Ferroptosis-induced Endoplasmic Reticulum Stress: Crosstalk Between Ferroptosis and Apoptosis

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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 crosstalk 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 lab of Dr. Brent R. Stockwell [1]. It is a unique iron-dependent form of non-apoptotic regulated cell death [1]. Ferroptosis is considered different from other types of cell death in various aspects. For example, ferroptosis does not result in morphological 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

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primarily as increased mitochondrial membrane density and mitochondrial shrinkage. Nevertheless, few studies have reported an interrelationship between ferroptosis and apoptosis: switching apoptosis to ferroptosis [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^+-independent cystine–glutamate X_c^- 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., desferrioxamine mesylate and deferoxamine) and lipid peroxidation inhibitors (e.g., zileuton, ferrostatin, and liproxstatin) are known to suppress ferroptosis and block pathological 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 post-translationally modify secretory proteins, the cells activate the unfolded protein 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 X_c^- 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 *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-eIF2α (eukaryotic initiation factor 2α)-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) [32]. Data from microassay studies reveal that the ferroptotic agent ART promotes

the expression of ATF4-dependent genes such as CHOP, TRIB3, and ASNS (Fig. 1). Previous studies show that CHOP binds to the promoter of the pro-apoptotic protein PUMA (p53 upregulated modulator of apoptosis) during ER stress and induces PUMA expression [33]. CHOP also induces several other pro-apoptotic proteins such as GADD34 (growth arrest and DNA damage-inducible protein), ERO1α (endoplasmic reticulum, oxidoreductin-1α), Bim (Bcl-2 [B-cell lymphoma 2]-like protein 11), and NOXA (Latin for *damage*) [34]. The ferroptotic agent ART induces PUMA expression, but not NOXA or BIM expression [3]. Interestingly, ferroptotic agent-induced PUMA expression does not induce apoptosis [3]. These studies suggest that ferroptosis and apoptosis are antagonistic.

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 (DRs) 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) [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 pro-apoptotic 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) [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 [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 crosstalk 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.

Acknowledgments

Abbreviations used in this paper

Apaf1	apoptosis signal-regulating kinase
ART	artesunate
ATF4	activating transcription factor 4
ATF6	activating transcription factor-6
Bak	Bcl-2 homologous antagonist killer
Bax	Bcl-2–associated X protein
Bcl-2	B-cell lymphoma 2
Bcl-xL	B-cell lymphoma-extra large
BH3	Bcl-2 homology
Bim	Bcl-2-like protein 11
Bid	BH3 interacting-domain death agonist
BSO	buthionine sulfoximine
C/EBP	CCAAT-enhancer-binding proteins
CHAC1	glutathione-specific gamma-glutamylcyclotransferase 1
CHOP	CCAAT-enhancer-binding protein homologous protein
DISC	death-inducing signaling complex
DR	death receptor

DR4	death receptor 4
DR5	death receptor 5
eIF2α	eukaryotic initiation factor 2α
ER	endoplasmic reticulum
ERO1α	endoplasmic reticulum, oxidoreductin-1α
FADD	Fas-associating protein with death domain
GADD34	growth arrest and DNA damage-inducible protein
GPX4	glutathione peroxidase 4
GSH	glutathione
IRE1	inositol requiring protein-1
NCOA4	nuclear receptor coactivator 4
PERK	protein kinase RNA (PKR)-like ER kinase
PUMA	p53 upregulated modulator of apoptosis
ROS	reactive oxygen species
tBid	truncated Bid
TRAIL	tumor necrosis factor-related apoptosis-inducing ligand
UPR	unfolded protein response
VDAC2/3	voltage-dependent anion channel 2/3

