Ferroptosis-Induced Endoplasmic Reticulum Stress: Cross-talk between Ferroptosis and Apoptosis

Young-Sun Lee1, Dae-Hee Lee2,3, Haroon A. Choudry1, David L. Bartlett1, and Yong J. Lee1

Abstract

Since its discovery in 2012, ferroptosis has been well characterized by the accumulation of lipid peroxides due to the failure of glutathione-dependent antioxidant defenses. It is known as an iron-dependent form of programmed cell death, which is distinct from other forms of cell death such as apoptosis and necrosis. Nonetheless, little is known about the ferroptotic agent-induced endoplasmic reticulum (ER) stress response and its role in cell death. Recent studies reveal that the ferroptotic agent-induced ER stress response plays an important role in the cross-talk between ferroptosis and other types of cell death. Ferroptotic agents induce the unfolded protein response and subsequently ER stress-mediated activation of the PERK–eIF2α–ATF4–CHOP pathway. CHOP (C/EBP homologous protein) signaling pathway-mediated p53-independent PUMA (p53 upregulated modulator of apoptosis) expression is involved in the synergistic interaction between ferroptosis and apoptosis. This review highlights the recent literature on ferroptotic and apoptotic agent interactions through the ER stress-mediated PERK–eIF2α–ATF4–CHOP–PUMA pathway and implicates combined treatment to effectively enhance tumoricidal efficacy as a novel therapeutic strategy for cancer.

Introduction

Ferroptosis

Ferroptosis was coined in 2012 by the laboratory of Dr. Brent R. Stockwell (1). It is a unique iron-dependent form of nonapoptotic regulated cell death (1). Ferroptosis is considered different from other types of cell death in various aspects. For example, ferroptosis does not result in morphologic changes like the loss of plasma membrane integrity that occurs during necrosis, the formation of double membrane-layered autophagic vacuoles that occurs during autophagy, or the chromatin condensation that occurs during apoptosis; instead, it manifests primarily as increased mitochondrial membrane density and mitochondrial shrinkage. Nevertheless, few studies have reported an interrelationship between ferroptosis and apoptosis; switching apoptosis (2) and ferroptotic agent-mediated sensitization of apoptosis (3).

Synthetic lethal screening studies have identified several genes responsible for ferroptosis, including those involved in lipid and amino acid metabolism (4–6). Chemical compounds inhibiting these genes trigger ferroptosis: the glutathione S-transferase inhibitor artesunate (ART), the glutathione-dependent antioxidant enzyme glutathione peroxidase 4 (GPX4) inhibitor (1S, 3R)-RSL3, the glutathione (GSH) synthesis inhibitor buthionine sulfoximine (BSO), and the Na+/dependent cystine–glutamate Xc− antiporter inhibitors sorafenib and erastin (1, 7–11). In the presence of ferroptosis-inducing agents, the iron storage protein ferritin and/or the ferritinophagy cargo receptor NCOA4 (nuclear receptor coactivator 4) are degraded via ferritinophagic degradation and release ferrous iron, which generates reactive oxygen species (ROS) through the Fenton reaction and subsequently induces lipid peroxidation (12, 13). The accumulation of lipid peroxidation and depletion of plasma membrane polyunsaturated fatty acids have been well known to result in this lethal event (1, 4, 14, 15). Genetic variation and complexity of cancer cells affect the pharmacodynamic response of ferroptosis-inducing agents. Functional p53 expression or high-level RAS–RAF–MEK pathway activity may elevate the generation of ROS through inhibition of cystine uptake or involvement of mitochondrial voltage-dependent anion channel 2/3 (VDAC2/3), respectively, and sensitize cancer cells to ferroptosis (16–21). Conversely, iron chelators (e.g., deferoxamine mesylate and deferoxamine) and lipid peroxidation inhibitors (e.g., zileuton, ferostatin, and liproxstatin) are known to suppress ferroptosis and block pathologic cell death events in the brain, kidney, and other tissues (10, 22–25).

Ferroptosis-induced ER stress

When endoplasmic reticulum (ER) lumenal conditions are altered or chaperone capacity is overwhelmed due to alterations in redox state, calcium levels, or failure to posttranslationally modify secretory proteins, the cells activate the unfolded protein

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1Department of Surgery, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania. 2Brain Korea 21 Program for Biomedicine Science, Korea University College of Medicine, Korea University, Seoul, Republic of Korea. 3Division of Oncology/Hematology, Department of Internal Medicine, College of Medicine, Korea University Medical Center, Korea University, Seoul, Republic of Korea.

