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Targeting ferroptosis to treat colorectal cancer

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Ferroptosis has emerged as a promising target for colorectal cancer (CRC) treatment. Although disrupting glutathione metabolism is the primary strategy for ferroptosis induction, additional key pathways link ferroptosis to CRC pathogenesis. Here, we discuss arachidonic acid (AA), energy metabolism, AMP-activated protein kinase (AMPK), phosphatidylinositol-3-kinase (PI3K)-protein kinase B (Akt)-mammalian target of rapamycin (mTOR), and Hippo signaling, summarize key findings, and propose new conceptual avenues for CRC treatment.

Ferroptosis in CRC and potential vulnerabilities

CRC is a malignant tumor of the colon or rectum that affects approximately 150 000 new individuals each year [1]. Current treatment strategies for CRC, such as chemotherapy, radiation therapy, immunotherapy, and surgery, face challenges ranging from cancer recurrence and drug resistance to overt toxicity. Therefore, more effective therapies are urgently needed for these patients. Metabolic and signaling pathways that regulate ferroptosis have been presented as potential therapeutic targets; ferroptosis inducers have been shown to eliminate CRC or enhance the effects of other therapies. This article discusses the emerging role for ferroptosis in CRC, novel targets for ferroptosis induction, and possible challenges for the clinical translation of these findings.

The basic mechanisms of ferroptosis have been reviewed by others previously and are summarized as part of Figure 1A. Briefly, solute carrier family 7 member 11 (SLC7A11) imports cystine for the synthesis of reduced glutathione (GSH), which is used as a cofactor by glutathione peroxidase 4 (GPX4) to reduce lipid peroxides and suppress ferroptosis. Labile iron contributes to this process by directly oxidizing lipids via Fenton chemistry and serving as a cofactor for several lipid-oxidizing enzymes. Inhibiting GPX4 or SLC7A11 induces ferroptosis, and these targets have been studied extensively over the past decade. Whether other proteins or molecules that regulate ferroptosis are potential targets for the treatment of CRC requires further research. We discuss these targets and their roles in metabolic and signaling pathways that regulate ferroptosis in the following section.

Energy metabolism regulates redox status and ferroptosis

Glycolysis and oxidative phosphorylation are two major metabolic pathways that provide energy for CRC cells. Oncogenes and tumor suppressors that are frequently mutated in CRCs such as KRAS, BRAF, and p53 have been shown to alter these pathways by driving the uptake and metabolism of metabolic substrates. Tumor cells undergoing ferroptosis exhibit decreased glycolytic activity due to downregulation of pyruvate kinase M2, hexokinase II, and platelet-type phosphofructokinase [2,3]. The cellular drivers of and justification for this metabolic shift remain unknown. Inhibition of glycolysis, however, can result in metabolic rewiring toward the pentose phosphate pathway (PPP), which regulates ferroptosis through multiple, antagonistic mechanisms. First, the PPP produces reduced NADPH which is a cofactor for the conversion of oxidized glutathione (GSSG) to GSH by GPX4, and for the regeneration of coenzyme Q_{10} (CoQ_{10}) for use by ferroptosis suppressor 1 (FSP1) [4] (Figure 1A). By contrast, NADPH can serve as an electron donor for NADPH oxidase, which produces superoxide radicals, promoting ferroptosis. The relative contributions of NADPH to overall redox status are likely context dependent and require additional investigation [4,5]. Disrupting both energy generation and the PPP could increase the ferroptosis sensitivity of tumor cells by hampering their antioxidative defense systems. Many chemicals that target these pathways exist; however, they have not yet been tested for this purpose.