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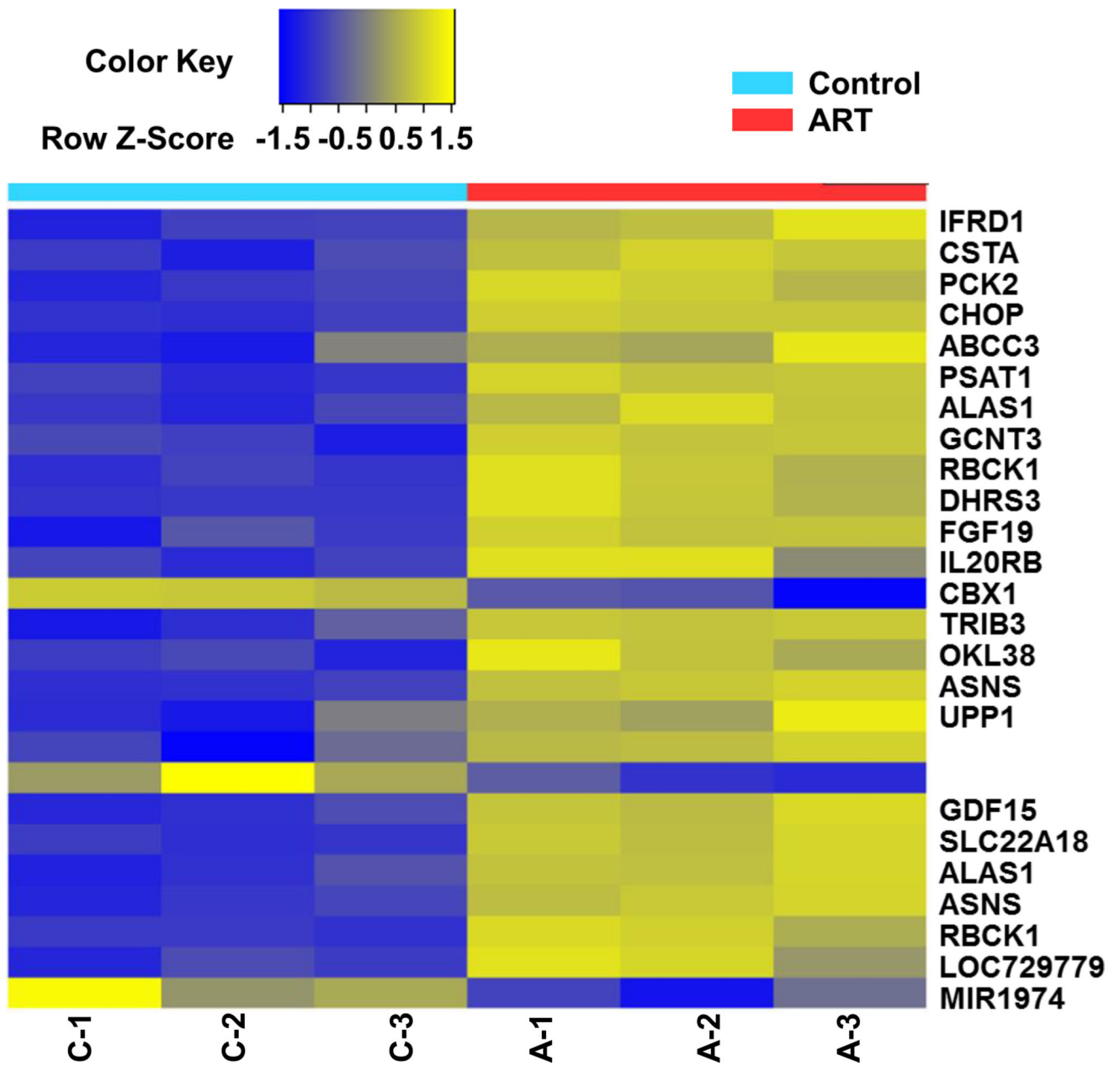49. Yu J, Zhang L. PUMA, a potent killer with or without p53. Oncogene. 2008; 27:S71–S83. [PubMed: 19641508]

