficiency [5].

3.2. FDX2's auxiliary role in lipid metabolism

While FDX2 ablation does not cause embryonic lethality, it reproduces key aspects of FDX1-mediated lipid metabolism dysregulation and increases susceptibility to spontaneous tumorigenesis in mice [58]. In human HCC cell lines (HLF and Huh 7), knockdown of FDX2 increases the transcriptional levels of SREBP1/2 target genes (such as FASN and SCD1) and enhances lipid droplet accumulation. Similar to FDX1, the activation of SREBP2 upon FDX2 deficiency is clearly documented; however, whether SREBP2 directly transcriptionally regulates FDX2 expression through promoter binding remains unexplored. This mechanistic ambiguity highlights a critical area for future research to elucidate potential cross-regulatory dynamics between FDX2 and SREBP2. The research team encountered challenges in constructing FDX2-knockout human cells, potentially due to compensatory mechanisms stemming from FDX1's dominant role in lipid metabolism [58]. Interestingly, FDX2 serves as a critical component of the mitochondrial iron-sulfur cluster (ISC) assembly machinery, specifically facilitating the incorporation of iron into the [4Fe–4S] cluster of lipoyl synthase (LIAS) [47]. This connection suggests a potential link between FDX2's role in ISC biogenesis and its secondary effects on lipid metabolism, though this relationship warrants further investigation.

Collectively, these findings delineate a complex picture where FDX1 serves as the primary regulator of lipid homeostasis, with its deficiency triggering embryonic lethality and severe metabolic syndrome, while FDX2 exerts auxiliary effects, with its dysregulation contributing to tumorigenesis. The potential for SREBP2 to directly bind and regulate the promoters of both FDX1 and FDX2 adds a layer of transcriptional complexity to this network, but this hypothesis awaits experimental validation through chromatin immunoprecipitation (ChIP) and promoter-reporter assays. The lipid metabolic disorders orchestrated by FDX deficiencies, including lipid accumulation and alterations in signaling lipids like ceramides, are known to create a cellular environment permissive to lipotoxicity and oxidative stress. As such, these disruptions provide a plausible mechanistic link between the metabolic functions of FDXs and the potential to influence cell survival decisions, which we will explore in the subsequent section.

4. Dysregulation of FDXs: from physiological regulators to pathological effectors

The precise control of FDX1 and FDX2 is paramount for mitochondrial integrity, extending beyond their core functions to encompass a

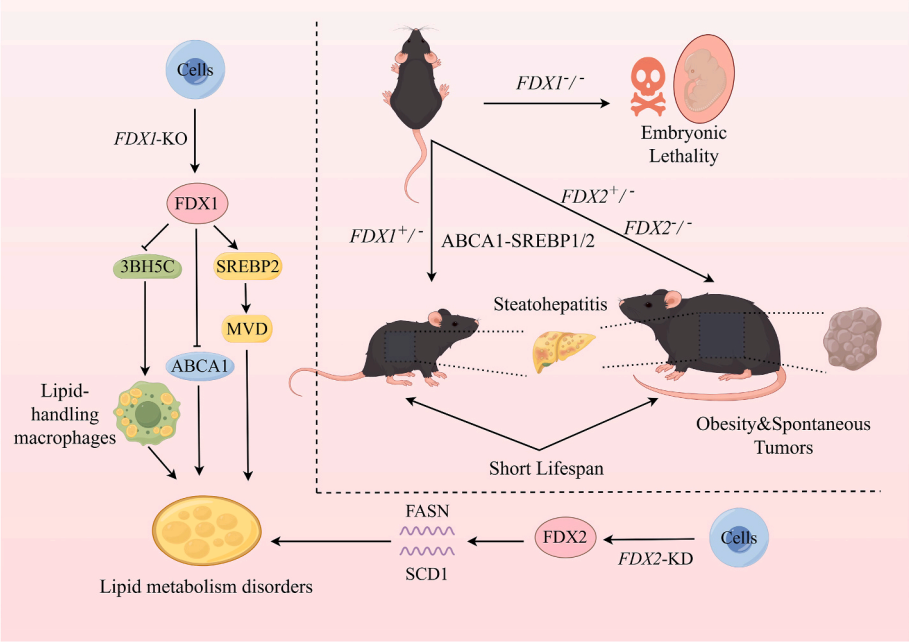

Fig. 2. Regulatory roles of FDX1/2 in lipid metabolism and embryonic development
FDX1 acts as a master regulator, suppressing SREBP1/2 to inhibit cholesterol/fatty acid synthesis and activating ABCA1 to promote the efflux. FDX2 defects influence lipogenesis via FASN/SCD1. FDX1 knockout causes embryonic lethality, while *Fdx1*$^{+/-}$ heterozygotes develop steatohepatitis and shortened lifespan. Deficiencies in FDX2 or combined *Fdx1*$^{+/-}$*Fdx2*$^{+/-}$ lead to obesity and the development of spontaneous tumors.

sophisticated, multi-layered regulatory network. This network operates at the transcriptional, post-transcriptional, and post-translational levels to dynamically fine-tune FDX abundance and activity in response to cellular cues. In addition to these dynamic mechanisms, FDX function is also influenced by genetic variations. These range from common polymorphisms that modulate disease susceptibility by affecting expression levels, to rare, inactivating mutations that directly cause severe monogenic disorders. The following sections detail this regulatory landscape, with the dysregulation of these layers being a hallmark of diverse pathological states.

4.1. *Multi-layered regulatory network governing FDXs*

An intricate network of regulatory mechanisms fine-tunes the functionality of FDXs. In terms of transcriptional and epigenetic control, the transcription of FDX1 is activated in colorectal cancer by the long noncoding RNA PVT1, which recruits transcription factors like SF1 to its promoter [59]. In glioma, FDX1 expression is upregulated through an m6A methylation-mediated mechanism, where the oncogene C-MYC enhances FDX1 expression via the reader protein YTHDF1 [60]. At the transcriptional level, the SREBP2, a master regulator of cholesterol synthesis, has been implicated in regulating *FDX*s expression, although direct binding to the *FDX*s promoter warrants further experimental validation [5,58]. Additionally, the transcription factor YY1 has been shown to suppress *FDX1* expression in hepatocellular carcinoma as part of the SEC14L3-ERK-YY1 axis [61]. Furthermore, common genetic polymorphisms can set the baseline for FDX1 expression. A prominent example is the rs10488763 variant, which disrupts transcription factor binding and reduces *FDX1* expression in macrophages, thereby increasing susceptibility to complex diseases such as coronary artery disease, as detailed in Section 6.3 [57,62]. Regarding post-transcriptional regulation, microRNAs enable rapid fine-tuning of FDX1 levels. For example, miR-3130–5p directly targets the 3′ untranslated region of FDX1 mRNA to trigger its degradation in hepatocellular carcinoma [63]. Additionally, RNA processing mechanisms contribute to FDX1 regulation; one such mechanism is lactate-induced NUDT21 lactylation, which promotes the formation of a longer 3′ untranslated region that is less efficiently translated, thereby suppressing FDX1 synthesis [64]. In the context of post-translational modification, these modifications can directly modulate FDX1 activity without changing its abundance. A key regulatory mechanism here is the phosphorylation of FDX1 at the Ser63 site by AKT1. This phosphorylation event inhibits FDX1's reductase activity, disrupts lipoylation processes, and leads to the induction of resistance to cuproptosis in triple-negative breast cancer [65]. Beyond transcriptional, post-translational, and direct inhibitory control, the human core iron-sulfur cluster (ISC) assembly machinery is also subject to a precise allosteric switch. A prime example is the FDX2-containing complex. Structural studies reveal a structural basis for this regulation, showing that FDX2 binds the NFS1-ISD11-ACP1 complex in two distinct conformations: a 'distal' state, where its [2Fe–2S] cluster is positioned ~23 Å from the ISCU2 active site, and a 'proximal' state, where key interactions bring the cluster within ~14 Å to enable efficient electron transfer for synthesis [30]. This conformational switch represents a natural allosteric system. Targeting these allosteric interfaces with small molecules designed to stabilize the inactive distal conformation provides a promising strategy to fine tune Fe–S cluster biogenesis. This strategy aims to modulate the synthesis rate in a context-dependent fashion, enabling precise therapeutic intervention while potentially circumventing the systemic toxicity that results from complete inhibition of this essential pathway.

4.2. *Pathological suppression of FDXs activity*

Pathological suppression of FDX activity transcends simple downregulation at the expression level. These microenvironments employ direct and indirect mechanisms to impair FDX function. In terms of inhibitory post-translational modifications, the hyperactivation of oncogenic signaling pathways in cancers directly inactivates FDX1, with AKT1-mediated phosphorylation serving as a typical example; this modification converts FDX1 from a pro-death protein into a pro-survival factor [65]. Under conditions of severe oxidative stress, another direct inhibitory mechanism comes into play: the iron-sulfur clusters within FDXs themselves can be damaged, resulting in the loss of their electron transfer capability. This damage creates a vicious cycle, where

mitochondrial dysfunction further exacerbates FDX impairment. Competitive ion inhibition represents yet another direct regulatory mechanism. As detailed in Section 2.1.2, zinc ions (Zn^{2+}) competitively inhibit FDX2's binding to the ISCU scaffold, directly hindering Fe–S cluster biogenesis [28]. This mechanism underscores how the dysregulation of other metal ions can specifically exert pathological suppression on FDX2 activity. Transcriptional silencing also contributes to the function suppression of FDXs. In addition to direct mechanisms, indirect inhibition occurs through energetic substrate depletion. Pathological conditions that deplete cellular NADPH pools, including metabolic syndrome and oxidative stress, indirectly cripple the entire FDXR-FDX electron transfer chain. This is because FDXR relies on NADPH as its primary electron donor, and the depletion of this substrate disrupts the chain's normal function.

Collectively, these mechanisms demonstrate that the pathological impairment of FDXs is an active process driven by the disease microenvironment. This acquired dysregulation, as well as underlying genetic alterations, can disrupt FDX homeostasis, with the consequences of rare loss-of-function mutations detailed in Section 6.5. This sets the stage for the execution of specific cell death pathways and the manifestation of diverse diseases, as elaborated in the following sections.

5. The role of FDXs in programmed cell death

FDX1 and FDX2 have specialized physiological roles in mitochondrial electron transfer, Fe–S cluster biogenesis, metal ion homeostasis and lipid regulation. These roles inherently position FDX1 and FDX2 as pivotal arbiters of cell fate. Crucially, the very pathways they sustain under physiological conditions can, when dysregulated, become powerful inducers of programmed cell death (PCD). This functional duality establishes FDXs as a central "metabolism-death axis" within the mitochondrion. The specific type of PCD initiated is directly determined by which FDX isoform and which metabolic process is compromised: FDX1, as the master of copper reduction and protein lipoylation, is the core molecular switch for cuproptosis. Copper overload leads to FDX1-mediated reduction of Cu^{2+} to Cu^{+}, triggering toxic aggregation of lipoylated TCA cycle proteins and proteotoxic stress. Both FDX1 and FDX2 are intimately linked to ferroptosis, but through distinct mechanisms rooted in their primary functions. FDX2 deficiency directly impairs Fe–S cluster biogenesis, causing mitochondrial iron overload and radical generation. FDX1 indirectly influences ferroptotic sensitivity by regulating the mitochondrial redox environment and membrane potential. FDX1 also modulates autophagy and apoptosis, often in a context-dependent manner, by influencing mitochondrial integrity, ROS production, and cellular metabolic status.

Thus, the regulation of PCD by FDXs is not a separate function but a pathological extension of their canonical mitochondrial roles. The following sections will dissect how the dysregulation of their specific physiological activities cascades into distinct cell death signaling pathways. We first summarize the distinct roles of FDX1 and FDX2 across major cell death pathways (Table 2), highlighting their unique contributions to cuproptosis regulation and shared functions in ferroptosis execution (Fig. 3).

5.1. The role of FDX1 in cuproptosis

Copper metabolism is an indispensable part of cellular physiological processes. The role of FDX in cellular copper metabolism has been described above. However, when copper homeostasis is disrupted, abnormal copper concentrations can affect normal physiological states. Copper ion depletion impairs mitochondrial ultrastructure. Lee et al. reported that the mitochondria-targeted copper chelator induces irreversible mitochondrial fragmentation in triple-negative breast cancer cells: the tubular network transforms into spherical morphology accompanied by cristae remodeling. Exogenous copper completely blocks this process, confirming that copper chelation is the direct cause of morphological changes [66]. Conversely, copper overload in Wilson's disease models also leads to mitochondrial damage, manifested as vacuolization, reduced cristae structure, and decreased activity of respiratory chain complexes I-III, all of which rely on functional Fe–S clusters [73]. Notably, mitochondrial morphological changes precede energy metabolism disturbances, which show a 50 % reduction in ATP, and apoptosis, representing an early event in copper depletion [74]. Obviously, excessive copper impedes the assembly of mitochondrial iron-sulfur proteins. The homeostasis imbalance of the accessible free Cu^{+} pool in the mitochondrial matrix directly impairs the maturation of iron-sulfur proteins. The molecular basis of this disruption involves a two-pronged mechanism whereby Cu^{+} both directly dismantles pre-assembled Fe–S clusters and potently inhibits their de novo biogenesis. In vitro reconstitution studies have demonstrated that Cu^{+} binds with high affinity to conserved cysteine residues on Fe–S cluster assembly proteins, such as Cys 144 and Cys 146 of ISCA2, thereby competitively excluding ferrous iron and blocking cluster transfer[75]. Moreover, Cu^{+} can directly attack the [2Fe–2S] clusters on GLRX5 and pre-formed [4Fe–4S] clusters, displacing iron and triggering cluster disintegration [75]. Our work further substantiates that copper overload inhibits the iron-binding capacity of core assembly proteins, including ISCA1, ISCA2, and ISCU, effectively halting de novo [2Fe–2S] cluster formation on ISCU [73]. This concerted attack culminates in a profound deficiency of functional Fe–S proteins, which ultimately leads to a significant reduction in the activity of key mitochondrial Fe–S enzymes, such as aconitase, and respiratory chain complexes I-III [73]. This body of evidence demonstrates that Cu^{+} disrupts Fe–S clusters through a direct, high-affinity interaction with cysteine ligands in the clusters and their assembly machinery, rather than through an indirect mechanism involving protease activation.