AMPK mediates energy homeostasis, regulating ferroptosis

AMPK is a major sensor of cellular energy stress that regulates metabolic pathways to balance nutrient supply with energy demand. Energy stress inhibits ferroptosis through the AMPK-dependent phosphorylation and inhibition of acetyl-coenzyme A (CoA) carboxylase (ACC), an enzyme necessary for the synthesis of polyunsaturated fatty acid (PUFA) substrates for lipid peroxidation [6]. Activation of AMPK with 5-aminoimidazole-4-carboxamide ribonucleotide blocks lipid peroxide formation and ferroptosis, while knockout of AMPK enhances erastin-induced ferroptosis. Liver kinase B1 (LKB1), a serine/threonine kinase and a tumor suppressor, can also cooperate with and activate AMPK to decrease PUFA synthesis and suppress ferroptosis (Figure 1B) [7]. Furthermore, LKB1-deficient cells fail to activate AMPK in the presence of energetic stress and continue to proliferate uncontrollably, leading to metabolic catastrophe and cell death. Although these findings collectively support a ferroptosis-suppressing role for AMPK, other studies report contradictory, ferroptosis-promoting AMPK activity. Beclin 1 (BECN1) directly binds to SLC7A11 to mediate erastin-induced ferroptosis, and this cannot occur without AMPK-mediated BECN1 phosphorylation [8]. The AMPK pathway is also intrinsically linked to nutrient supply and mTOR signaling. AMPK is switched on to inhibit