Figure 1. Microarray assay for detection of ART-induced gene expression
Human colon cancer HCT116 cells were treated with 50 µM ART for 24 h and triplicate
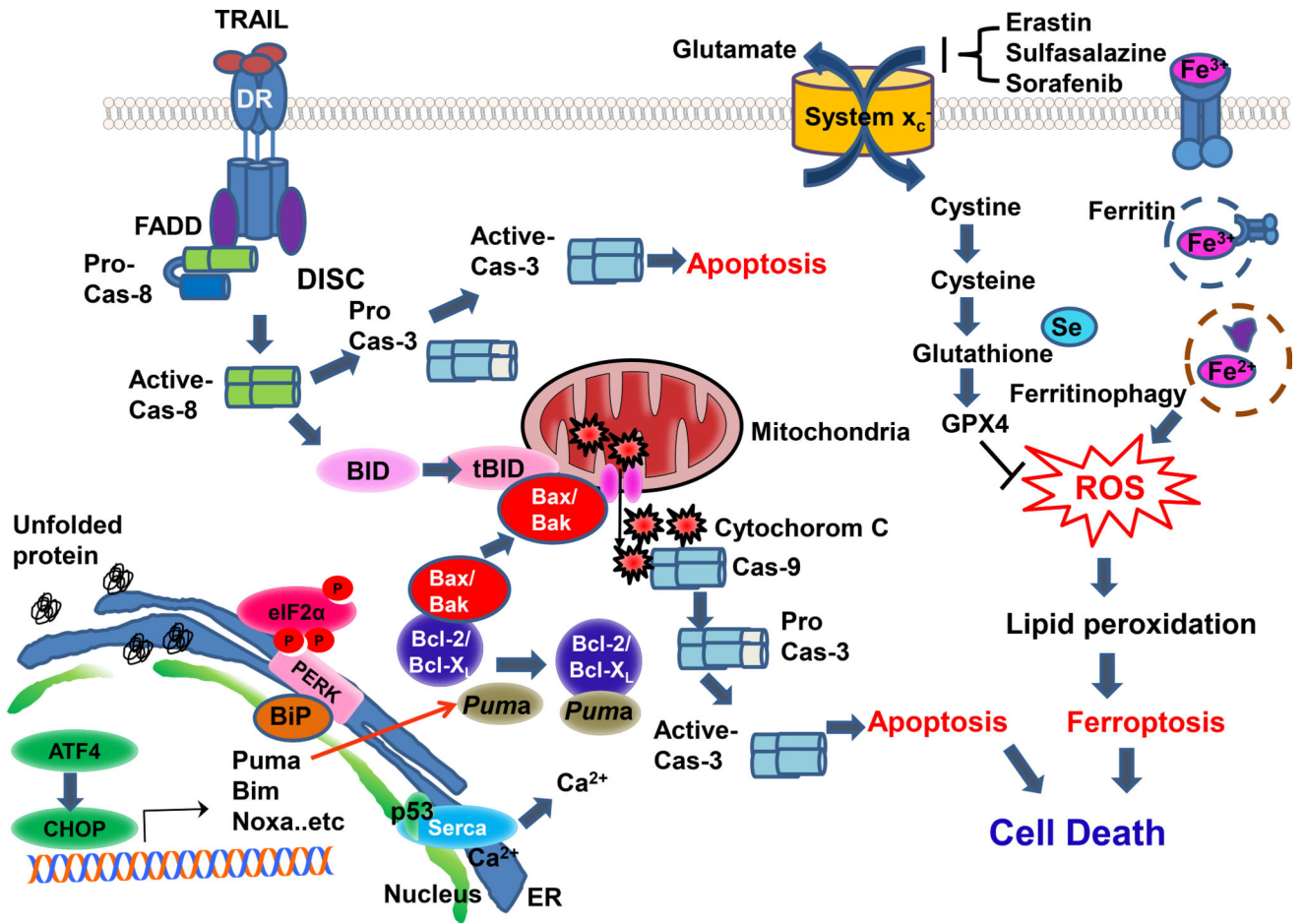Illumina gene expression microarrays were performed with BeadArray microarray
technology.

Figure 2. Schematic diagram of ferroptotic agent-induced ER stress and its role in the interplay between ferroptosis and apoptosis

Schematic diagram of ferroptotic agent-induced ER stress and its role in the interplay between ferroptosis and apoptosis