In fact, elevated copper levels function as an inducer of cytotoxic cell death [76,77]. This novel form of copper-dependent cell death was

Table 2
Mechanistic roles of FDXs in programmed cell death.

Cell Death Type	FDX1	FDX2	Reference
Cuproptosis	Core regulator: Reduces Cu^{2+} to Cu^{+} via reductase activity; Induces oligomerization of lipoylated proteins (DLAT/DLST); Exacerbate HSP70-mediated proteotoxic stress; Knockout confers resistance to copper ionophores.	Not reported.	[22,25,26,66–68]
Ferroptosis	Regulates mitochondrial membrane potential; PM2.5-induced upregulation promotes ROS and Fe^{2+} accumulation.	Knockout causes Fe–S protein (SDHB, MUTYH) deficiency; Regulates p53-dependent IRP2 activation – iron influx; Deficiency increases SLC39A14-mediated iron influx.	[12,14,47,69–72]
Autophagy	Promotes autophagic flux in PCOS granulosa cells (upregulates ATG3/7); Inhibits mitophagy in glioma (downregulates LC3-II).	Not reported.	[13,60]
Apoptosis	Loss in ovarian aging induces granulosa cell apoptosis (ROS-PI3K/AKT pathway); PM2.5 exposure triggers Leydig cell apoptosis.	Deficiency causes p53-dependent apoptosis; Fe–S cluster defects inactivate DNA repair enzymes (MUTYH, NTHL1).	[12,69,70]

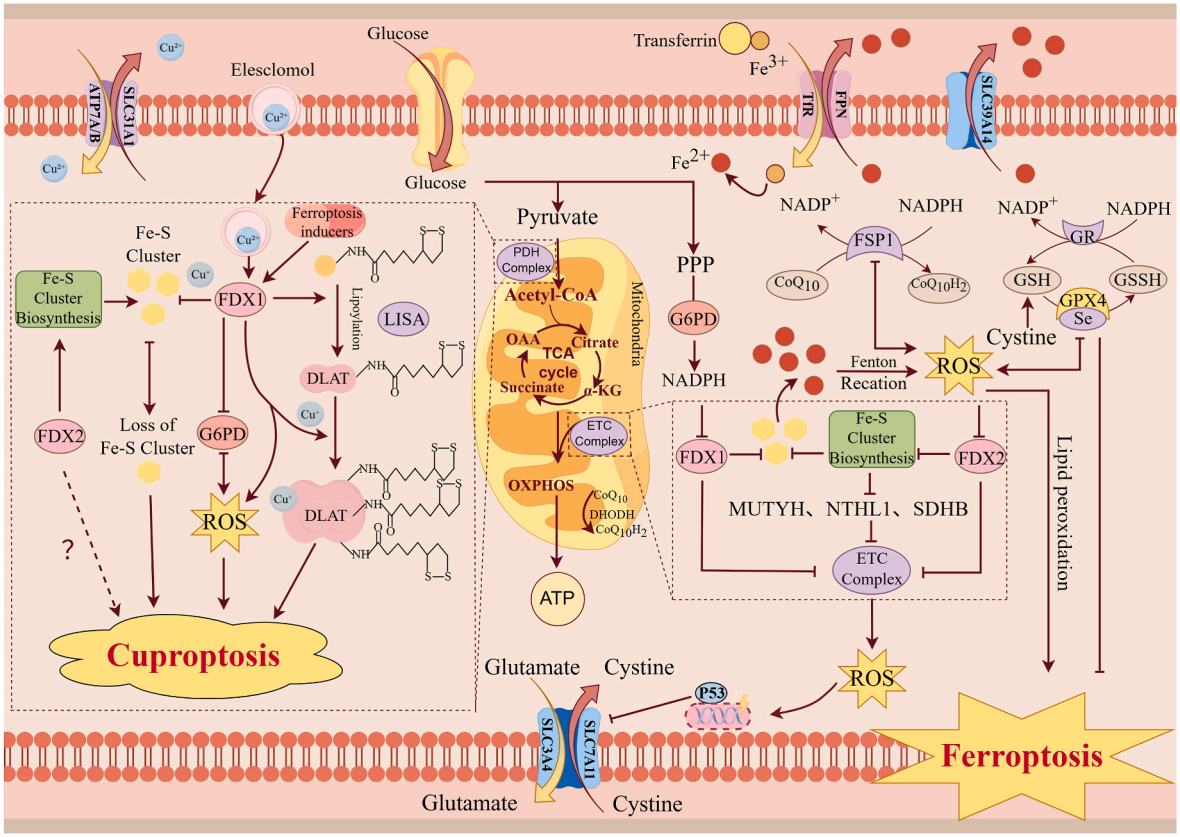

Fig. 3. FDX1-mediated the crosstalk of cuproptosis and ferroptosis Mitochondrial copper overload triggers FDX1 reduction of Cu^{2+} to Cu^{+}, inducing oligomerization of lipoylated TCA proteins (e.g., DLAT) and Fe–S cluster destabilization, culminating in proteotoxic stress and cuproptosis. FDX2 deficiency impairs Fe–S biogenesis, triggering SLC39A14-dependent mitochondrial iron retention and cytosolic Fe^{2+} release. Fe^{2+} triggers Fenton-reaction-mediated lipid peroxidation, which is further amplified by GPX4 suppression, ultimately inducing ferroptosis. The content of NADPH is crucial for the normal operation of the redox system, while FDX1-G6PD interaction disrupts NADPH regeneration, sensitizing to cuproptosis. FDX1's reductase function bridges copper toxicity and iron dysregulation, positioning it as a therapeutic target for OXPHOS-dependent cancers.

discovered by Tsvetkov et al. in 2022 and defined as cuproptosis [22]. In recent years, cuproptosis has attracted widespread attention. Specifically, it is a cell death triggered by copper-dependence and mitochondrial respiration regulation [78]. The core driving factor of this process is the accumulation of copper within cells, including Cu^{+} generated by the reduction of Cu^{2+} catalyzed by FDX1. Once these copper ions exceed the threshold, they become the decisive inducement for cuproptosis. Subsequently, copper ions execute a dual-pronged attack. As detailed in the preceding paragraph, Cu^{+} directly instigates the instability and loss of Fe–S cluster-containing proteins via its high-affinity binding to cysteine ligands in the clusters and their assembly machinery. Concurrently, copper ions target lipoylated components in the tricarboxylic acid cycle, such as DLAT in the PDH complex and DLST in the KGDH complex. Upon binding to the lipoic acid moieties of these proteins, copper induces abnormal oligomerization and aggregation. The combined loss of Fe–S clusters and aggregation of lipoylated proteins triggers severe mitochondrial dysfunction and proteotoxic stress, as marked by elevated HSP70. These interconnected processes constitute the defining hallmarks of cuproptosis. [22,26]. Cuproptosis is associated with the occurrence and development of various human diseases, which will be discussed in detail in the following sections.

FDX1 plays a core regulatory role in cuproptosis, which is essentially a pathological extension of its mitochondrial functions. FDX1 drives cuproptosis through a cascade of reductase activity, lipoylated protein oligomerization, and mitochondrial Fe–S cluster instability. This stands in sharp contrast to the protective role of FDX2 in Fe–S cluster biosynthesis, thus highlighting the unique position of FDX1 in copper metabolism toxicity.

As a key regulator of cuproptosis, FDX1 functions primarily as a reductase that leverages the mitochondrial NADPH-FDXR electron transport chain to reduce Cu^{2+} to the more redox-active Cu^{+}. This conversion initiates the hallmark events of cuproptosis: Firstly, it directly promotes the oligomerization of lipoylated proteins, such as DLAT and DLST in the PDH/KGDH complexes. Secondly, Cu^{+} concurrently disrupts Fe–S cluster proteins (e.g., ACO2, SDHB). These parallel actions collectively lead to metabolic collapse [22]. Moreover, the presence of FDX1 further exacerbates the proteotoxic stress caused by the upregulation of HSP70 [22]. FDX1 is also an essential upstream factor for maintaining protein lipoylation, as its absence results in a complete loss of lipoylated proteins and dysfunction of the TCA cycle [22]. Furthermore, FDX1 interacts with glucose-6-phosphate dehydrogenase (G6PD) in endometriosis, disrupting its structure and impairing the production of NADPH and GSH, thereby perturbing cellular redox homeostasis [79].

The indispensability of FDX1 is confirmed by functional evidence: its knockout significantly enhances cellular resistance to copper ionophores such as elesclomol and disulfiram, which has been verified in various cell lines, including OVISE, PSN1, K562, ABC1, and A549 [22,67,68]. Finally, the occurrence of cuproptosis specifically requires both the FDX1-lipoylation axis and active mitochondrial respiration, while inhibition of glycolysis can significantly increase cellular sensitivity to the process [20,80,81].

In terms of cross-interaction, the mitochondrial Fe–S cluster instability caused by FDX1-mediated cuproptosis is different from the Fe–S cluster defects caused by FDX2 deficiency (see section 2.2.2). When copper is overloaded, FDX1-reduced Cu^{+} disrupts Fe–S clusters such as

ACO2 and SDHB, forming a vicious cycle of lipoylated protein aggregation, Fe–S cluster disintegration and mitochondrial respiratory chain collapse. This association between copper metabolism and mitochondrial dysfunction makes FDX1 a key vulnerability in cancers dependent on oxidative phosphorylation, and therapeutic strategies targeting FDX1 may enhance the efficacy of copper-dependent cell death in treating refractory malignant tumors [80,81]. Currently, there are no reports on the role of FDX2 in cuproptosis, and future studies can further explore whether FDX2 compensates for FDX1 deficiency or antagonizes cuproptosis by maintaining Fe–S clusters.

5.2. The role of FDXs in ferroptosis

Ferroptosis is an iron-dependent non-apoptotic programmed cell death mode proposed by Dixon in 2012 [82]. It is characterized by iron overload-induced lipid peroxidation and GPX4 inhibition, both converging to cause membrane damage and cell death. Mechanistically, intracellular free Fe^{2+} catalyzes free radical generation through the Fenton reaction, the reaction between Fe^{2+} and H_2O_2, thereby driving lethal lipid peroxidation [82–84]. Morphologically, ferroptotic cells exhibit distinct mitochondrial abnormalities: condensation/swelling, increased membrane density, cristae loss, and outer membrane rupture [85–88]. GPX4 serves as the master regulator of ferroptosis. This antioxidant enzyme converts toxic lipid peroxides to harmless alcohols [88–90]. Its inhibition via genetic, pharmacological, or glutathione-depletion pathways causes catastrophic lipid peroxide accumulation, overwhelming cellular defenses and executing ferroptosis [91,92].

FDX1 and FDX2, which participate in mitochondrial Fe–S cluster biosynthesis and electron transfer, play crucial roles in regulating intracellular iron homeostasis and antioxidant responses [29,30]. Their involvement in ferroptosis is rooted in their specialized mitochondrial functions, with Fe–S cluster homeostasis serving as a central node linking their activities to the iron-dependent oxidative cascade. The molecular mechanism of ferroptosis follows a cascade of Fe–S cluster dysfunction-iron metabolism imbalance-lipid peroxidation, and the roles of FDX1 and FDX2 in this process are directly tied to their intrinsic mitochondrial functions.

FDX1 exerts indirect regulation by maintaining mitochondrial membrane potential. FDX1 deficiency disrupts mitochondrial respiratory chain function, leading to abnormal membrane potential elevation that further promotes Fe^{2+} release from mitochondria [14]. This is consistent with observations in ovarian cancer cell lines, where FDX1 knockdown increases mitochondrial membrane potential and lipid peroxidation, enhancing ferroptosis sensitivity [14]. In specific contexts such as PM2.5 exposure, FDX1 upregulation directly promotes ROS and Fe^{2+} accumulation-this effect is notably evident in Leydig cells, where PM2.5-induced FDX1 elevation triggers ferroptosis through ROS and Fe^{2+} overload [69].

FDX2 directly drives ferroptosis through its dual roles in Fe–S cluster synthesis and systemic iron metabolism regulation. As a high-affinity binding partner of the NFS1-ISD11-Acp1 complex, FDX2 is critical for the maturation of [2Fe–2S] to [4Fe–4S] clusters [31]. Its deficiency blocks Fe–S cluster assembly, impairing mitochondrial iron sequestration and causing massive release of free Fe^{2+} [12]. In human ovarian cancer cell lines, FDX2 knockout leads to widespread downregulation of Fe–S proteins (e.g., MUTYH, NTHL1, SDHB), triggering mitochondrial dysfunction, energy metabolism disorders, and intracellular free Fe^{2+} overload [12]. FDX2's regulatory role extends beyond iron-sulfur cluster biosynthesis through its involvement in the IRP2-mediated pathway. In HCT116 colon cancer cells and mouse embryonic fibroblasts (MEFs), FDX2 deficiency increases IRP2 expression, altering the expression of iron transporters (e.g., TfR1) and ferritin (FTH1) to enhance iron uptake [70,71]. This regulation occurs through p53-dependent ferroptosis pathways, where FDX2 depletion leads to mitochondrial iron overload and oxidative stress-induced DNA damage [69,70]. In HCC cells, FDX2 knockdown strongly activates the iron starvation response, upregulating the metal transporter SLC39A14 to promote iron influx and subsequent lipid peroxidation accumulation [72]. The accumulated Fe^{2+} catalyzes lipid peroxide generation through the Fenton reaction, while Fe–S cluster defects induced by FDX2 deficiency impair Fe–S-dependent enzymes such as aconitase and respiratory chain complexes [12,47], rendering cells unable to clear peroxides. All these promote the production of ROS [93,94]. Consequently, FDX2-deficient cells exhibit heightened sensitivity to ferroptosis when GPX4/FSP1-mediated antioxidant pathways are inhibited [95].

The pathway specificity of FDX1 and FDX2 in ferroptosis reflects the functional differentiation of their mitochondrial roles. FDX1's regulation is indirect, centered on maintaining mitochondrial redox balance and membrane potential, with minor, context-dependent effects on Fe^{2+} levels that primarily modulate ferroptosis sensitivity [14]. FDX2 directly controls Fe–S cluster biosynthesis and iron transport, driving substantial Fe^{2+} accumulation that is universally observed and critical for initiating ferroptosis [12,72].

