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REVIEW

Implication of Ferroptosis in Cholangiocarcinoma: A Potential Future Target?

Mingyu Yang*, Meng Li*, Zhuozhen Lyu, Zhen Yang

Department of Infectious Diseases, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, Shandong, 25000, People's Republic of China

*These authors contributed equally to this work

Correspondence: Zhen Yang, Shandong Provincial Hospital Affiliated to Shandong First Medical University, No. 324, JingWu Road, Jinan, Shandong, 25000, People's Republic of China, Tel +86 15168867123, Email yangzhen@sdfmu.edu.cn

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Abstract: Cholangiocarcinoma (CCA), the second most common liver neoplasm, has a poor overall 5-year survival rate of less than 10%. A deeper understanding of the molecular pathogenesis contributing to CCA progression is essential for developing better therapeutic approaches to manage this disease. Ferroptosis, an oxidative iron-dependent form of regulated cell death, has been reported to be involved in tumorigenesis and progression. In particular, ferroptosis and inflammation, which are common issues in cholangiocarcinogenesis and CCA development, might be in concert with disease progression. Notably, the key feature of cancer cells is "iron addiction", which is crucial for the high metabolic demand in carcinogenesis and cancer progression. Additionally, iron metabolism is of great importance in ferroptosis. Moreover, that cancer cells are vulnerable to ferroptosis might be a possible mechanism of CCA development. Although the underlying mechanism of how ferroptosis is implicated in CCA development requires further investigation, developing a new strategy combined with a pro-ferroptotic treatment would be an exciting CCA treatment approach in the future.

Keywords: cholangiocarcinoma, ferroptosis, inflammation, iron metabolism, treatment target

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Introduction

Cholangiocarcinoma (CCA), the second most common primary liver tumor after hepatocarcinoma, has rare therapeutic options and a high mortality rate.[1] It can be divided into intrahepatic, perihilar, and distal CCA. Surgical resection and liver transplant are the potential treatment options in the early stages of CCA; however, early symptoms are not always impressive. For example, extrahepatic CCA may be observed as the tumor develops, blocking the bile tract system and causing jaundice. As a result, less than a third of patients may have the chance to undergo surgery. Furthermore, the risks of local neoplasm recurrence and distant metastasis are as high as 60%.[2] Tumor markers used for early CCA diagnosis, prognosis, and treatment are considerably limited, with unexpectedly low sensitivity.[3] Therefore, it would be crucial to investigate the underlying mechanism controlling the disease progression.

Ferroptosis, an iron-dependent non-apoptosis cell death, is characterized by iron overload and lipid peroxidation.[4] Compared with apoptosis and autophagy, ferroptosis has distinct differences in morphology, biochemical characteristics, and regulating mechanisms. Recently, it has been shown that iron metabolism regulates various tumors by enhancing oxidative stress and cell death control.[5] In addition, ferroptosis is also implicated in tumorigenesis and progression,[6] highlighting the potential of ferroptosis in CCA treatment. Thus, it would be of great importance to further explore the role of ferroptosis in CCA.

Cholangiocarcinoma

Cholangiocarcinoma (CCA), the second most common liver neoplasm, arises from the malignant transformation of bile duct epithelial cells.[7] CCA is anatomically classified as intrahepatic (iCCA), perihilar (pCCA), or distal extrahepatic (dCCA).[8] The incidence rate of CCA varies greatly, ranging from 0.45 to 3.36 per 100,000 population, owing to genetic

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differences and geographical variations in the risk factors.[9] The highest incidence rates of CCA are observed in Asia because of liver fluke infections and correspondingly oncogenic effect of the associated chronic biliary tract inflammation.[10] Surgical resection and liver transplantation are potentially curative therapeutic approaches for all three CCA subtypes only at an early stage; nevertheless, the median 5-year survival rate after R0 resection is only approximately 30%.[11] CCA still remains a highly lethal disease with a poor overall 5-year survival rate of less than 10%.[2] Therefore, a deeper understanding of the molecular pathogenesis contributing to CCA progression is of primary importance for developing better therapeutic approaches to tackle this disease.