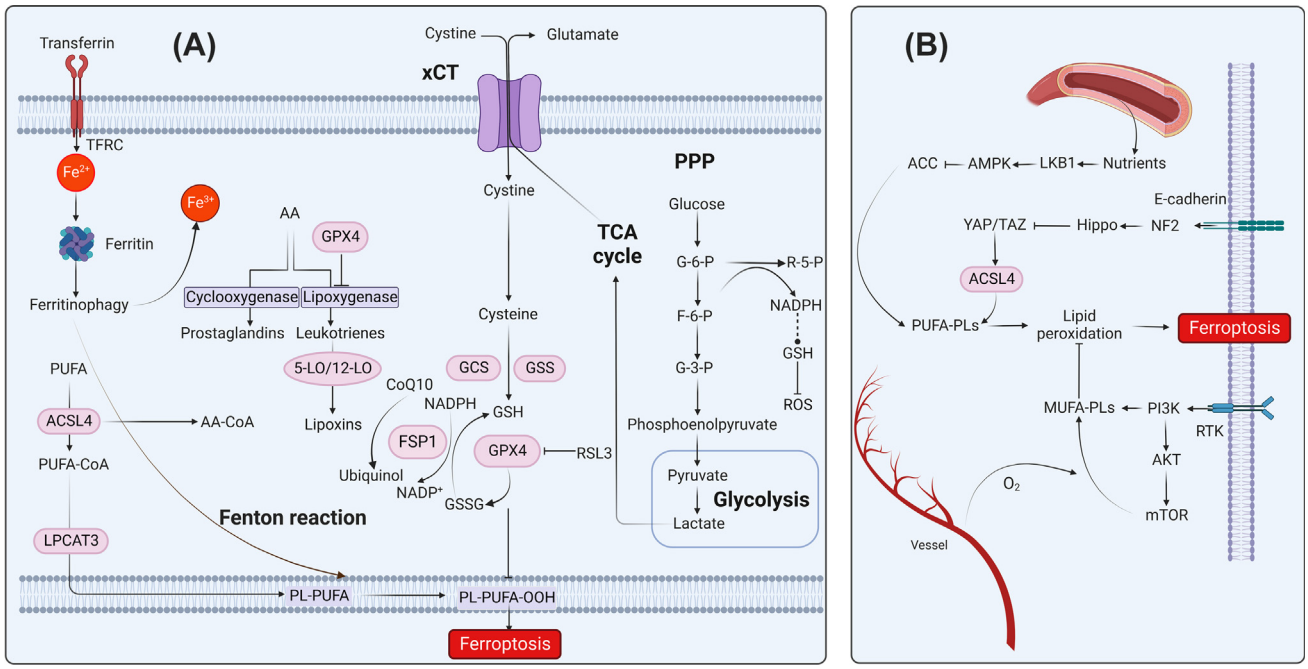

Figure 1. Key molecular contributors to ferroptosis and selected ferroptosis signaling pathway in CRC. (A) The iron-dependent accumulation of lipid peroxides at the cell membrane leads to ferroptosis. GPX4, the master suppressor of ferroptosis, consumes GSH to reduce lipid peroxides. GSH is synthesized from cyst(e)ine, which can be imported as cystine by xCT or generated by the transsulfuration pathway. Substrates for lipid peroxidation during ferroptosis are phospholipids with polyunsaturated fatty acid tails (PL-PUFAs) because of their intrinsic susceptibility to peroxidation. These PL-PUFAs are generated by enzymes such as ACSL4 and LPCAT3 that activate and incorporate free PUFAs into phospholipids. PUFAs can be scavenged from the environment and dietary sources and can be synthesized from the basic building block acetyl coenzyme A. Once PL-PUFAs are incorporated into membrane environments, iron-dependent enzymes and labile iron use molecular oxygen (O_2) to perform a peroxidation reaction, generating polyunsaturated-fatty-acid-containing-phospholipid hydroperoxides (PL-PUFA-OOH). Labile iron is imported through the TFRC and stored in ferritin. Ferritin can be degraded through an autophagy-like process known as ferritinophagy, which releases labile iron and facilitates the peroxidation reaction driving ferroptosis. Cellular energy metabolism activities such as glycolysis, PPP, and TCA cycle are involved in the regulation of key ferroptosis markers such as reduced NADPH, GSH, and ROS, therefore imposing potential regulatory roles in ferroptosis. Metabolites downstream of AA metabolism like prostaglandins, leukotrienes, and lipoxins regulate inflammatory and immune responses in cancer cells. (B) Activation or inhibition of these pathways can affect the ferroptosis sensitivity in CRC. Lipid metabolism is a complex process regulated by many signaling pathways, and the activation or suppression of these pathways can therefore modulate sensitivity to ferroptosis. AMPK phosphorylates and inhibits activities of ACC. One of the ways Hippo senses cell–cell contact is through the adhesion protein E-cadherin. Overexpression of constitutively active YAP or TAZ mutants could also sensitize these cells to ferroptosis. YAP and TAZ increase sensitivity to ferroptosis by altering expression of ferroptosis-promoting proteins like ACSL4. 'T' means suppress. Abbreviations: 5-LO, arachidonate 5-lipoxygenase; 12-LO, arachidonate 12-lipoxygenase; AA, arachidonic acid; ACC, acetyl-CoA carboxylase; ACSL4, acyl coenzyme A synthetase long-chain family member 4; AMPK, AMP-activated protein kinase; AKT, protein kinase B; CoA, coenzyme A; CoQ_{10}, coenzyme Q_{10}; Fe, iron; F-6-P, fructose 6-phosphate; G-3-P, glyceraldehyde 3-phosphate; G-6-P, glucose-6-phosphate; GCS, g-glutamylcysteine synthetase; GLS, glutaminase; GPX4, glutathione peroxidase 4; GSH, reduced glutathione; GSS, glutathione synthetase; GSSG, oxidized glutathione; LKB1, liver kinase B1; LPCAT3, lysophosphatidylcholine acyltransferase 3; mTOR, mammalian target of rapamycin; MUFA, monounsaturated fatty acid; NF2, neurofibromatosis type 2; PI3K, phosphatidylinositol-3-kinase; PL, phospholipid; PPP, pentose phosphate pathway; PUFA, polyunsaturated fatty acid; R-5-P, ribulose 5-phosphate; ROS, reactive oxygen species; RSL3, RAS-selective lethal 3; RTK, receptor tyrosine kinase; TAZ, transcriptional coactivator with PDZ-binding motif; TCA, tricarboxylic acid; TFRC, transferrin receptor; YAP, yes-associated protein.