Cross-interactions between ferroptosis and other cell death pathways arise from the multifunctionality of FDXs in mitochondria, with Fe–S clusters acting as key hubs. A prominent example is the synergy with cuproptosis: recent studies have revealed a significant crosstalk between cuproptosis and ferroptosis, two distinct forms of regulated cell death with promising therapeutic potential in cancer [96,97]. This interplay is exemplified by metformin, which has been shown to enhance both cuproptosis and ferroptosis in breast cancer concurrently. A retrospective clinical analysis demonstrated that breast cancer patients treated with metformin exhibited significantly elevated expression of FDX1 and the ferroptosis marker acyl-CoA synthetase long-chain family member 4 (ACSL4). Notably, correlation analysis revealed a positive association between ACSL4 and FDX1 levels ($R = 0.51$, $P = 0.045$), suggesting a mechanistic link between these pathways [98]. Further supporting this crosstalk, ferroptosis inducers (e.g., sorafenib, erastin) were found to potentiate cuproptosis in HCC cells through FDX1 protein stabilization and promotion of copper-dependent lipoylated protein aggregation [99]. Our research team has extended these findings by demonstrating that in myelodysplastic syndromes (MDS), the combination of cuproptosis inducers (DSF/Cu or ES-Cu) with ferroptosis inducers (erastin, sorafenib, or simvastatin) synergistically enhances FDX1-mediated cuproptosis [100,101]. Specifically, during cuproptosis, Cu^{+} reduced by FDX1 directly disrupts the stability of iron-sulfur cluster proteins assembled by FDX2 [22], releasing Fe^{2+} that amplifies oxidative stress via the Fenton reaction. This forms a cascade of cuproptosis activation, Fe–S cluster disintegration and ferroptosis enhancement, which underlies the efficacy of such combinatorial therapies.

In summary, FDX1 and FDX2 form a hierarchical environment regulation-substrate supply mechanism in ferroptosis through their specialized mitochondrial functions. FDX1 indirectly regulates ferroptosis sensitivity by membrane potential, while FDX2 directly drives Fe^{2+} accumulation and Fenton reactions via Fe–S cluster synthesis defects and iron transport regulation. This division of Fe–S clusters serves as a core hub linking ferroptosis with cuproptosis and lipid metabolism, providing a theoretical basis for subtype-specific FDX targeting. For instance, FDX2-deficient tumors may exhibit increased sensitivity to GPX4 inhibitors, while FDX1-high tumors could be targeted with copper ionophores to enhance ferroptosis.

5.3. The role of FDX1 in autophagic cell death

Autophagy is an evolutionarily conserved degradation and recycling mechanism that maintains cellular homeostasis by eliminating damaged components or recycling nutrients through lysosomal degradation [102–104]. There are three main types of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy [105]. Among them, macroautophagy is the most prevalent form. In this process, a double-membrane-structured autophagosome encapsulates substances

in the cytoplasm and transports them to the lysosome for degradation [105]. Among these, mitophagy, a selective form of autophagy, is critical for mitochondrial quality control: it clears dysfunctional mitochondria to prevent oxidative stress and maintain cellular integrity, relying on pathways such as PINK1/Parkin-mediated recognition and engulfment of damaged mitochondria, though PINK1/Parkin-independent mechanisms also contribute to this complex process [102,106–109].

In autophagy, FDX1 exerts its function mainly by regulating mitochondrial quality control, serving as a bridge between mitochondrial status and autophagic signaling. In polycystic ovary syndrome (PCOS), FDX1 upregulation in theca cells correlates with increased transcription of autophagy-related genes (ATG3, ATG7, BECN1, LC3-II) in both clinical samples and DHEA-induced mouse models, while its knockdown suppresses autophagic flux in human ovarian granulosa cells [13]. This suggests FDX1 promotes autophagy in PCOS, likely by maintaining mitochondrial redox homeostasis and energy metabolism to support autophagic processes. Conversely, in achondroplasia (HCH), mutant FGFR3 (G382D) upregulates FDX1, which associates with Drp1-mediated mitochondrial fission, impairing chondrocyte autophagy, indicating FDX1 can also inhibit autophagy when linked to abnormal mitochondrial dynamics [98].

In HCC, FDX1 functions as a tumor suppressor by regulating redox homeostasis and mitophagy: its deficiency increases mitochondrial ROS levels, activating mitophagy and the PI3K/AKT pathway, while scavenging ROS attenuates these effects [41]. The PI3K/AKT axis serves as a common node in FDX1-mediated mitophagy regulation across different cancer types [110]. This connection is further supported by findings in LUAD, where FDX1 expression is significantly downregulated and correlates with poor patient survival [111]. Intriguingly, FDX1 overexpression in LUAD cells suppresses migration and invasion, while xenograft models demonstrate that FDX1 upregulation inhibits tumor growth. Mechanistically, FDX1 was found to upregulate GPRIN2 and inhibit the PI3K pathway [111]. Although this study did not explicitly link FDX1's effects to mitophagy, the conserved involvement of PI3K/AKT signaling across these contexts raises the possibility that FDX1 may influence LUAD progression, at least in part, through mitochondrial quality control.

In myocardial ischemia-reperfusion injury, Phillygenin (PHI) treatment upregulates autophagy-related genes (Beclin-1, ATG12, ATG13), activates the autophagy-lysosome pathway, and promotes CTR1 copper transporter degradation to suppress FDX1 expression, ultimately alleviating myocardial damage [112]. This highlights FDX1's role in tuning autophagy in response to mitochondrial stress beyond cancer contexts.

FDX1's regulation of autophagy exhibits striking context dependence, shaped by tissue-specific mitochondrial demands and microenvironmental cues. In PCOS, where ovarian cells require robust energy metabolism for steroidogenesis, FDX1 promotes autophagy to support mitochondrial health; in HCH, where chondrocyte function depends on stable mitochondrial dynamics, FDX1 upregulation disrupts autophagy by altering fission [13,98]. This specificity extends to disease states: HCC relies on FDX1 to limit excessive mitophagy triggered by ROS, and glioma employs FDX1 upregulation to inhibit mitophagy and downregulate LC3-II. LUAD uses FDX1-mediated PI3K inhibition to modulate autophagic flux, and myocardial cells employ FDX1 suppression to enhance protective autophagy [41,60,111,112].

Collectively, these findings indicate that FDX1 is crucial for autophagy regulation, with its effects dictated by mitochondrial status and cellular context. By balancing mitochondrial quality control with autophagic signaling, FDX1 influences cell survival and disease progression across diverse pathologies, including PCOS, cancer, and cardiovascular disorders. Future studies should explore FDX2's potential role in autophagy-including functional interactions with FDX1-and clarify the molecular mechanisms underlying FDX1's context-dependent effects, offering new therapeutic targets for autophagy-related diseases.

5.4. The role of FDXs in apoptosis

The mechanism of apoptosis involves multiple molecular pathways and signal transduction pathways. The initiation of apoptosis is usually triggered by endogenous and exogenous signals, ultimately leading to the orderly death of cells. The extrinsic apoptotic pathway is mainly mediated by death receptors. Firstly, death receptors such as Fas and tumor necrosis factor receptor (TNFR), upon binding to their ligands, activate downstream caspase-8, which in turn activates effector caspases such as caspase-3, resulting in the occurrence of apoptosis [113]. The intrinsic apoptotic pathway is mainly mediated by mitochondria. Intracellular stress signals cause an increase in the outer mitochondrial membrane permeability, leading to the release of cytochrome *c* into the cytoplasm. Cytochrome *c* then binds to Apaf-1 to form an apoptosome, which activates caspase-9. Subsequently, caspase-9 activates caspase-3, ultimately triggering apoptosis [114,115]. The Bcl-2 family tightly regulates this process, with anti-apoptotic members like Bcl-2 and Bcl-xL inhibiting pro-apoptotic proteins such as Bax and Bak to preserve mitochondrial integrity [116]. ROS, often a byproduct of mitochondrial stress, further amplifies apoptotic signals by disrupting mitochondrial membrane potential [117].

Mitochondria play a central role in apoptosis regulation, with their dysfunction frequently triggering programmed cell death through increased ROS production [118]. The FDX protein family emerges as a key regulator of mitochondrial-dependent apoptosis through multiple interconnected mechanisms. As essential mediators of Fe–S cluster biogenesis in mitochondria, FDXs maintain ETC functionality and oxidative phosphorylation efficiency, thereby directly influencing mitochondrial redox homeostasis [119].

FDX1's influence on apoptosis is closely linked to its role in maintaining mitochondrial homeostasis. In PCOS, elevated FDX1 expression in granulosa cells directly promotes apoptosis by upregulating autophagy-related proteins ATG3 and ATG7, leading to caspase-3 activation. Consistently, FDX1 knockdown reduces both basal and DHEA-induced apoptosis rates in KGN cells, confirming its pro-apoptotic role [13]. In ovarian aging, age-related decline in FDX1 expression reflects mitochondrial dysfunction rather than directly triggering apoptosis. FDX1 deficiency compromises TCA cycle and ETC activity, resulting in lipid peroxidation and granulosa cell vulnerability [120,121]. Restoring FDX1 levels via DHEA, coenzyme Q10, or T3 supplementation enhances mitochondrial respiratory efficiency, thereby indirectly mitigating oxidative stress-induced apoptosis [120,121]. These findings highlight that FDX1-mediated mitochondrial health dictates apoptotic susceptibility in a pathology-specific manner: an active apoptosis driver in PCOS, but a biomarker of mitochondrial decline in aging ovaries.

FDX2, as a critical regulator of Fe–S cluster biosynthesis, supports the function of Fe–S cluster-dependent DNA repair enzymes such as MUTYH and NTHL1. Its deficiency impairs DNA repair, accumulating ROS-mediated DNA damage and activating p53-dependent apoptosis in ovarian cancer cells [12]. In HCT116 cells and MEFs, FDX2 modulates IRP2 activity to influence p53 expression; its deficiency reduces p53 and p21 levels, weakening the DNA damage response and apoptotic capacity [70]. These findings connect FDX2's role in Fe–S cluster metabolism to genomic stability and apoptotic signaling.

The regulatory roles of FDX1 and FDX2 in apoptosis exhibit distinct contextual specificities. FDX1's effects are tied to metabolic demands, varying in contexts like PCOS, where autophagy-apoptosis crosstalk predominates, and ovarian aging, where metabolic decline drives apoptosis [13,121]. FDX2, by contrast, acts primarily through genomic stability pathways, with its deficiency consistently linked to DNA repair defects and p53 activation across cell types [12,70]. Cross-interactions between FDX-mediated pathways and other cellular processes further highlight their integrative roles. FDX1's regulation of autophagy in PCOS creates a link between metabolic stress and apoptotic outcomes [13], while FDX2's impact on DNA repair connects Fe–S cluster metabolism to genomic integrity checkpoints [12]. FDX1 and FDX2 intersect

with ROS signaling, albeit through different mechanisms: FDX1 via mitochondrial dysfunction, and FDX2 via impaired DNA repair, forming a redox-sensitive network modulating apoptotic thresholds.

Collectively, these findings demonstrate that FDX1 and FDX2 are key regulators of apoptosis, with FDX1 linking mitochondrial function and metabolic state to apoptotic pathways, and FDX2 connecting Fe–S cluster-dependent genomic stability to apoptotic signaling. Understanding these mechanisms provides insights into apoptotic dysregulation in diseases such as PCOS, ovarian aging, and cancer, and highlights potential therapeutic targets for modulating apoptosis through FDX-mediated pathways.

6. The role of FDXs in diseases

As the central hub of cellular energy metabolism, the maintenance of mitochondrial functional homeostasis highly depends on the precise regulation of FDX1 and FDX2 (Section 2). When the functions of FDXs are abnormal, the core functions of mitochondria will undergo a cascade of disorders, promoting the disease process by triggering mitochondrial-dependent cell death pathways such as cuproptosis and ferroptosis (Section 5). As key mitochondrial-targeted Fe–S proteins, FDX1 and FDX2, through their specialized functions, become the core nodes connecting mitochondrial functions and disease phenotypes: Abnormalities in FDX1 disrupt the mitochondrial copper ion reduction balance and lipoylation modification, triggering proteotoxic stress and metabolic reprogramming. Defects in FDX2 directly impede mitochondrial Fe–S cluster assembly, leading to the collapse of the electron transport chain and iron metabolism imbalance.

This section systematically elaborates on how FDXs participate in disease development by regulating mitochondrial functions: (i) In cancer, the cuproptosis regulation of FDX1 and the Fe–S cluster-dependent function of FDX2 jointly affect tumor metabolism and the immune microenvironment; (ii) In neurodegenerative diseases, mitochondrial copper toxicity and lipoylation defects mediated by FDX1 exacerbate neuronal damage, while FDX2 deficiency impairs Fe–S cluster biogenesis and respiratory chain activity; (iii) In cardiovascular diseases, the abnormal lipid metabolism regulatory function of FDX1 disrupts vascular homeostasis and myocardial energy supply; (iv) In endocrine diseases, the steroidogenic function of FDX1 and the cuproptosis pathway jointly participate in metabolic disorders; (v) In genetic diseases, the copper metabolism defect of FDX1 and the Fe–S cluster synthesis disorder of FDX2 respectively lead to specific mitochondrial dysfunction syndromes (Fig. 4). These contents will reveal the disease-regulating mechanisms of FDXs as the "gatekeepers" of mitochondrial functions, providing a theoretical basis for therapeutic strategies targeting the mitochondrial-FDXs axis.