Ferroptosis

The term "ferroptosis" was derived from the word "ptosis" (falling) and "ferrum" (iron). It is defined as a unique form of regulated cell death (RCD) induced by erastin in 2012.[12] Apoptosis has long been considered the only form of RCD; however, traditional understanding has been challenged by the discovery of several forms of RCD, among which ferroptosis has attracted considerable attention because of its involvement in various pathological processes. Ferroptosis is an oxidative, iron-dependent form of RCD with an accumulation of reactive oxygen species (ROS) and lipid peroxidation products at lethal levels.[13,14] Ferroptotic cells exhibit typical necrotic morphology with abnormal mitochondrial characteristics.[14,15] Moreover, the ballooning phenotype can be observed in cells undergoing ferroptosis.[16] Furthermore, ferroptotic cells induce harmful peroxidation of polyunsaturated fatty acids (PUFAs) owing to the accumulation of intracellular Fe^{2+}.[17] In particular, ferroptosis is genetically driven by several genes associated with iron metabolism and oxidative stress pathways.[18,19] The abnormal expression of these genes has been regarded as a biomarker of ferroptosis, such as acyl-CoA synthetase long-chain family member 4 (ACSL4) with inducing-ferroptosis activity, and glutathione peroxidase 4 (GPX4) with anti-ferroptosis activity. However, it is reported that ACSL4-independent ferroptosis and GPX4-independent ferroptosis could still occur.[18,20] Of special interest, ferroptosis has been found to be implicated in tumorigenesis and progression,[6,21,22] highlighting the potential of ferroptosis in cancer treatment.

Inflammation, CCA, and Ferroptosis

CCA and Inflammation

Several different risk factors are involved in cholangiocarcinogenesis; however, most have a common issue in eliciting chronic inflammation. Inflammatory inducers cause progressive mutations in tumor suppressor genes and proto-oncogenes, leading to cell transformation, cholangiocarcinogenesis, and disease development. Therefore, inflammation is of great importance in CCA development.[23]

PUFAs and their metabolic enzymes are necessary for regulating important cellular processes during inflammation.[24] Arachidonic acid (AA), a ώ-6 PUFA, is mainly present in the form of phospholipids. The cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 pathways have been implicated in AA metabolism.[25] COX is the most crucial rate-limiting enzyme for converting AA to prostaglandins. There are two isoforms: COX-1 and COX-2. Recent studies have found that COX-2 has pro-inflammatory potential and facilitates the transformation of inflammatory sites into precancerous microenvironments.[26,27] Upregulation of COX-2 stimulates CCA proliferation, whereas COX-2 inhibitors induce apoptosis and suppress cell growth.[28,29] Furthermore, COX-2 is modulated by inducible nitric oxide synthase (iNOS), which produces large amounts of nitric oxide (NO) in response to inflammatory mediators, causing oxidative DNA damage.[30] Moreover, iNOS and NO can activate the *Notch1* gene, and upregulated *Notch1* was discovered in iCCA and eCCA.[31–33]

Interleukin-6 (IL-6), a well-known pleiotropic pro-inflammatory cytokine, is involved in numerous pathways that facilitate cholangiocarcinogenesis. IL-6 is remarkably upregulated in CCA cell lines and specimens.[34] However, IL-6 induces JAK-STAT signaling in normal cholangiocytes and then promotes the transcription of the cytokine suppressor, SOCS-3, to form a negative feedback loop of IL-6.[23] Moreover, SOCS-3 knockdown in CCA suppresses negative feedback.[35] Another inflammatory cytokine, tumor necrosis factor α (TNF-α), can induce activation-induced cytidine deaminase (AID), an enzyme that causes DNA mutations. A recent study indicated that AID is rarely found in normal livers but is detectable in 80% of primary sclerosing cholangitis and 93% of CCA cases.[36] In particular, accumulating evidence has shed light on the role of macrophages in Wnt-signaling activation in cholangiocarcinogenesis, with induced cell proliferation and decreased apoptosis.[37–39] Thus, all these findings support the role of inflammation in cholangiocarcinogenesis.

Ferroptosis and Inflammation

It is well known that ferroptosis is involved in various pathogeneses that act as pro-inflammatory factors. A complicated relationship has been found between ferroptosis, AA metabolism, and eicosanoid biosynthesis. Ferroptosis is directly correlated with the upregulation of prostaglandin-endoperoxide synthase 2 as a COX-2 encoding gene, acceleration of AA metabolism, and induced secretion of inflammatory signaling molecules.[40] Reactive AA transfer between cells plays an important role in this pathway, and it has been proposed that cells undergoing ferroptosis could be regarded as AA donors for the trans-cellular eicosanoids biosynthesis.[41] In addition, unlike apoptotic cells, ferroptotic cells are characterized by the release of inflammatory cytokines and danger-associated molecular patterns and reprogramming of the pro-inflammatory microenvironment.[42] Different types of cancer cells undergoing ferroptosis have been discovered following a massive release of high mobility group 1 (HMGB1), which could be implicated in inflammation pathogenesis, amplifying inflammation.[43–46] HMGB1 exhibits immunostimulatory behavior as an adjuvant and facilitates the activation of the immune system and inflammatory response when it is released from the cells. Furthermore, HMGB1 neutralizing antibodies inhibit inflammatory response induced by ferroptotic cells.[44] Moreover, ferroptosis can also induce an inflammatory response by releasing IL-33 or other pathways.[13] Some inflammatory cytokines (such as TNF-α, prostaglandin E2, and IL-6) have been shown to affect GPX4 in cancer cells.[47] Cells exposed to TNF-α show a sustained decrease in GPX4, which then induces ferroptosis.[42] LOXs also trigger ferroptosis through pro-inflammatory metabolites derived from LOX.[48]

Collectively, these results provide evidence that ferroptosis and inflammation might be in concert in carcinogenesis and progression and may be promising therapeutic targets for CCA.

