RESEARCH HIGHLIGHT

Cuproptosis: a copper-triggered modality of mitochondrial cell death

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Various heavy metals can induce regulated cell death through different subroutines. A recent study published in *Science* found that intracellular copper accumulation triggers the aggregation of mitochondrial lipoylated proteins and the destabilization of Fe–S cluster proteins, leading to a unique type of cell death termed cuproptosis.

Beyond classical apoptosis, several forms of regulated cell death (RCD) have been identified. These RCD subroutines differ in the initiating stimuli, intermediate activation events, and end effectors.[1] Heavy metal ions are essential micronutrients, but either insufficient or excessive abundance of metals can trigger cell death. For example, ferroptosis has been defined as an iron-dependent form of oxidative cell death caused by unrestricted lipid peroxidation.[2] Surprisingly, a recent study by Tsvetkov and colleagues showed that intracellular copper (Cu) induces a novel form of RCD that is different from oxidative stress-related cell death (e.g., apoptosis, ferroptosis, and necroptosis) and has been termed "cuproptosis".[3] In contrast, mitochondrial stress, especially the aggregation of lipoylated mitochondrial enzymes and the loss of Fe–S cluster proteins, ignites cuproptosis (Fig. 1).

First, the authors examined whether the toxicity of Cu ionophores, especially elesclomol (ES), depended on the induction of known cell death modalities. Pharmacological or genetic inhibition of apoptosis (using the caspase inhibitor Z-VAD-FMK or double knockout of *BAK* and *BAX*), ferroptosis (using ferrostatin-1 and liproxstatin-1), and necroptosis (using necrostatin-1) failed to suppress cell death induced by the ES–Cu complex in multiple cancer cell lines. Interestingly, the hydrophilic antioxidant glutathione (GSH) blocked the toxicity of ES–Cu by chelating intracellular Cu. Other antioxidants, such as N-acetylcysteine, α-tocopherol, ebselene, and JP4-039, failed to rescue the growth inhibition by ES–Cu, indicating that reactive oxygen species (ROS), including mitochondrial ROS, are not essential for cuproptosis.

Second, the authors found that NCIH2030 lung cancer cells that rely on galactose-mediated mitochondrial respiration were nearly 1000-fold more sensitive to ES–Cu-induced growth inhibition than cells that rely on glucose-induced glycolysis. Consistently, inhibitors of respiratory chain complexes I and III (rotenone and antimycin A, respectively), an inhibitor of mitochondrial pyruvate uptake (UK5099), or genetic suppression of complex I limited cuproptosis. Real-time measurements of oxygen consumption rate indicated that basal or adenosine 5′-triphosphate-linked respiration in cells is not affected by ES–Cu. Hypoxia (1% O_2), which obliges cells to rely on glycolysis rather than on oxidative phosphorylation, also reduced cuproptosis sensitivity. This distinguishes cuproptosis from ferroptosis, which requires glucose uptake and pyruvate oxidation.[4, 5] Thus, cuproptosis and ferroptosis are coupled to distinct alterations of energy metabolism and mitochondrial function.

Third, the authors identified two mitochondrial proteotoxic stress pathways that mediate cuproptosis using a genome-wide CRISPR/Cas9 knockout screen combined with metabolic and biochemical assays. Of note, Cu increased mitochondrial protein lipoylation, a post-translational modification of lysine that occurs in four enzymes (dihydrolipoamide branched chain transacylase E2 (DBT), glycine cleavage system protein H (GCSH), dihydrolipoamide S-succinyltransferase (DLST), and dihydrolipoamide S-acetyltransferase (DLAT)) that regulate carbon entry into the tricarboxylic acid (TCA) cycle. Cu directly bound to DLAT, promoting disulfide bond-dependent aggregation of lipoylated DLAT. Notably, ferredoxin 1 (FDX1) turned out to be a novel effector of lipoylation, which contributes to the accumulation of toxic lipoylated DLAT and subsequent cuproptotic cell death. In addition, mass spectrometric proteomics revealed that the FDX1-dependent degradation of Fe–S cluster proteins might favor cuproptosis. However, it is unclear how Cu ionophores selectively trigger the simultaneous aggregation and degradation of distinct sets of mitochondrial proteins.[3]