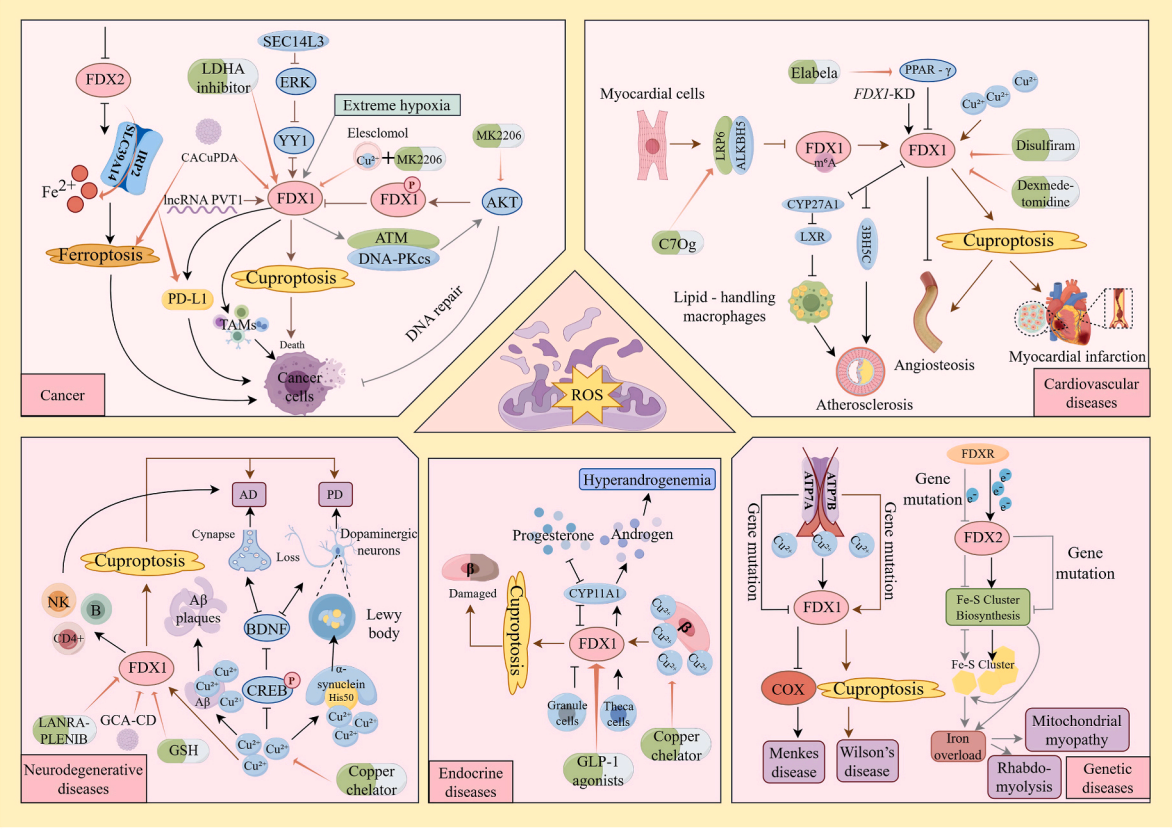

Fig. 4. FDXs as central regulators of mitochondrial pathology across disease spectrums
As core regulators of mitochondrial homeostasis, FDX1 and FDX2 control mitochondrial homeostasis and cell death through multiple pathways, thus linking mitochondrial dysfunction to major diseases. In cancer, FDX1 drives cuproptosis by mediating copper-dependent lipoylation of mitochondrial proteins, while FDX2 regulates Fe–S cluster assembly to control iron metabolism and ferroptosis. Their interplay amplifies oxidative stress, linking tumor metabolism to cell death pathways. In neurodegenerative diseases, FDX1 mediates copper toxicity in AD and PD via impaired copper reduction and lipoylation defects. Copper overload disrupts mitochondrial function and induces cuproptosis, exacerbating neurodegeneration. In cardiovascular diseases, FDX1 dysregulation disrupts lipid metabolism and causes copper-induced cuproptosis in ischemic cardiomyopathy and atherosclerosis. Mitochondrial dysfunction further destabilizes vascular homeostasis. In endocrine diseases, FDX1 integrates steroidogenesis and copper-dependent β-cell cuproptosis in diabetes. Tissue-specific roles in PCOS and T2DM highlight its dual functions. In genetic diseases, FDX1 defects cause copper dyshomeostasis in Menkes/Wilson disease, while FDX2 mutations disrupt Fe–S cluster biogenesis, leading to mitochondrial myopathies and lactic acidosis. Treatment interventions target copper chelation, iron-sulfur cluster restoration, or mitochondrial protection, highlighting FDXs as key nodes linking the regulation of mitochondrial function to disease outcomes.

6.1. The role of FDXs in cancer

Cancer remains one of the most formidable global public health challenges, with escalating incidence and mortality rates significantly impeding improvements in life expectancy worldwide [122]. Despite advances in oncology, effective cancer treatment continues to face substantial challenges due to the intrinsic complexity of malignant tumors. Tumorigenesis is driven by multiple hallmark biological processes, including: (i) sustained proliferative signaling, (ii) evasion of growth suppression, (iii) resistance to apoptotic cell death, (iv) replicative immortality, (v) angiogenesis induction, (vi) activation of invasion and metastasis, (vii) metabolic reprogramming, and (vii) immune evasion. Furthermore, the dynamic tumor microenvironment adds another layer of complexity to cancer pathogenesis and therapeutic resistance [123].

At the core of these pathological processes lies mitochondrial function – a hub for energy metabolism, cell death regulation, and redox balance – where members of the ferredoxin family, FDX1 and FDX2, exert unique and critical roles. Their core biological functions are intimately linked to mitochondrial integrity: FDX1 is involved in copper metabolism and lipoylation of mitochondrial proteins [124]. Emerging evidence highlights cuproptosis, a copper-dependent, mitochondria-mediated cell death pathway, as a critical modulator of tumor immunity and clinical outcomes across various cancer types, with FDX1 serving as its central mediator [125]. In contrast, FDX2 influences mitochondrial function, iron metabolism, and genomic stability in cancer through its key involvement in iron-sulfur cluster biogenesis. Collectively, FDX1 and FDX2 connect mitochondrial function to cancer hallmarks such as metabolic reprogramming, resistance to cell death, and immune evasion, making their mitochondria-centered mechanisms pivotal for understanding cancer pathogenesis and developing targeted therapies.

6.1.1. The role of FDX1 in cancer

As an essential mitochondrial iron-sulfur protein, FDX1 exerts diverse roles in cancer through mechanisms deeply rooted in mitochondrial function. Its biological activities, spanning lipoylation of mitochondrial enzymes, regulation of metabolic pathways, and mediation of cuproptosis, are all tightly linked to mitochondrial homeostasis, forming a functional network that extends from basic mitochondrial processes to the complex pathogenesis of tumors. Exploring these roles reveals how FDX1's mitochondrial functions shape cancer development, therapeutic responses, and immune interactions. At the mitochondrial level, FDX1 mediates lipoylation of mitochondrial enzymes (e.g., DLAT), a process critical for TCA cycle function and mitochondrial metabolic integrity. This lipoylation capacity directly underlies its role as the central executor of cuproptosis [126]. In cancer, FDX1-mediated cuproptosis exhibits a dual role shaped by its mitochondrial lipoylation function. In KIRC/LUAD, downregulated FDX1 impairs lipoylated protein oligomerization, reducing cuproptosis sensitivity and promoting tumor survival. In STAD/GBM, upregulated FDX1 enhances lipoylation-dependent cuproptosis, increasing sensitivity to chemotherapy (e.g., 5-FU) [111,125,127,128]. The molecular basis for these opposing expression patterns appears to be rooted in the distinct metabolic adaptations of different tumors to their microenvironment. In GBM, which is characterized by severe hypoxia and a reliance on mitochondrial respiration for invasion, FDX1 is upregulated, potentially as an adaptive response to maintain electron transfer and OXPHOS under metabolic stress [129]. Conversely, in KIRC, where VHL loss leads to constitutive HIF activation and a pseudo-hypoxic state, there may be a selective pressure to downregulate FDX1. This adaptation could serve to decouple the lipoylation-dependent TCA cycle flux from the risk of FDX1-mediated cuproptosis, thereby evading a metabolic vulnerability that might otherwise be exacerbated by the hypoxic tumor milieu [125,130]. This duality is further regulated by mitochondrial redox and copper metabolism: FDX1 reduces Cu^{2+} to Cu^{+} in mitochondria, and the FDX1-Cu^{+} complex drives lipoylated protein aggregation, triggering cuproptosis [111,131]. Therapeutically, this mitochondrial mechanism is targetable. Copper ionophores like ES may enhance FDX1-mediated cuproptosis in FDX1-high cancers [131,132]. However, AKT1-mediated phosphorylation of FDX1 at Ser63 disrupts its mitochondrial function, reducing DLAT lipoylation and shifting metabolism from mitochondrial oxidative phosphorylation to glycolysis, thereby resisting cuproptosis [65]. This resistance is reversible: Combining AKT inhibitor MK2206 with ES restores FDX1's mitochondrial lipoylation capacity, re-sensitizing TNBC to cuproptosis in vitro and in vivo [65].

Additionally, multiple regulatory axes control FDX1 activity in cuproptosis. In esophageal squamous cell carcinoma (ESCC), L-lactate induces NUDT21 lactylation via AARS1, promoting 3′ UTR lengthening of FDX1 mRNA and translational suppression, correlating with poor prognosis in LDHA/NUDT21-high tumors [64]. In HCC, copper activates SEC14L3, which suppresses ERK/YY1 signaling to elevate FDX1 expression, forming a feed-forward loop that enhances cuproptosis sensitivity and establishes the SEC14L3-ERK-YY1-FDX1 axis [61]. MiR-3130–5p inhibits FDX1 by binding its 3′ UTR, worsening HCC prognosis [63]. In colorectal cancer, lncRNA PVT1 activates FDX1 transcription via direct promoter binding (with H3K27ac deposition) and recruiting transcription factor SF1 [59]. In OC, FDX1 expression governs sensitivity to ES-Cu-driven cuproptosis in a dose-dependent manner; its depletion confers resistance, whereas enforced overexpression heightens susceptibility. This same axis also modulates the cellular response to cisplatin [132]. In pancreatic cancer (PC), chlorophyllin (CHL) induces cuproptosis sequentially: depletes GSH/elevates ROS, releases free Cu^{2+}, which binds FDX1 to drive DLAT oligomerization. It is important to note that this synergistic activity, mediated through the FDX1 pathway, has been demonstrated in preclinical studies including cell-based assays and mouse xenograft models, and its efficacy in human patients remains to be evaluated [133]. In STAD, FDX1 knockdown increases 5-FU/cisplatin IC50, confirming its role in chemosensitivity [127]. In multiple myeloma, elesclomol overcomes proteasome inhibitor resistance by disrupting the mitochondrial Fe–S cluster pathway [67].

FDX1's mitochondrial functions extend to metabolic regulation, linking mitochondrial metabolism to tumor progression. Its role in the TCA cycle regulation influences cancer cell metabolic reprogramming, a hallmark of malignancy. In KIRC, FDX1 expression correlates with genes involved in the TCA cycle and OXPHOS, indicating its role in maintaining mitochondrial energy metabolism [125,130,134]. Pan-cancer analyses further link FDX1 to fatty acid metabolism and PPAR signaling pathways intertwined with mitochondrial lipid metabolism [41,135–137]. In OC cells, FDX1 activation during chronic cuproptosis induction upregulates mitochondrial cholesterol biosynthesis genes, elevating total cholesterol levels. This suggests cross-talk between FDX1's mitochondrial function and cholesterol metabolism, as cholesterol depletion mitigates cuproptosis cytotoxicity [132]. Building upon the observation that FDX1 upregulation enhances chemosensitivity in STAD, comprehensive bioinformatic analyses provide deeper mechanistic and clinical insights. A landmark study by Zuo et al. integrating data from TCGA, GTEx, and GEO, established FDX1 as a pivotal cuproptosis regulator in this malignancy. They demonstrated that high FDX1 expression is a significant prognostic factor associated with improved survival. It was significantly correlated with elevated tumor mutation burden (TMB, $r = 0.240$, $p < 0.001$) and microsatellite instability (MSI, $r = 0.247$, $p < 0.001$), suggesting enhanced tumor immunogenicity. However, this coexisted with an overall reduction in immune cell infiltration, presenting a complex interplay. Crucially, their drug sensitivity analysis confirmed that FDX1-high tumors exhibit significantly increased sensitivity to first-line chemotherapeutic agents, including cisplatin ($p = 0.012$) and 5-fluorouracil ($p = 0.034$). These findings collectively position FDX1 as a multifaceted biomarker with utility in prognostic prediction and chemotherapy guidance, underscoring its value in formulating individualized treatment strategies for

STAD patients [127]. Under extreme hypoxia, glioblastoma cells upregulate FDX1 to preserve mitochondrial function. FDX1 interacts with DNA damage response kinases (ATM/DNA-PKcs) in mitochondria, activating AKT and enhancing DNA repair, enabling tumor cells to survive hypoxic stress and resist radiation [129]. These metabolic roles directly affect therapeutic responses. High FDX1 expression increases sensitivity to 17-AAG (a mitochondrial HSP90 inhibitor) in pan-cancer analyses, while conferring cisplatin resistance in ovarian/cervical cancers and enhancing oxaliplatin sensitivity in HCC [14,25,138,139].

FDX1's immune regulatory roles in cancer are indirectly linked to its mitochondrial functions, as mitochondrial metabolism shapes the tumor microenvironment (TIME). Single-cell sequencing shows FDX1 is highly expressed in tumor-associated macrophages (TAMs), cells dependent on mitochondrial metabolism for function [134]. In KIRC, low FDX1 correlates with abnormal immune infiltration and advanced disease [140]. In STAD, high FDX1 associates with favorable profiles: activated $CD4^+$ T memory cells, follicular helper T cells, M1 macrophages, and negatively correlates with resting NK cells/monocytes [127]. FDX1 upregulates PD-L1 (CD274), likely via mitochondrial redox-dependent transcriptional control. In KIRC, LUSC, glioma, and melanoma, FDX1 expression positively correlates with PD-L1 [125,141,142]. In gliomas, FDX1 regulates PD-L1 via NOD-like receptor signaling activation, pathways influenced by mitochondrial stress [141]. In gliomas, this is mediated by NOD-like receptor signaling activation, pathways associated with mitochondrial stress [141]. Copper-doped nanoparticles (CACuPDA) were found to effectively compromise mitochondrial function and trigger dual FDX1-dependent cuproptosis and ferroptosis through depletion of the GSH/GPX4 antioxidant axis. This dual cell death mechanism causes extensive tumor lysis and significantly reprograms the immunosuppressive microenvironment, as evidenced by increased cytotoxic T cells and reduced regulatory T cells. Notably, this approach synergizes remarkably with *anti*-PD-L1 therapy, enhancing tumor suppression by approximately 5-fold compared to *anti*-PD-L1 monotherapy [143]. This synergy highlights how FDX1's mitochondrial-mediated cell death modulates immune responses.