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Corresponding Author: Yong J. Lee, Department of Surgery, University of Pittsburgh, Hillman Cancer Center, 3117 Centre Avenue Room 146C, Pittsburgh, PA 15213. Phone: 412-623-5268; Fax: 412-623-7709; E-mail: leeyj@upmc.edu

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response (UPR) to these ER stresses (26). ER stress is sensed by three upstream signaling proteins: IRE1 (inositol requiring protein-1), ATF6 (activating transcription factor-6), and PERK [protein kinase RNA (PKR)-like ER kinase]. The activation of these three signaling pathways induces apoptosis (26, 27).

System Xc\(^{-}\) is an amino acid antiporter that typically mediates the exchange of extracellular cystine for intracellular glutamate (28). Previous studies have shown that inhibition of cystine-glutamate exchange by ferroptotic agents leads to activation of an ER stress response and upregulation of the \(\text{CHAC1}\) (glutathione-specific gamma-glutamylcyclotransferase 1) gene (29, 30). The ER stress indicator ATF4 (activating transcription factor 4) is known to be a basic leucine zipper transcription factor that regulates several UPR target genes (31). It is well known that the ER stress response mediated by the PERK–eIF2\(\alpha\) (eukaryotic initiation factor 2\(\alpha\))–ATF4 pathway is involved in the regulation of the expression of several target genes such as CHOP [C/EBP (CCAAT-enhancer-binding protein) homologous protein; ref. 32].

When ferroptosis meets apoptosis: TRAIL-induced apoptosis and synergistic interaction between ferroptotic agent and TRAIL

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induces apoptosis through initiating the extrinsic pathway by binding to its respective death receptors (DR) such as DR4 and DR5 (Fig. 2). Ligation of TRAIL to DRs results in trimerization of DRs and leads to the recruitment of Fas-associating protein with death domain (FADD) and procaspase-8 and then the formation of the death-inducing signaling complex (DISC; ref. 35). Procaspase-8 is activated through two cleavage events at the DISC (36). Activated caspase-8 leads to further activation of downstream executioner caspase-3, -6, and -7, which culminates in apoptotic death (37). Activated caspase-8 also cleaves a proapoptotic Bcl-2 homology (BH3) interacting-domain death agonist (Bid) into truncated Bid (tBid), which translocates to the mitochondria and induces insertion and oligomerization of Bax (Bcl-2–associated X protein) and Bak (Bcl-2 homologous antagonist killer; refs. 38, 39). Insertion of homo-/hetero-oligomerized Bax and Bak into the mitochondrial outer membrane culminates in pore formation, membrane permeabilization, and depolarization of the mitochondria, which leads to cytochrome \(c\) release (40, 41).

Released cytochrome \(c\) binds to Apaf1 (apoptosis signal-regulating kinase) and facilitates the formation of the apoptosome, which activates caspase-9 and subsequently caspase-3 (42). Recent studies reveal that TRAIL-induced cytotoxicity can be modulated by various agents—not only chemotherapeutic drugs (43–45), ionizing radiation (46), other cytokines (47), and matrix metalloprotease inhibitors (48), but also ferroptotic agents (3). Synergistic interaction between ferroptotic agents and the apoptotic agent TRAIL may be mediated through ER stress–induced p53-independent PUMA expression (ref. 3; Fig. 2). Previous biochemical studies indicate that PUMA induces apoptosis by...
activating the multidomain proapoptotic protein Bax and/or Bak through its interaction with antiapoptotic Bcl-2 family members such as Bcl-2 (B-cell lymphoma 2) and Bcl-xL (B-cell lymphoma-extra-large), thereby triggering mitochondrial dysfunction, cytochrome c release, and caspase activation (49).

Conclusion

Ferroptosis is a recently recognized form of programmed cell death that is dependent on iron and characterized by the accumulation of lipid peroxidation through generation of ROS by the Fenton reaction. It is considered genetically and biochemically distinct from other forms of regulated cell death. However, emerging evidence suggests that ferroptosis often shares common pathways with other types of cell death. In light of recent studies, ferroptotic agents induce ER stress and elevate expression of the proapoptotic molecule PUMA through the ER stress-mediated PERK–eIF2α–ATF4–CHOP pathway without inducing apoptosis. Ferroptotic agent–induced PUMA plays an important role in the cross-talk between ferroptosis and apoptosis. Much work is still needed to understand how ferroptotic agent–induced PUMA sustains a biochemically inactive state during treatment with ferroptotic agent alone. Furthermore, such studies should examine how PUMA switches from an inactive to an activate state during combinatorial treatment with ferroptotic agent and the apoptotic agent TRAIL.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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