Finally, the authors investigated whether natural Cu stress (without ES) would induce a similar phenomenon to ES–Cu.[3] The supplementation with $CuCl_2$ preferentially caused a cuproptotic phenotype in cells that were manipulated to overexpress the Cu importer solute carrier family 31 member 1 (SLC31A1). The depletion of GSH by buthionine sulfoximine (BSO), a potent inhibitor of the enzyme gamma-glutamyl-cysteine synthetase, also increased susceptibility to cuproptosis in A549 lung cancer cells. In addition, the authors used a Wilson disease mouse model in which the deletion of the copper exporter ATPase copper transporting beta (*Atp7b*) resulted in excess Cu accumulation and cuproptotic damage in aging livers.[3] At this point, however, it

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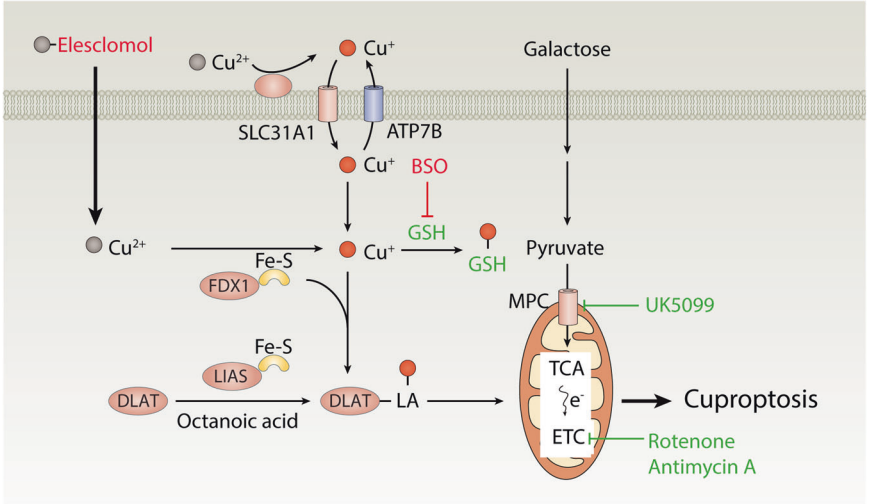

Fig. 1 Signaling and mechanism of cuproptosis. Elesclomol binds copper (Cu^{2+}) in the extracellular environment and transports it to intracellular compartments. Increased Cu accumulation causes cuproptosis mainly through FDX1-mediated mitochondrial proteotoxic stress. On the one hand, FDX1 reduces Cu^{2+} to Cu^{+}, facilitating the lipoylation (LA) and aggregation of enzymes (especially DLAT) involved in the regulation of mitochondrial TCA cycle. On the other hand, FDX1 causes the destabilization of Fe–S cluster proteins. In addition to Cu ionophores, Cu importers (e.g., SLC31A1) and exporters (e.g., ATP7B) regulate cuproptosis sensitivity by affecting intracellular Cu^{+} levels. GSH functions as a thiol-containing copper chelator that blocks cuproptosis, whereas BSO promotes cuproptosis by depleting GSH. The mitochondrial pyruvate carrier (MPC) inhibitor UK5099 and electron transport chain (ETC) complex I/III inhibitors (e.g., rotenone and antimycin A) attenuate elesclomol-induced cuproptosis.

is unclear whether the depletion of FDX1 would prevent *Atp7b* deficiency-driven Wilson disease and copper-induced hepatic damage.

Taken together, the present findings not only reinforce the notion that mitochondria are multifaceted regulators of cell death,[6] including copper-induced cell death,[7] but also challenge the conventional view that oxidative stress is a fundamental molecular mechanism of metal-induced toxicity.[8] Despite these major advances, they have also drawn additional attention to the processes and consequences of cuproptotic death.[1] For example, it is unclear whether cuproptosis is required to activate specific copper enzymes, many of which are involved in oxygen activation and reduction. Mitochondrial stress can cause a profound loss of mitochondrial membrane potential, which occurs in apoptosis and ferroptosis. The effect of ES–Cu on mitochondrial membrane potential and mitochondrial dynamics needs to be further clarified.[1] It is conceivable that the activation of mitochondrial quality control systems, such as mitochondrion-specific autophagy or mitophagy, limits cuproptosis. Regardless, how the protein degradation machinery (e.g., autophagy and the ubiquitin–proteasome system) regulates proteotoxic stress to control cuproptosis deserves further study.[9] Given that some forms of cell death may be more inflammatory than others, understanding how cuproptosis is initiated, propagated, and ultimately executed may have important implications for therapeutic interventions and possible combination treatments. For example, because glycolysis is essential for the proliferation of cancer cells, inhibition of glucose metabolism would not only enfeeble their malignant potential,[10] but also sensitize them to treatment with Cu ionophores.

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ADDITIONAL INFORMATION

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