The expression patterns of FDX1 are closely related to its mitochondrial functions. In KIRC, LUAD, LIHC, and CRC, FDX1 is downregulated, impairing mitochondrial lipoylation/cuproptosis and promoting survival [125,130]. In KIRC, reduced FDX1 correlates with higher grades, advanced TNM stages, and poor survival, partly via EMT promotion through FMR1-ALCAM dysregulation [140,144]. In OC, FDX1 mRNA is decreased in tumors vs. normal ovarian epithelium [132]. Conversely, FDX1 is overexpressed in glioblastoma, gastric adenocarcinoma, and endometrial carcinoma, potentially supporting mitochondrial metabolism for proliferation [25,145]. In STAD, high FDX1 associates with better survival, 5-FU/cisplatin sensitivity, and higher tumor mutational burden (TMB) and microsatellite instability (MSI) [127]. In glioma, FDX1 is part of a cuproptosis-related gene signature with prognostic value [128]. The activity of FDX1 is regulated by phosphorylation, lactylation, and multiple mechanisms at the transcriptional and epigenetic levels. These mechanisms act together on mitochondrial function, providing entry points for targeted interventions [59,61,63–65]. For example, LDHA inhibitors targeting lactate-mediated FDX1 suppression could restore mitochondrial-dependent cuproptosis in ESCC [64]. Collectively, FDX1's roles in cancer stem from mitochondrial functions, connecting lipoylation, metabolism, cuproptosis, immune modulation, and clinical outcomes, supporting its potential as a mitochondrial-centric therapeutic target.

6.1.2. The role of FDX2 in cancer metabolism is rooted in Fe–S cluster biogenesis and iron homeostasis

As the master regulator of Fe–S cluster biogenesis, FDX2 contributes to cancer pathogenesis by playing a fundamental role in mitochondrial function, iron metabolism, and the maintenance of genomic stability. Its function is critical for the activity of numerous mitochondrial and nuclear proteins, including ETC complexes, key metabolic enzymes, and DNA repair machinery [12]. Deficiency in FDX2 disrupts Fe–S cluster biogenesis, leading to a cascade of detrimental effects: impaired mitochondrial ETC function reduces ATP production, dysregulated iron homeostasis causes mitochondrial Fe^{2+} overload, and subsequent oxidative stress elevates ROS. This convergence of metabolic and genotoxic stress is a hallmark of cancer cell vulnerability. In ovarian cancer cells, loss of FDX2 creates a state of heightened sensitivity to ferroptosis. The resulting iron overload and lipid peroxidation predispose cells to this iron-dependent form of cell death. This vulnerability is profoundly exposed when combined with pharmacological inhibition of the GPX4 or FSP1 pathways [12]. This synthetic lethal interaction underscores that FDX2 deficiency, while manageable under basal conditions, pushes cells beyond a survivable threshold upon disruption of parallel antioxidant systems. Consequently, FDX2 status emerges as a critical biomarker for predicting sensitivity to therapeutic ferroptosis inducers.

FDX2-deficient mice develop lipid metabolism disorders, spontaneous tumors (lymphomas, sarcomas), and steatohepatitis; lipidomics shows disrupted cardiolipin and ceramide metabolism, which may promote malignancy by altering membrane dynamics and signaling [58]. FDX2 maintains genomic stability, as Fe–S cluster-containing proteins are essential for DNA repair. Impaired Fe–S cluster formation due to FDX2 dysfunction can lead to genomic instability, a driver of tumor initiation. Mendelian randomization and co-localization analyses highlight the 19q13.32 region tagged by rs78295726, a locus adjacent to FDX2, as contributing to multiple myeloma risk specifically in the context of COVID-19 hospitalization. This genomic convergence implicates COVID-19-driven cellular stress and potentially perturbed Fe–S cluster homeostasis in myelomagenesis [146]. FDX2's role in cancer is context-dependent: its deficiency can promote tumorigenesis in some cases, while in others, it may act as a tumor suppressor by maintaining mitochondrial and genomic integrity. Elucidating the precise regulatory networks governing FDX2 in different cancer types, and exploring its synergistic potential with existing modalities, represent critical avenues for future research that could unlock novel, FDX2-directed therapeutic strategies.

6.1.3. Cross-talk between FDX1 and FDX2 in cancer

In tumors, FDX1 and FDX2 crosstalk through Fe–S cluster homeostasis: FDX1-mediated cuproptosis disrupts Fe–S clusters assembled by FDX2, amplifying oxidative stress and synergizing with ferroptosis inducers (e.g., CHL in PC) [133]. Beyond cuproptosis, they interact through iron and copper metabolism. FDX2's role in Fe–S cluster biogenesis affects iron availability, which influences FDX1-mediated copper homeostasis. Conversely, FDX1-regulated copper levels may impact FDX2's Fe–S cluster synthesis, as copper can displace iron in some metalloproteins. In ovarian cancer, FDX2 deficiency-induced ferroptosis can be enhanced by FDX1-mediated copper accumulation, with both pathways contributing to lipid peroxidation and mitochondrial damage [12].

Mitochondrial metabolism is another area of cross-talk. FDX1's involvement in the TCA cycle and FDX2's role in ETC function coordinate to maintain mitochondrial integrity. Disruption of either leads to mitochondrial dysfunction, and combined dysregulation exacerbates metabolic stress, driving tumor cells toward death or enhancing dependence on alternative energy sources. In hypoxic tumor microenvironments, FDX1 upregulation supports survival through DNA repair and metabolic adaptation, while FDX2 ensures functionality of Fe–S cluster-containing enzymes involved in hypoxia responses [35,129]. Their cross-talk extends to immune modulation in the TIME. FDX1's regulation of PD-L1 and immune cell infiltration can be influenced by FDX2-mediated Fe–S cluster metabolism, as immune cell function depends on Fe–S cluster-containing proteins [147]. FDX2-induced ferroptosis can release immunogenic cell death markers, which may synergize with FDX1-dependent cuproptosis to enhance anti-tumor immune responses. This is seen in copper-doped nanoparticles (CACuPDA),

which induce FDX1-dependent cuproptosis and ferroptosis, reprogramming the tumor immune microenvironment and enhancing *anti*-PD-L1 therapy efficacy [143]. Combining copper ionophores that trigger FDX1-dependent cuproptosis with inhibitors of Fe–S cluster synthesis, which impair FDX2 activity, could induce synthetic lethality in tumors reliant on both pathways. This dual-targeting strategy may enhance therapeutic efficacy across diverse cancers. Looking forward, the emerging technology of single-cell spatial transcriptomics holds immense potential to advance our understanding of FDX-mediated intercellular crosstalk fundamentally. This approach could directly interrogate the proposed metabolic symbiosis within the TME by simultaneously mapping the transcriptional profiles of cancer and stromal cells while preserving their spatial context. A critical hypothesis to test is whether 'FDX1-high' tumor cells, which are primed for cuproptosis and reliant on oxidative phosphorylation, coexist in specific niches with 'FDX2-high' TAMs that possess enhanced capacity for Fe–S cluster biogenesis. Such spatial association would suggest a previously unrecognized form of metabolic cooperation, potentially mediated by the exchange of iron/copper-containing metabolites or other signaling molecules. Decoding this intricate communication network could unveil novel therapeutic vulnerabilities. For instance, simultaneously targeting FDX1 in tumor cells to induce cuproptosis while disrupting the FDX2-supported pro-tumor functions of TAMs, thereby achieving a synergistic combined therapy that remodels the entire TME.

6.2. The role of FDXs in neurodegenerative diseases

Neurodegenerative diseases (NDs), including Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic Lateral Sclerosis (ALS), and Friedreich's Ataxia (FRDA), are driven by protein misfolding, mitochondrial dysfunction, and disrupted metal homeostasis [148]. These processes are tightly linked to the roles of FDX1 in copper reduction and lipoylated protein stability, as well as the role of FDX2 in Fe–S cluster biogenesis, making FDXs key players in the pathogenesis of NDs.

6.2.1. The role of FDX1 in AD and PD

Experimental evidence demonstrates that chronic copper exposure induces oxidative stress by generating ROS, impairing mitochondrial membrane potential, and exacerbating dopaminergic neuron loss in PD [149–151]. Mechanistically, abnormal FDX1 activity reduces GSH antioxidant capacity, leading to copper overload and cuproptosis [149–151], which is consistent with FDX1's role in mediating cuproptosis through lipoylated protein aggregation as described in earlier sections.

In AD, copper ions significantly accelerate amyloid-β (Aβ) aggregation. Under copper overload conditions, redox cycling promotes Aβ aggregation and plaque formation, generating ROS that damages mitochondrial membranes and proteins. This further depletes mitochondrial iron-sulfur clusters, impairs complex IV activity, and compromises ATP synthesis and mitochondrial function [152]. Studies in mice show that copper overload exacerbates cognitive deficits, causing cerebral and urinary copper accumulation. It also upregulates FDX1, DLAT, and HSP70 while reducing iron-sulfur cluster protein expression, collectively leading to neuronal degeneration and oxidative damage [153].

Single-cell transcriptomics has revealed cell-type-specific expression patterns of FDX1 in NDs. For example, while some studies report downregulated FDX1 mRNA in peripheral blood mononuclear cells (PBMCs) of AD patients, qPCR analysis of whole-blood samples shows upregulation, a discrepancy likely due to methodological differences and cell-sorting effects [154,155]. Importantly, FDX1 upregulation is specifically observed in B cells, NK cells, and $CD4^+$ T cells, suggesting its potential involvement in AD pathogenesis via immune-regulatory mechanisms [154]. As a critical gene in cuproptosis, FDX1 is intricately linked to mitochondrial function, particularly oxidative phosphorylation, and contributes to immune microenvironment remodeling. This makes FDX1 a promising therapeutic target, with potential drugs like LANRAPLENIB already identified as candidates for AD research [154]. Though direct evidence linking FDX1 to copper-induced Aβ aggregation is lacking, its roles in mitochondrial function and cuproptosis suggest it may indirectly influence Aβ oligomerization by modulating copper ion distribution and activity, warranting further investigation [152,153]. Additionally, copper-FDX1 interactions in AD impair synaptic energy metabolism, contributing to cognitive decline [155].

Mitochondrial dysfunction emerges as a convergent mechanism across NDs [156,157]. This is particularly evident in PD, copper disrupts α-synuclein (α-Syn) homeostasis, facilitating Lewy body formation via interactions with copper-binding residues (e.g., His 50) [158–160]. FDX1 expression is upregulated in PD, where it acts as a core molecular switch linking mitochondrial dysfunction, oxidative stress, and α-Syn pathology by driving the cuproptosis pathway – emerging as a novel target for PD treatment. Specifically, FDX1 overexpression directly induces dopaminergic neuron death and motor function impairment, confirming its critical role in PD pathogenesis [159]. This axis represents a promising therapeutic target, as guanidinium-modified calixarene/cyclodextrin (GCA-CD) nanomedicine rescues motor deficits in Parkinson's disease models by inhibiting FDX1/LIAS-mediated cuproptosis, reducing α-synuclein aggregation, and restoring mitochondrial function [159].

Copper homeostasis dysregulation, which is associated with the pathogenesis of both AD and PD, triggers a series of harmful events. In AD models under copper stress, for example, upregulation of FDX1 is associated with aggregation of lipoylated proteins (e.g., DLAT) and induction of oxidative stress; simultaneously, copper overload inhibits CREB phosphorylation and BDNF expression, leading to impaired synaptic plasticity [153,155,157]. In PD, copper homeostasis dysregulation similarly activates FDX1 to induce cuproptosis, promotes α-Syn aggregation, and depletes GSH, jointly exacerbating proteotoxic stress and the loss of dopaminergic neurons. Importantly, CREB inactivation is also a key feature of PD, driving dopaminergic neurodegeneration [153,161]. This dual pathway of cuproptosis and CREB inhibition highlights the common mechanisms of neurodegeneration in AD and PD.

6.2.2. Research gaps and potential mechanisms of FDXs in FRDA and ALS

FRDA is the most common autosomal recessive ataxia. It is caused by GAA repeat expansion in the FXN gene, which encodes frataxin, a mitochondrial protein involved in ISC biogenesis [162]. FRDA is characterized by the degeneration of large sensory neurons and spinocerebellar tracts, as well as an increased incidence of cardiomyopathy and diabetes [163]. A deficiency in FXN protein impairs the biosynthesis of Fe–S clusters [164]. FXN acts as a kinetic activator, accelerating persulfide transfer from NFS1 to ISCU. FXN deficiency slows persulfide transfer by ~6-fold, leading to toxic accumulation and impaired [2Fe–2S] cluster assembly on ISCU. This disrupts FDX2-dependent electron transfer, causing mitochondrial failure and oxidative stress. Indirectly, Fe–S defects impair lipoylation via LIAS dysfunction, contributing to metabolic disturbances. A key research gap is understanding the precise role of FDX2 in FRDA pathogenesis and whether it can be targeted therapeutically. While FXN is the primary defect, FDX2 dysfunction exacerbates pathology, as Zn^{2+} dysregulation may further inhibit FDX2 under FXN-deficient conditions. This dual hit of FXN deficiency and zinc imbalance amplifies mitochondrial dysfunction [28,52]. Additionally, calcitriol (1,25-dihydroxyvitamin D3) has shown potential in treating FRDA. Studies have demonstrated that calcitriol supplementation significantly increases frataxin protein levels and restores mitochondrial function in dorsal root ganglion (DRG) neurons, cardiomyocytes with frataxin deficiency, and lymphoblasts from FRDA patients [165]. Mechanistically, calcitriol may bind to the promoter region of the frataxin gene via the vitamin D receptor (VDR) to enhance its transcription. Meanwhile, calcitriol restores the level of FDX1, an electron donor for the calcitriol-synthesizing enzyme CYP27B1. It also improves the expression of the mitochondrial calcium exchanger (NCLX)

and mitochondrial membrane potential (ΔΨm), reduces oxidative stress and calcium dysregulation, and decreases apoptosis and neurite degeneration. Ultimately, these effects lead to increased cell survival. These findings support calcitriol as an accessible and affordable therapeutic strategy for FRDA [165]. However, the direct involvement of FDX2 in this process remains unclear, highlighting another area for investigation. Future studies should also examine whether targeting FDX2 activation or zinc chelation could modulate disease progression, and how FDXs interact with other ISC biogenesis components in FRDA models.