cell growth when nutrient supply is low, while mTOR promotes cell growth in nutrient surplus conditions. In CRC cells, 2-imino-6-methoxy-2H-chromene-3-carbothioamide (IMCA) induces ferroptosis by stimulating AMPK phosphorylation, which inhibits mTOR activity and downregulates SLC7A11 [9]. Although studying the specificity of this compound remains necessary, its structural derivatives could provide drug candidates for this pathway. As with other ferroptosis regulators, it is probable that the actions of AMPK on ferroptosis are heavily context dependent. For example, AMPK-driven suppression of PUFA synthesis is likely not a relevant mechanism of ferroptosis suppression in cells with low basal ACC activity. Furthermore, AMPK-mediated BECN1 activation is required for ferroptosis triggered by SLC7A11 but not GPX4 inhibition, highlighting the importance of the mode of ferroptosis induction in these results.

Inhibition of the PI3K-Akt-mTOR pathway activates ferroptosis

The PI3K-Akt-mTOR pathway has also been linked to ferroptosis independently

from AMPK. Inhibition of PI3K, AKT, or mTOR has been shown to sensitize cancer cells to ferroptosis by decreasing sterol regulatory element-binding protein 1/stearoyl-CoA desaturase-1-mediated synthesis of monounsaturated fatty acids, which inhibit ferroptosis (Figure 1B) [10]. Moreover, several small molecules have been found to potently inhibit CRC by downregulating the PI3K-AKT-mTOR pathway. It is unlikely that PI3K, AKT, or mTOR inhibitors alone are sufficient for CRC treatment, as the WNT/β-catenin and mitogen-activated protein kinase (MAPK) signaling pathways can drive drug resistance. However, identifying combination regimens that circumvent or overcome these resistance mechanisms could provide a breakthrough. The dual PI3K/HDAC inhibitor BEBT-908, for instance, promotes immunogenic ferroptosis and enhances the efficacy of immunotherapy [11]. Targeting ferroptosis to stimulate antitumor immune responses is discussed in more detail in the next section.

AA metabolism links ferroptosis to antitumor immunity

AA is a PUFA that can undergo lipid peroxidation to trigger ferroptosis. AA is metabolized by cyclooxygenases (COXs), lipoxygenases (LOXs), and cytochrome P450 enzymes to a broad swath of downstream mediators including prostaglandins (Figure 1A), leukotrienes, epoxyeicosatrienoic acids, dihydroxyeicosatetraenoic acid, eicosatetraenoic acids, and lipoxins. Prostaglandin E2 (PGE2), in particular, has been associated with cancer immune evasion and chemotherapy resistance. Nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit the production of PGE2 by COX2, have been used for CRC chemoprevention for decades, and more recent data show their promise as immunomodulatory agents. The effect of these drugs on AA abundance, LOX and GPX4 activity, and ferroptosis sensitivity, however, has not been extensively tested.

A recent study reported that AA coordinates with interferon-gamma (IFNγ) secreted by $CD8^+$ T cells to induce ferroptosis in cancer cells, and treatment with AA inhibited tumor growth and enhanced responses to αPD-L1 [12]. This finding builds upon previous work from the same group showing that radiotherapy and immunotherapy independently induce ferroptosis in cancer cells through suppression of SLC7A11 by ataxia-telangiectasia mutated (ATM) and IFNγ, respectively [13]. Since both these studies demonstrated that inhibiting ferroptosis limits responses to these treatments, they also introduce the possibility that ferroptosis suppression mediates radiotherapy and immunotherapy resistance in CRC and other cancers. Consequently, additional knowledge of differences in CRC patient metabolic programs or metabolite abundance might help identify patients with higher likelihoods of responding to these or other ferroptosis-targeted therapies.