ALS is a fatal neurodegenerative disorder characterized by the progressive degeneration of motor neurons in the brain and spinal cord, leading to muscle weakness, paralysis, and ultimately respiratory failure within 3–5 years of onset [166]. Superoxide dismutase 1(SOD1) is a classic pathogenic gene for ALS. Mutations in this gene play a key role in the pathogenesis of ALS by mediating toxic gain-of-function mechanisms, including protein aggregation and oxidative stress [167]. A significant research gap exists in understanding the direct role of FDXs, particularly FDX2, in ALS pathogenesis. While mutations in SOD1 are known to cause familial ALS by promoting toxic gain-of-function, including aberrant peroxidase activity and protein aggregation [168], the connection to mitochondrial Fe–S cluster damage warrants further investigation. Studies have shown that the superoxide radical ($O_2^{\bullet-}$) can oxidize [4Fe–4S] clusters to [3Fe–4S] clusters in neural tissues of the SOD1G93A ALS rat model, concurrently releasing iron [169]. This Fe–S cluster damage is associated with mitochondrial dysfunction and is considered a potential source of redox-active iron contributing to oxidative stress in ALS [169]. This presents a critical research gap regarding whether the observed Fe–S cluster deficiency in ALS models is related to impaired FDX2 function. As the primary electron donor for Fe–S cluster biogenesis, FDX2 dysfunction could directly lead to the collapse of [4Fe–4S] clusters in critical mitochondrial complexes (I, II, III) and enzymes like aconitase, mirroring the damage induced by superoxide [29,169]. Future studies should investigate whether mutant SOD1 or the resulting oxidative stress directly or indirectly impacts FDX2 expression, activity, or its interaction with the Fe–S cluster assembly machinery (e.g., NFS1-ISD11-ACP1 complex, ISCU, ISCA1-ISCA2). Establishing a link between FDX2 dysfunction and the Fe–S cluster pathology observed in ALS would identify a novel mechanistic pathway and highlight FDX2 as a potential therapeutic target for stabilizing mitochondrial function in ALS.

Despite diverse genetic and clinical presentations in neurodegenerative diseases, mitochondrial dysfunction mediated by FDX1 and FDX2 represents a unifying pathogenic mechanism. FDX1 dysregulation disrupts copper homeostasis and triggers cuproptosis, whereas FDX2 deficiency impairs Fe–S cluster biogenesis, compromises respiratory chain activity, and heightens ferroptosis susceptibility. Both pathways converge on extensive mitochondrial ROS accumulation, promoting neuronal death via oxidative damage and energy failure [152,156]. Importantly, crosstalk exists between FDX1-modulated copper metabolism and FDX2-driven Fe–S cluster assembly: copper ions disrupt Fe–S cluster stability, and impaired Fe–S biogenesis aggravates iron dyshomeostasis, creating a self-reinforcing vicious cycle. Ultimately, FDX dysfunction culminates in respiratory chain failure and ATP depletion.

Interventional strategies targeting FDXs, such as metal ion chelators and supplementation with the antioxidant GSH, provide potential therapeutic directions for various neurodegenerative diseases by stabilizing metal ion homeostasis and redox balance [158]. Future therapies should consider cell-type-specific FDX modulation to ameliorate neuronal loss and neuroinflammation simultaneously. Current research still needs to address key gaps, particularly the role of FDX2 in amyotrophic lateral sclerosis-related Fe–S cluster deficiency and Friedreich's ataxia pathogenesis, as well as the potential value of FDXs in other neurodegenerative diseases.

6.3. The role of FDX1 in cardiovascular diseases

Cardiovascular diseases (CVDs) are one of the major causes of death and disability worldwide [170]. FDX1's role in cardiovascular health is deeply rooted in its core functions spanning lipid metabolism regulation and mitochondrial redox balance maintenance, with copper homeostasis serving as a critical intersecting factor [171]. Experimental studies highlight how disruptions in these functions drive vascular and myocardial damage through interconnected pathways.

FDX1's involvement in lipid metabolism is central to atherosclerotic processes. Functional validation shows FDX1 knockout decreases 3BH5C secretion by 82–90 % in hepatocytes and reduces macrophage cholesterol efflux by 15 %, while overexpression rescues these phenotypes [57]. This aligns with its role in regulating cholesterol transport, as single-cell analyses link FDX1 to CD52-high lipid-handling macrophage subpopulations in plaques, where it drives reverse cholesterol transport via CYP27A1 and LXR signaling [57]. Genetic association analyses identify an atherosclerosis-risk locus near *FDX1* (rs10488763) where the minor allele(T) disrupts AP-1/CEBPβ-mediated transcriptional activation, reducing FDX1 expression by 13 % in lipid-loaded macrophages [62]. This impairment compromises mitochondrial Fe–S cluster biogenesis, resulting in depressed respiratory chain activity and attenuated lipid clearance. The variant confers significant coronary artery disease risk, mechanistically linking FDX1 deficiency to foam cell formation in atherosclerotic plaques [62]. Metabolomics studies have shown that FDX1 influences the atherosclerotic process by regulating the synthesis of 3BH5C, a cholesterol metabolite in the acidic pathway, thereby affecting cholesterol efflux. A study of 8124 Asian individuals revealed that the risk allele rs10488763-T exhibits a significant population-specific effect. The impact of the FDX1-3BH5C pathway, marked by this allele, on coronary artery disease is substantially stronger in Asian populations compared to European populations, with a 5- to 6-fold difference in the effect size. This disparity highlights the population-specific regulatory mechanisms of the cholesterol metabolic pathway and reflects the unique pathophysiological characteristics of Asian populations in FDX1-mediated metabolism [57].

Mitochondrial dysfunction, another core FDX1 function, underpins multiple CVD pathologies. In ischemic cardiomyopathy (IC), FDX1 downregulation correlates with mitochondrial respiratory chain defects and oxidative damage, underscoring its potential as an immunobiomarker [172]. Pharmacological activation of FDX1 (e.g., dexmedetomidine) mitigates cerebral ischemia-reperfusion injury by reducing copper accumulation and preserving mitochondrial membrane potential, while FDX1 deficiency disrupts ETC activity, amplifying ROS production and lipid peroxidation in endothelial cells to destabilize atherosclerotic plaques [173,174]. This mitochondrial vulnerability extends to myocardial infarction (MI), where copper ion overload in infarcted tissue triggers FDX1-mediated cuproptosis, a process involving copper-dependent aggregation of lipoylated proteins (refer to Section 5.1). Cardiomyocytes respond through LRP6 nuclear translocation, which interacts with ALKBH5 to inhibit m^6A modification of FDX1, exacerbating copper toxicity [175]. Therapeutic strategies targeting this axis show promise: tribromobimane (TLB) improves cardiac function in doxorubicin-induced cardiotoxicity (DIC) by binding FDX1, reducing mitochondrial oxidative stress and cuproptosis [176]. DSF also protects against cerebral ischemia-reperfusion injury by inhibiting FDX1, regulating copper homeostasis, and alleviating inflammation via the HSP70/TLR4/NLRP3 pathway [177]. Broadly, alleviating oxidative stress and inhibiting cuproptosis exert cardioprotective effects [176,178,179].

FDX1's dual functions in lipid metabolism and mitochondrial health also influence vascular calcification. In vascular smooth muscle cells (VSMCs), elevated FDX1 and Slc31a1 levels coincide with increased copper levels and decreased Elabela expression. Administration of Elabela hinders calcification progression in VitD3-overloaded models by activating PPAR-γ signaling, which suppresses FDX1 expression and

rescues mitochondrial function [180]. Similarly, in post-MI contexts, chrysin-7-*O*-glucuronide (C7Og) reduces myocardial damage by inhibiting LRP6, thereby modulating FDX1-dependent mitochondrial stability [175].

These findings position FDX1 as a pleiotropic regulator of cardiovascular health, with its core functions in lipid metabolism and mitochondrial homeostasis driving pathogenesis across atherosclerosis, ischemia, and calcification. Targeting FDX1's context-specific roles through lipid metabolism modulation, mitochondrial protection, or cuproptosis inhibition offers diverse therapeutic avenues for CVD.

6.4. The role of FDX1 in endocrine diseases

FDX1 exerts pivotal effects in endocrine diseases through its dual core functions: steroidogenesis and copper metabolism-mediated cell death. These functions converge in conditions like type 2 diabetes mellitus (T2DM) and polycystic ovary syndrome (PCOS), driving pathological processes through tissue-specific mechanisms.

FDX1's role in steroid hormone synthesis, rooted in its function as an electron transporter for cytochrome P450 enzymes, underpins its tissue-specific effects in PCOS. As a key component of the FDXR-FDX1-P450scc complex, it supports cholesterol side-chain cleavage via CYP11A1, a rate-limiting step in steroidogenesis [181,182]. This process exhibits dysregulated tissue specificity in PCOS: in granulosa cells, FDX1 downregulation reduces CYP11A1 activity, impairing progesterone synthesis and disrupting the hormonal balance; conversely, in theca cells, FDX1 upregulation enhances mitochondrial steroidogenesis, accelerating cholesterol-to-androgen conversion and exacerbating hyperandrogenemia [183,184]. This imbalance in steroidogenesis, driven by FDX1's tissue-specific expression, directly contributes to PCOS pathophysiology. Therapeutically, GLP-1 agonists like liraglutide improve PCOS symptoms by upregulating FDX1 and mitigating oxidative stress, restoring the balance between androgen and progesterone synthesis [185].

In T2DM, FDX1's role in copper homeostasis and cuproptosis takes center stage, mirroring its cell death mechanisms in cancer but with distinct pathological consequences. Serum copper metabolism imbalance leads to mitochondrial copper accumulation in β-cells, activating FDX1-dependent cuproptosis [186,187]. This cuproptotic pathway impairs insulin secretion by compromising β-cell viability, a key feature of T2DM progression. The pathological reach of FDX1-mediated copper dysregulation extends beyond β-cells. In diabetes-associated cognitive dysfunction (DACD), neuronal copper overload exacerbates cuproptosis, as evidenced by reduced expression of FDX1, LIAS, and DLAT under high glucose/palmitic acid conditions [188]. Mitochondrial ROS production amplifies β-cell oxidative damage, while in diabetic retinopathy, FDX1 downregulation is linked to STAT1-mediated SLC31A1 overexpression, promoting microglial inflammation under hyperglycemic conditions [189,190]. Therapeutic strategies targeting FDX1's copper metabolic function show promise. Peripheral mitochondrial transplantation (Mito-PLt) suppresses cuproptosis by restoring copper homeostasis and neuronal function, while copper chelators or FDX1 agonists may preserve β-cell function by rebalancing copper levels [187,188,190]. These interventions align with FDX1's core role in regulating copper-dependent cell death, highlighting its potential as a target for T2DM and its complications.

The dual functions of FDX1, steroidogenesis and copper metabolism, position it as a critical regulator of endocrine-metabolic disorders. In PCOS, its role in steroidogenesis drives hormonal imbalance, while in T2DM, its mediation of cuproptosis disrupts β-cell function. These processes, though distinct, share a reliance on FDX1's mitochondrial localization and interaction with metal ions, linking back to its fundamental mechanisms outlined in earlier sections. Future research should focus on tissue-specific modulation of FDX1 to target these distinct pathways, optimizing therapeutic strategies for endocrine diseases.

6.5. The role of FDXs in genetic diseases

FDX1 and FDX2 contribute to distinct genetic disorders through their core functions – copper metabolism and Fe–S cluster biogenesis – both converging on mitochondrial integrity. Disruption of either pathway impairs mitochondrial function, driving distinct genetic disorders while sharing a common root in mitochondrial dysfunction.

6.5.1. FDX1-related genetic diseases

FDX1's role in mitochondrial copper metabolism is pivotal in two copper-related genetic disorders, with pathogenicity rooted in impaired mitochondrial copper utilization and redox homeostasis.

In Menkes syndrome, an X-linked recessive disorder from *ATP7A* mutations, FDX1's ability to reduce Cu^{2+} to Cu^{+} (a step critical for mitochondrial copper import) is compromised [191]. This impairs mitochondrial copper-dependent processes, including COX activity, a key component of the respiratory chain. Research shows FDX1-dependent reduction of ES-Cu^{2+} to ES-Cu^{+} enables mitochondrial copper release and respiratory function recovery; FDX1 knockout exacerbates mitochondrial dysfunction (e.g., decreased COX activity) and diminishes the therapeutic effect of ES, highlighting its role in sustaining mitochondrial respiratory chain function [20]. Conversely, Wilson's disease, caused by *ATP7B* mutations, leads to copper accumulation in mitochondria [192]. FDX1 expression is upregulated here, with its copper-reducing activity amplifying toxic Cu^{+} levels in the mitochondrial matrix. This overload disrupts redox balance, inducing mitochondrial ROS production and impairing respiratory chain function. Clinical studies show FDX1 levels decrease after treatment with sodium dimercaptosulphonate, linking its activity directly to mitochondrial copper toxicity [193]. FDX1 dysfunction disrupts mitochondrial copper homeostasis in both Menkes and Wilson's diseases through opposing mechanisms. In Menkes disease, impaired FDX1 activity leads to mitochondrial copper deficiency, starving respiratory enzymes of their essential cofactor. Conversely, in Wilson's disease, excessive FDX1-mediated Cu^{2+} reduction results in toxic Cu^{+} overload. Both scenarios ultimately cause mitochondrial respiratory failure.

6.5.2. FDX2-related genetic diseases

FDX2's role in mitochondrial Fe–S cluster biogenesis makes it critical for maintaining mitochondrial enzyme activity, with mutations disrupting this process and inducing mitochondrial energy failure.

Mutations in FDX2 disrupt [4Fe–4S] cluster synthesis, a process essential for mitochondrial respiratory chain complexes I-III and Fe–S-dependent enzymes like aconitase [48]. This directly impairs mitochondrial energy metabolism: homozygous FDX2 c.431C > T (p.P144L) mutations cause childhood-onset optic atrophy, myopathy, and leukoencephalopathy, with muscle biopsies showing SDH/COX deficiency (key mitochondrial enzymes) and iron deposition [45,194]. Other mutations (e.g., c.1A > T) reduce aconitase activity by 75 %, while c.12G > T and c.10A > T mutations induce rhabdomyolysis and lactic acidosis, all reflecting mitochondrial energy metabolism collapse due to Fe–S cluster depletion [195–197]. The interaction between FDX2 and FDXR is critical for electron transfer in Fe–S cluster synthesis: FDXR mutations (e.g., R392W, c.1174C > T) impair electrostatic interactions with FDX2, reducing electron transfer efficiency to just 30 % of normal levels [198–200]. This disruption in electron supply to FDX2 hinders Fe–S cluster biogenesis, leading to mitochondrial iron overload, complex I/II deficiencies, and lactate accumulation [199–201]. Concurrently, this electron transfer dysfunction compromises mitochondrial membrane potential and exacerbates ROS production, further amplifying mitochondrial damage [199–201]. Diagnostic markers such as elevated serum FGF21 and impaired respiratory chain enzyme activity further reflect mitochondrial stress induced by FDX2 defects [45,198,199]. Antioxidant therapies target mitochondrial ROS to mitigate this damage [195,202]. It is important to note that comprehensive epidemiological data, such as the population frequency of FDX2 mutations,

remain undefined due to the extreme rarity of these disorders. The current clinical understanding is built upon a constellation of consistent findings from individual case reports. These have firmly established that specific serum biomarkers, most notably lactic acid and FGF21, are consistently elevated in affected individuals and serve as reliable hallmarks of the severe mitochondrial dysfunction caused by FDX2 deficiency.

FDX1 and FDX2 drive genetic diseases through distinct pathways but converge on mitochondrial impairment. FDX1's copper metabolism defects disrupt mitochondrial redox balance and respiratory chain activity, while FDX2's Fe–S cluster defects cripple mitochondrial enzymes and energy production. Both pathways induce mitochondrial stress through mechanisms such as copper toxicity, Fe–S cluster depletion, or ROS overproduction, which underscores mitochondria as the central effector in FDX-related genetic disorders. This convergence highlights the need for therapies that target mitochondrial resilience while also modulating FDX1/FDX2 in an isoform-specific manner, as such approaches can address the root causes of these conditions.

As summarized in Sections 6.1-6.5, FDX1 and FDX2 exert distinct yet interconnected roles in disease pathogenesis, stemming from their core biological functions. FDX1, through its roles in copper metabolism and lipid metabolism, drives cuproptosis in malignancies and modulates metabolic balance in cardiovascular diseases and endocrine disorders. Its dysfunction disrupts mitochondrial redox homeostasis, linking copper overload/toxicity to pathologies like Wilson's disease and β-cell cuproptosis in T2DM. FDX2, centered on Fe–S cluster biogenesis, maintains mitochondrial enzyme function; its deficiency triggers Fe–S cluster defects, leading to mitochondrial myopathies, genomic instability, and tumor susceptibility. These mechanistic insights have accelerated targeted therapy development: strategies range from restoring FDX activity (e.g., copper chelators in Wilson's disease) to exploiting FDX-dependent vulnerabilities (e.g., cuproptosis inducers in chemotherapy-resistant cancers). To systematize these advances, we summarize disease-specific FDX dysregulation mechanisms and clinical associations, and overview FDX-targeting drugs with their mechanisms and therapeutic impacts (Tables 3 and 4). Together, these analyses highlight FDXs as pivotal nodes bridging mitochondrial metabolism and cell death pathways, underscoring their potential in precision medicine. Future efforts should prioritize isoform-specific drug design – leveraging FDX1's copper/lipid metabolism vs. FDX2's Fe–S cluster roles – and combinatorial strategies to address context-dependent functions across tissues.

7. Conclusions and prospects

The FDXs family, as a key hub linking mitochondrial metabolic reprogramming and programmed cell death, embodies its co-evolution from a basic electron transport role to disease-specific regulatory functions. This review clarifies how FDX proteins dynamically coordinate mitochondrial homeostasis through metabolic remodeling and precisely regulate multiple cell death signaling pathways, thus playing an important role in various diseases. The functions of FDX1 and FDX2 are both specific and synergistic, profoundly reflecting their integrated role in cell physiology.

First, the functional differentiation between FDX1 and FDX2 reveals their complex roles in coordinating metabolism with cell death pathways. As a core metabolic sensor, FDX1 directly regulates TCA cycle flux through protein lipoylation while governing copper metabolism and cuproptosis induction. FDX2, as the primary mediator of iron-sulfur cluster biogenesis, maintains respiratory chain function by supporting [4Fe–4S] cluster assembly in complexes I-III. Their interaction forms a central "metabolism-death axis" where FDX1-driven cuproptosis triggers Fe–S cluster dysfunction through Cu^{+}-mediated protein aggregation, while FDX2 deficiency promotes ferroptosis via iron overload and impaired antioxidant defenses due to loss of Fe–S enzymes like Aconitase 1. Beyond these pathways, FDX1 modulates apoptosis in granulosa

Table 3
Molecular mechanisms and clinical associations of FDXs-related diseases.

Disease Type	FDX1-Related Mechanisms	FDX2-Related Mechanisms	References
Cancer	Tissue-specific expression patterns (decreased in KIRC/LUAD/LIHC/CRC; increased in GBM/STAD); Regulates EMT through FMR1-ALCAM axis in KIRC; Induces cuproptosis via DLAT lipoylation; AKT1-mediated phosphorylation at Ser63 confers resistance; Correlates with PD-L1 expression and immune infiltration.	Deficiency causes lipid metabolism disorders and spontaneous tumors; Disrupts cardiolipin/ceramide metabolism; Induces ferroptosis through mitochondrial Fe^{2+} overload; rs78295726 locus associated with multiple myeloma risk.	[22,25,65,67,125,127,128,130,132,140,144]
Neurodegenerative Diseases	Upregulated in specific immune cells (B cells, NK cells, $CD4^{+}$ T cells)in AD; Mediates dopaminergic neuron death in PD; mpairs synaptic plasticity via CREB/BDNF suppression.	FDX2 dysfunction may contribute to ALS-related Fe–S cluster damage; In FRDA, FDX2 electron transfer is disrupted by FXN deficiency, impairing Fe–S cluster assembly.	[22,148–151,153,155–160,165,169]
Cardiovascular Diseases	rs10488763 polymorphism affects macrophage lipid metabolism; LRP6-ALKBH5 axis regulates m6A modification post-MI; Elabela/PPAR-γ axis regulates vascular calcification.	Not directly implicated.	[57,62,170–180]
Endocrine Diseases	T2DM: Induces β-cell cuproptosis through copper overload; PCOS: Differentially regulates steroidogenesis in granulosa/theca cells; Diabetic retinopathy: Associated with STAT1/SLC31A1 pathway.	Not directly implicated.	[181–190]
Genetic Disorders	Menkes disease: Mediates ES-Cu redox cycling; Wilson disease: Expression decreases after chelation therapy.	Mutations cause: Optic atrophy, Mitochondrial myopathy, Rhabdomyolysis (elevated CK), Leukoencephalopathy; Impairs FDXR interaction (reduced to 30 % electron transfer).	[20,45,48,191–202]

Table 4
Drugs targeting FDXs.

Drug/Intervention	Disease	Mechanism	Impact/Findings	Reference
Elesclomol-Cu	Ovarian cancer, TNBC, HCC	FDX1-mediated Cu^{2+}-Cu^{+} reduction.	First-in-class cuproptosis inducer; overcomes cisplatin resistance (synergizes with AKT inhibitor MK2206).	[22,25,65,67,68,132]
Disulfiram-Cu	MDS	FDX1-mediated Cu^{2+}-Cu^{+} reduction – cuproptosis induction.	Synergizes with ferroptosis inducers (erastin); penetrates the blood-brain barrier.	[100,101]
Chlorophyllin	Pancreatic cancer	Depletes GSH – activates FDX1-Cu^{+}.	Shows synergistic anti-tumor activity with gemcitabine in mouse models; clinical efficacy remains unknown.	[133]
Erastin/Sorafenib	Ovarian cancer	Exploits FDX2 deficiency-induced lipid peroxidation.	In FDX2-deficient cells, the sensitivity to GPX4 inhibitors can be greatly enhanced.	[12,99]
Simvastatin	MDS	Suppresses the mevalonate pathway.	Upregulates FDX1 to enhance cuproptosis sensitivity.	[101]
Liraglutide	PCOS	GLP-1 receptor-mediated FDX1 upregulation.	Improves granulosa cell mitochondrial function; reduces hyperandrogenemia.	[185]
DHEA/CoQ10	Ovarian aging	Restores FDX1-dependent steroidogenesis.	Rescues oocyte quality in animal models by restoring mitochondrial metabolism.	[120,121]
Guanidinium-modified calixarene/cyclodextrin	Parkinson's disease	Inhibits FDX1-LIAS cuproptosis axis.	Reduces α-synuclein aggregation; improves motor function in PD models.	[159]
Phillygenin	Myocardial ischemia-reperfusion	CTR1 degradation reducing FDX1 activity.	Protects cardiomyocytes from copper overload-induced damage.	[112]
Tribromobimane	Doxorubicin cardiotoxicity	Direct binding to FDX1 inhibiting cuproptosis.	Dose-dependently improves cardiac function (15 % LVEF increase).	[176]
Elabela	Vascular calcification	PPARγ-FDX1 axis regulation.	Inhibits vascular smooth muscle calcification by suppressing FDX1 overexpression.	[180]
Copper chelators (e.g., Penicillamine)	Wilson's disease	Copper load reduction modulating FDX1.	Normalizes FDX1 expression post-treatment; reduces liver copper accumulation.	[193]
Mitochondrial transplantation	Diabetes-associated cognitive dysfunction	Functional FDX1 replenishment.	Rescues neurons from cuproptosis; improves cognitive function in diabetes models.	[188]
Hypoxia-targeted radiotherapy	Glioblastoma	Inhibition of FDX1-ATM/DNA-PKcs pathway.	Doubles radiosensitivity in FDX1-knockout hypoxic tumor cells.	[129]
CACuPDA nanoparticles	Lung cancer	Concurrent induction of cuproptosis and ferroptosis.	5-fold enhanced tumor suppression when combined with *anti*-PD-L1 therapy.	[143]

cells by disrupting mitochondrial membrane potential, and FDX2 deficiency induces p53-dependent apoptosis in ovarian cancer through impaired DNA repair resulting from MUTYH/NTHL1 dysfunction. The regulatory dichotomy extends to autophagy-FDX1, which promotes autophagic flux in PCOS theca cells while suppressing it in cardiomyocytes during ischemia-reperfusion. This bidirectional regulatory network exerts its effects through mitochondrial redox balance, and its regulation may be related to shared substrates such as NADPH or the cross-regulation of ROS levels.

Second, the clinical significance of FDX biology is increasingly prominent across multiple disease areas. In cancer, the differential expression patterns of FDX1 across tumor types and its role in regulating cuproptosis sensitivity offer opportunities for targeted therapy, with copper ionophores like ES showing promise for overcoming therapeutic resistance. Furthermore, the emerging role of FDX1 in regulating the tumor immune microenvironment, such as its influence on PD-L1 expression, highlights its significant potential as an immunotherapeutic target. Nevertheless, it is essential to carefully balance its pro-death efficacy against potential pro-survival effects. Beyond oncology, FDX dysregulation contributes to diverse pathologies through distinct mechanisms. In neurodegenerative diseases, FDX1-mediated copper toxicity drives neuronal damage in Alzheimer's and Parkinson's diseases, suggesting copper homeostasis modulation as a potential intervention strategy. Cardiovascular disorders reveal another dimension, where FDX1 polymorphisms impair lipid metabolism in macrophages, exacerbating atherosclerosis, while its overexpression in vascular smooth muscle cells promotes calcification. The endocrine system demonstrates FDX1's dual role in POCS – enhancing androgen production in theca cells while suppressing progesterone synthesis in granulosa cells. Genetic disorders further highlight this dichotomy: FDX1 deficiency worsens Menkes disease through impaired copper utilization, whereas its overexpression contributes to Wilson's disease pathology. Meanwhile, FDX2 mutations cause severe mitochondrial myopathies and leukoencephalopathy through defective Fe–S cluster assembly. Notably, FDX2's clinical relevance extends beyond rare genetic disorders to cancer metabolism and therapeutic targeting. As the cornerstone of Fe–S cluster biogenesis, FDX2 deficiency creates a unique vulnerability in tumors, sensitizing them to ferroptosis induction through mitochondrial iron overload and impaired antioxidant defense. This synthetic lethality with GPX4 inhibition positions FDX2 as both a biomarker for patient stratification and a potential indirect target for combinatorial therapies. Future clinical investigations should prioritize defining FDX2 expression patterns across cancer subtypes and validating its utility in predicting response to ferroptosis-inducing regimens. This spectrum of disease associations underscores the necessity of precise, tissue-specific therapeutic targeting to maximize efficacy while minimizing off-target effects.

Third, previous sections have detailed the essential mechanistic role of FDX1 in copper-dependent cell death and mitochondrial metabolism. To evaluate the clinical potential of targeting FDX1, the following summarizes key clinical trial data. These studies investigate FDX1-directed therapeutics across specific oncology indications, focusing on copper-induced cytotoxicity and phosphorylation modulation mechanisms. The compiled data confirm the effectiveness of FDX1 as a viable clinical target. They provide key insights into the therapeutic breakthroughs of FDX1-targeted cuproptosis in solid tumors through copper ion release and phosphorylation regulation. They also reveal the clinical value and potential for future expansion to other diseases, as well as deeper research into regulatory mechanisms (Table 5).

Fourth, the tissue-specific expression of FDX1 and FDX2 is influenced by functional demands, but the molecular mechanisms are distinct and not fully elucidated for both isoforms. For FDX1, which specializes in steroidogenesis, expression is directly regulated by cell type-specific transcription factors such as SF1, as demonstrated in colorectal cancer, where the lncRNA PVT1 activates FDX1 by recruiting SF1 to its promoter and depositing the H3K27ac mark. For FDX2, which supports Fe–S cluster biogenesis and iron metabolism, its enrichment in metabolically active tissues (e.g., liver, heart) suggests a response to high metabolic demands, but the specific regulatory factors remain less clear. While iron-sensing regulators like IRP1/2 are involved in systemic iron

Table 5
Summary of clinical trials involving FDX1.