Hippo signaling regulates ferroptosis in a cell density-dependent manner

Hippo signaling is an evolutionarily conserved pathway that controls tissue homeostasis and organ size (Figure 1B). Hippo signaling has also been linked to ferroptosis sensitivity through its downstream effectors, yes-associated protein (YAP), and transcriptional coactivator with PDZ-binding motif (TAZ), but their actions on ferroptosis are dependent on cell density. Under low density, the Hippo pathway is switched off; YAP and TAZ are dephosphorylated and translocate to the nucleus where their downstream effects on target genes such as acyl-CoA synthetase long-chain family member 4 (*ASCL4*) and transferrin receptor (*TRFC*) render cells vulnerable to ferroptosis [14]. Under this condition, YAP drives the expression of genes that increase cell proliferation and metastasis. It is thus thought to be oncogenic in CRC. At higher cell densities, the Hippo pathway is switched on, and YAP and TAZ are phosphorylated by large tumor suppressor 1/2 (LATS1/2), restricting them to the cytoplasm. This has also been linked to ferroptosis resistance. E-cadherin-induced neurofibromatosis 2 (NF2) signaling recruits mammalian Ste20-like kinases 1/2 (MST1/2) which activates LATS1/2 by complexing with Salvador family WW domain-containing protein 1 (SAV1). Under these conditions, the oncogenic activity of YAP is inhibited. However, YAP also has been shown to have a tumor suppressor function in CRC, expression of YAP in CRC xenografts restricted their growth, and decreased expression of LATS1 is associated with increased tumor development and progression in human CRC patients. Finally, Hippo signaling intersects with other pathways that are important in CRC, such as WNT signaling [15]. Adenomatous polyposis coli (APC), a key tumor suppressor gene mutated in CRC, is a downstream regulator of the WNT pathway. Although testing this first requires more rigorous evaluation of Hippo-WNT crosstalk in CRC, it is possible that APC mutations alter WNT and Hippo signaling in CRC and regulate ferroptosis. In summary, CRCs with activated YAP may be sensitive to ferroptosis and therefore responsive to therapeutics that target ferroptosis. To date, it has proved difficult to pharmacologically target key Hippo pathway components, but approaches that aim to prevent the interaction of YAP and TAZ with each other or a key downstream molecule appear most promising.

The CRC tumor microenvironment is uniquely suited to influence ferroptosis

In addition to the metabolic and signaling pathways mentioned previously, there are other aspects of the tumor, such as the tumor microenvironment (TME), which are important to consider. The CRC TME consists of a complex milieu of host, immune, and microbial cells. The microbiome, which encompasses a diverse array of species and genomes, produces metabolites

that can cause DNA damage, impact tumor metabolism, and alter antitumor immune responses in CRC. Metabolic crosstalk between host cells, microbiota, and the immune system could influence the progression of CRC and response to therapeutics. When the TME is under stress such as during tumor growth and hypoxia, nutrient supply can be limiting, resulting in changes to tumor metabolism and signaling to enable cell survival. In addition, hypoxia stabilizes hypoxia-inducible factor 1α (HIF-1α) which activates the transcription of many genes involved in energy metabolism and those that protect from ferroptosis. Given that nutrient supply is important to signaling pathways such as AMPK, PI3K-Akt-mTOR, and energy/redox pathways and that hypoxia may protect cells from ferroptosis, it is likely that ferroptosis is dependent on the nutrient status of the TME. In addition, many of the metabolites involved in the pathways that link ferroptosis to CRC pathogenesis can be co-metabolized by the microbiome; therefore, the role of the microbiome will be an important consideration for future targeting of ferroptosis in CRC.

Concluding remarks

The evolving field of ferroptosis offers the prospect of developing new treatment approaches for CRC. Although ferroptosis clearly exists at the nexus of metabolism, tumorigenesis, and antitumor immunity, several gaps remain in our understanding of this interplay that currently stand in the way of exploiting these pathways to induce or increase sensitivity to ferroptosis in CRC. Some of these gaps relate to whether results from other contexts translate to CRC, especially considering recent findings related to the heavy context dependence of ferroptosis. Closing other gaps will depend on the application of scientific techniques such as metabolic tracer studies, phosphoproteomics, and *in vivo* CRISPR screens that have remained largely absent from ferroptosis research so far. Lastly, targeting certain ferroptosis pathways in CRC might require integrating drug development strategies, such as proteolysis-targeting chimeras (PROTACs), to target previously 'undruggable' proteins.

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Declaration of interests

The authors declare no competing interests.

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