Disease	Medicine	Targets/Mechanism of Action	NCT	Phase	Conclusion of NCT
Solid tumors	Elesclomol	FDX1-dependent copper ion release induces cuproptosis.	NCT00827203	I	The drug is well-tolerated, and its toxicity profile is similar to that of paclitaxel alone.
Solid Tumors	Elesclomol + Paclitaxel	FDX1-dependent copper ion release induces cuproptosis and improves chemotherapy resistance.	NCT00088114	I	Unknown
Ovarian Epithelial Cancer, Fallopian Tube Cancer, or Primary Peritoneal Cancer	Elesclomol + Paclitaxel	FDX1-dependent copper ion release induces cuproptosis and improves chemotherapy resistance.	NCT00888615	II	The drug combination was well-tolerated but lacked sufficient promise to merit further investigation.
Melanoma	Elesclomol + Paclitaxel	FDX1-dependent copper ion release induces cuproptosis and improves chemotherapy resistance.	NCT00084214	II	The combination regimen not only achieved a statistically significant doubling of the median PFS but also had an acceptable toxicity profile, while the OS data were also encouraging.
Melanoma	Elesclomol + Paclitaxel	FDX1-dependent copper ion release induces cuproptosis and improves chemotherapy resistance.	NCT00522834	III	The combination of elesclomol and paclitaxel did not significantly improve PFS, but this combination therapy improved PFS in patients with normal serum LDH levels.
Relapsed or Refractory Acute Myeloid Leukemia	Elesclomol	FDX1-dependent copper ion release induces cuproptosis.	NCT01280786	I	Unknown
Soft Tissue Sarcomas	Elesclomol + Paclitaxel	FDX1-dependent copper ion release induces cuproptosis and improves chemotherapy resistance.	NCT00087997	II	Unknown
Stage IIIB or Stage IV Non-Small Cell Lung Cancer (NSCLC)	Elesclomol+ Paclitaxel + Carboplatin	FDX1-dependent copper ion release induces cuproptosis and improves chemotherapy resistance.	NCT00088088	II	Unknown

homeostasis, the article does not provide direct evidence that IRP1/2 control FDX2 expression; instead, FDX2 may be regulated by other mechanisms related to mitochondrial function or stress responses. In summary, FDX expression likely involves a collaborative interplay where tissue-specific contexts activate transcription factors and epigenetic machinery, but this model is more firmly established for FDX1, and further research is needed to define the precise pathways for FDX2.

Fifth, FDX function is dynamically regulated by PTMs, and an intriguing frontier is the potential cell cycle dependency of these modifications. It remains an open question whether key regulatory events, such as the AKT1-mediated phosphorylation of FDX1 at Ser63, are dynamically regulated across the cell cycle—for instance, being enhanced during the G2/M phase to meet the high bioenergetic and biosynthetic demands of mitosis. Investigating the temporal dynamics of FDX PTMs in synchronized cell models could reveal novel mechanisms through which mitochondrial function is coupled to cell division. Elucidating this link would not only answer a fundamental biological question but also have profound implications for cancer therapy, as it could identify a metabolic vulnerability in rapidly proliferating cells.

Sixth, FDXs function as central hubs that integrate the mitochondrial metal ion landscape. Directly, Cu^{+}/Cu^{2+} and Fe^{2+} serve as their primary redox substrates, while Zn^{2+} acts as a competitive inhibitor. Beyond these direct substrates, the broader ionic milieu, particularly Ca^{2+} and Mg^{2+}, may exert indirect metabolic influence. Calcium signaling fluctuations could modulate FDX efficiency by altering membrane potential and redox balance, while Mg^{2+} is crucial for powering the FDXR-FDX system by sustaining NADPH production. These plausible models of indirect regulation await validation, representing a promising frontier for understanding FDX function in physiology and disease. While direct binding evidence between Ca^{2+}/Mg^{2+} and FDXs is currently lacking, their profound influence on overall mitochondrial energy and redox metabolism positions them as key components of the ionic environment that modulates FDX activity. Investigating how FDX function is tuned within altered ionic microenvironments, especially under pathological conditions characterized by Ca^{2+} signaling dysregulation, represents a compelling and fruitful avenue for future research.

Seventh, the potential role of FDX2 in copper metabolism remains a core unanswered question. Currently, no experimental evidence indicates that FDX2 participates in the metallation of copper-binding proteins like COX17, nor has functional redundancy with FDX1 in copper redox cycling been observed. In fact, a paradigm of strict functional separation is well-established: FDX1 is dedicated to copper reduction, while FDX2 specializes in iron-sulfur cluster biogenesis. This division is strongly supported by their distinct redox potentials and specific protein interactomes. Therefore, shifting the research focus from seeking redundancy to precisely defining functional boundaries is more valuable. A key direction for the future lies in precisely defining the non-redundant role of FDX2 in copper biology through strategies such as analyzing the interaction between FDX2 and copper chaperone proteins, or evaluating FDX2 function under copper metabolism stress conditions, thereby more completely depicting the regulatory network of mitochondrial metal ion homeostasis.

Eighth, the tissue-specific and developmental regulation of FDX1-FDX2 homeostasis requires further investigation, as elucidating these mechanisms could identify novel therapeutic opportunities. From a therapeutic perspective, the development of isoform-specific modulators is both a challenge and an opportunity. For example, in-depth analysis of unique structural features such as the electronegative surface of FDX1 and the interaction interface between FDX2 and ISCA1/2, as well as how dynamic subcellular localization regulates the crosstalk between metabolic plasticity and death signals, can provide guidance for rational drug design. Exploring tissue-targeted delivery systems to improve specificity is also crucial. Combining multi-omics approaches with advanced structural biology techniques is essential for unraveling the complex regulatory networks centered on FDX proteins.

In conclusion, the co-evolution of FDXs embodies the functional expansion of mitochondria from energy factories to signaling hubs, and their ability to intricately integrate metabolic reprogramming with the execution of cell death is of great significance. As a critical nexus linking mitochondrial stress to the pathogenesis of human diseases, future research on FDXs should prioritize the integration of multi-omics stratification, the design of engineered biological agents targeting isoform-specific interfaces, and the clinical validation of dual-target strategies to decipher the spatiotemporal specificity of FDX networks – a crucial step in translating these mechanistic insights into precision therapies for cancer, cardiovascular diseases, neurodegenerative diseases, metabolic diseases, and hereditary diseases.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors agree to publish.

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CRediT authorship contribution statement

Yajuan Lu: Writing – original draft. **Yunyi Wu:** Data curation. **Chen Yang:** Writing – original draft. **Yi Zhou:** Writing – original draft. **Xueying Ren:** Visualization. **Haoran Li:** Methodology. **Hangqi Huang:** Writing – original draft. **Feifan Pan:** Writing – original draft. **Aoli Deng:** Writing – original draft. **Yi Lu:** Visualization. **Jiayi Yang:** Resources. **Xiangmin Tong:** Conceptualization, Visualization. **Yanchun Li:** Funding acquisition, Supervision. **Jing Du:** Conceptualization, Writing – review & editing.

Declaration of competing interest

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Abbreviations

FDXs, Ferredoxins; Fe–S, iron-sulfur; PCD, programmed cell death; FDX1, ferredoxin 1; FDX2, ferredoxin 2; FDXR, ferredoxin reductase; OXPHOS, oxidative phosphorylation; TCA, tricarboxylic acid; ETC, electron transport chain; CYP11A1, cytochrome P450 11A1; CYP11B1, cytochrome P450 11B1; Adx, adrenodoxin (FDX1); AdR, adrenodoxin reductase (FDXR); PTM, post-translational modification; LIAS, lipoic acid synthase; Kd, dissociation constant; ES, elesclomol; COX, cytochrome *c* oxidase; EPR, electron paramagnetic resonance; NFS1, cysteine desulfurase; ISD11, iron-sulfur cluster assembly protein (LYRM4); Acp1, acyl-carrier protein 1; ISCU, iron-sulfur cluster scaffold protein; FXN, frataxin; ISCA1, iron-sulfur cluster assembly 1; ISCA2, iron-sulfur cluster assembly 2; IBA57, iron-sulfur cluster assembly factor 57; RNAi, RNA interference; NMR, nuclear magnetic resonance; GDC, glycine decarboxylase complex; PDH, pyruvate dehydrogenase; KGDH, α-ketoglutarate dehydrogenase; BCKDH, branched-chain α-ketoacid dehydrogenase; ISR, integrated stress response; GDF15, growth differentiation factor 15; HEK293, human embryonic kidney 293 cells; CRISPR-Cas9, clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9; ROS, reactive oxygen species; HCC, hepatocellular carcinoma; NDUFS1, NADH dehydrogenase [ubiquinone] iron-sulfur protein 1; SDHB, succinate dehydrogenase complex iron-sulfur subunit B; SOD2, superoxide dismutase 2; SREBP1/2, sterol regulatory element-binding protein 1/2; ABCA1, ATP-binding cassette transporter A1; MVD, mevalonate diphosphate decarboxylase; FASN, fatty acid synthase; SCD1, stearoyl-CoA desaturase 1; ISC, iron-sulfur cluster; Cu^{2+}, copper ion (oxidized form); Cu^{+}, copper ion (reduced form); DLAT, dihydrolipoamide S-acetyltransferase; DLST, dihydrolipoamide S-succinyltransferase; HSP70, heat shock protein 70; G6PD, glucose-6-phosphate dehydrogenase; GPX4, glutathione peroxidase 4; MUTYH, MutY DNA glycosylase; NTHL1, endonuclease III-like protein 1; IRP2, iron regulatory protein 2; FTH1, ferritin heavy chain 1; TfR1, transferrin receptor 1; SLC39A14, solute carrier family 39 member 14; Fe^{2+}, ferrous iron; ATM, ataxia telangiectasia mutated; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; EGFR, epidermal growth factor receptor; LC3-II, microtubule-associated protein 1A/1B-light chain 3-II; PINK1, PTEN-induced kinase 1; Bcl-2, B-cell lymphoma 2; Bcl-xL, B-cell lymphoma-extra large; Bax, Bcl-2-associated X protein; Bak, Bcl-2 homologous antagonist/killer; caspase-3, cysteine-aspartic protease 3; caspase-8, cysteine-aspartic protease 8; caspase-9, cysteine-aspartic protease 9; Apaf-1, apoptotic protease activating factor 1; Fas, Fas receptor (CD95); TNFR, tumor necrosis factor receptor; RSI, reactive species interactome; RNS, reactive nitrogen species; RSS, reactive sulfur species; NO, nitric oxide; $ONOO^{-}$, peroxynitrite; H_2S, hydrogen sulfide; $O_2\bullet^{-}$, superoxide anion; Zn^{2+}, zinc ion; AD, Alzheimer's disease; PD, Parkinson's disease; Aβ, amyloid-β; α-Syn, α-synuclein; CREB, cAMP response element-binding protein; BDNF, brain-derived neurotrophic factor; PBMCs, peripheral blood mononuclear cells; CVD, cardiovascular disease; 3BH5C, 3β-hydroxy-5-cholestenoate; CYP27A1, cytochrome P450 27A1; LXR, liver X receptor; CAD, coronary artery disease; IC, ischemic cardiomyopathy; LVEF, left ventricular ejection fraction; TLB, tribromobimane; DSF, disulfiram; VSMCs, vascular smooth muscle cells; Elabela, apelin receptor early endogenous ligand; PPAR-γ, peroxisome proliferator-activated receptor gamma; Ca^{2+}, calcium ion; Mg^{2+}, magnesium ion; PCOS, polycystic ovary syndrome; T2DM, type 2 diabetes mellitus; GLP-1, glucagon-like peptide-1; Menkes disease, ATP7A-related copper deficiency disorder; Wilson disease, ATP7B-related copper overload disorder; CK, creatine kinase; TMB, tumor mutational burden; MSI, microsatellite instability; lncRNA PVT1, long non-coding RNA plasmacytoma variant translocation 1; miR-3130–5p, microRNA-3130–5p; CHL, chlorophyllin; MK2206, AKT inhibitor; 17-AAG, 17-allylamino-17-demethoxygeldanamycin (HSP90 inhibitor); 5-FU, 5-fluorouracil; ALS, Amyotrophic Lateral Sclerosis; FRDA, Friedreich's Ataxia; MDS, myelodysplastic syndromes; TNBC, triple-negative breast cancer; OC, ovarian cancer; GBM, glioblastoma; STAD, stomach adenocarcinoma; KIRC, kidney renal clear cell carcinoma; LUAD, lung adenocarcinoma; LIHC, liver hepatocellular carcinoma; CRC, colorectal cancer; ESCC, esophageal squamous cell carcinoma; PC, pancreatic cancer; NSCLC, non-small cell lung cancer; DIC, doxorubicin-induced cardiotoxicity; Mito-PLt, Mitochondrial transplantation; DACD, diabetes-associated cognitive dysfunction; CACuPDA, Copper-doped nanoparticles; FSP1, ferroptosis suppressor protein 1; NCLX, mitochondrial calcium exchanger; ΔΨm, mitochondrial membrane potential; SEC14L3, SEC14-like 3; ERK, extracellular regulated protein kinases; YY1, Yin Yang 1; AKT1, AKT serine/threonine kinase 1; NUDT21, nudix hydrolase 21; AARS1, alanyl-tRNA synthetase 1; LDHA, lactate dehydrogenase A; HIF, hypoxia-inducible factor; EMT, epithelial-mesenchymal transition; FMR1, fragile X mental retardation 1; ALCAM, activated leukocyte cell adhesion molecule; PD-L1, programmed death ligand 1; CD274, CD274 molecule; NOD-like receptor, nucleotide-binding oligomerization domain-like receptor; LRP6, low-density lipoprotein receptor-related protein 6; ALKBH5, alkB homolog 5; m^6A, N^6-methyladenosine; VitD3, vitamin D3; FGF21, fibroblast growth factor 21; STAT1, signal transducer and activator of transcription 1; SLC31A1, solute carrier family 31 member 1; ATP7A, ATPase copper transporting alpha; ATP7B, ATPase copper transporting beta; GPRIN2, G protein regulated inducer of neurite outgrowth 2; GCACD, guanidinium-modified calixarene/cyclodextrin; CTR1, copper transporter 1; TLR4, toll-like receptor 4; NLRP3, NLR family pyrin domain containing 3; NCT, National Clinical Trial; PFS, progression-free survival; OS, overall survival; IC50, half-maximal inhibitory concentration; GSH, glutathione; NADPH, nicotinamide adenine dinucleotide phosphate hydrogen; CoQ10, coenzyme Q10; T3, triiodothyronine; DHEA, dehydroepiandrosterone.

Data availability

No data was used for the research described in the article.

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