Iron Metabolism, CCA, and Ferroptosis

Iron is a critical element for the proliferation, cell cycle control, and genomic integrity of cancer cells. Normally, intracellular iron levels are tightly regulated to maintain homeostasis. In the systemic iron pool, iron binds to transferrin (TF). Next, iron-loaded TF and transferrin receptor 1 (TfR-1) form a complex on the plasma membrane.[49,50] The most active form of iron is utilized in various physiological processes. Imported iron is stored and transferred in iron–protein form, mainly with ferritin, which is an iron-containing protein with multiple functions. It has been shown that ferritin is upregulated in the plasma of cancer cells, and its higher expression is associated with poor prognosis.[51,52] Another common form of iron utilization is iron-sulfur biogenesis. As members of the novel, iron-sulfur protein family, NEET proteins are involved in maintaining the iron balance. NAF-1 and mitoNEET have been shown to accelerate cell proliferation and metastasis via mitochondrial iron accumulation. It establishes a crucial regulatory link that increases the levels of iron and ROS in cancer cells.[53,54] Furthermore, ferroportin (FPN), the only known iron exporter for iron efflux control, exports intracellular iron to maintain an intricate balance.[55,56] However, in various types of cancer, FPN is remarkably reduced.[57] FPN downregulation in breast cancer promotes epithelial-mesenchymal transition with enhanced E-cadherin and weakened N-cadherin expression.[58] Notably, the key feature of cancer cells is "iron addiction",[12,59–63] which describes the over-accumulation of iron in various cancers.[12,63] Although the underlying mechanism leading to "iron addiction" needs further investigation, it is conceivable that excess iron is essential for supporting the high metabolic demands in cancer cells; in addition, iron is a co-factor of many different proteins with various functions.[12,64] Iron metabolism is of great significance in ferroptosis. Accumulating evidence has demonstrated cancer cells with upregulated iron levels in the active form and with a high metabolism rate. Moreover, iron accumulation leads to ROS release; consequently, cancer cells are vulnerable to ferroptosis.[65–67] Depletion of iron-responsive element binding protein 2, which encodes the key regulator of iron metabolism, dramatically suppresses induced ferroptosis.[68] In addition, a critical role for the Ras/Raf/MEK signaling pathway has been revealed in ferroptosis vulnerability.[69] A possible explanation might be the intracellular iron overload induced by oncogenic Ras with TfR upregulation and ferritin downregulation, which has been found in many cancers.[66,70,71] As a result, therapeutic strategies targeting "iron addiction" in cancer cells would be promising and can enable ferroptosis-mediated cancer treatment.[12,59,63,67,72–75]

Tissue microarray profiles of CCA indicate enhanced TfR and reduced ferritin and FPN expression compared to those in para-cancer tissue. In addition, ferrous iron is significantly depleted in eCCA compared to that in controls, suggesting that dysregulated iron metabolism and ferroptosis might be possible mechanisms underlying eCCA.[3] The IDH1/2 mutation was verified as a supportable predictor and correlated with iron metabolism in iCCA.[76] In particular, iron regulatory proteins and iron discrimination have also been investigated in the development of liver fluke-associated CCA. The results show that iron is strongly stained in cancerous tissues and that high iron accumulation is associated with poor prognosis. TfR-1 is upregulated in CCA tissues, and TfR-1 inhibition leads to decreased levels of intracellular labile iron pool (LIP) with suppressed CCA proliferation and migration abilities. Therefore, strong TfR-1 expression leading to iron uptake facilitates increased LIP, which contributes to CCA progression with clinical deterioration.[77] Carbonylation is the oxidative modification of proteins. In CCA tissues, higher carbonylation of serotransferrin, heat shock protein 1 (HSP70.1), and α1-antitrypsin (A1AT) was confirmed. Thus, serotransferrin carbonylation leads to iron overload and oxidative stress imbalance through the Fenton reaction, while carbonylated HSP70.1 and A1AT may become dysfunctional, resulting in CCA progression.[78]

As shown in Figure 1, ferroptosis has been implicated in CCA development owing to its role in inflammation and iron metabolism pathway. The collective research data suggests that certain molecules involved in ferroptosis might serve as biomarkers or targets to manage this disease.

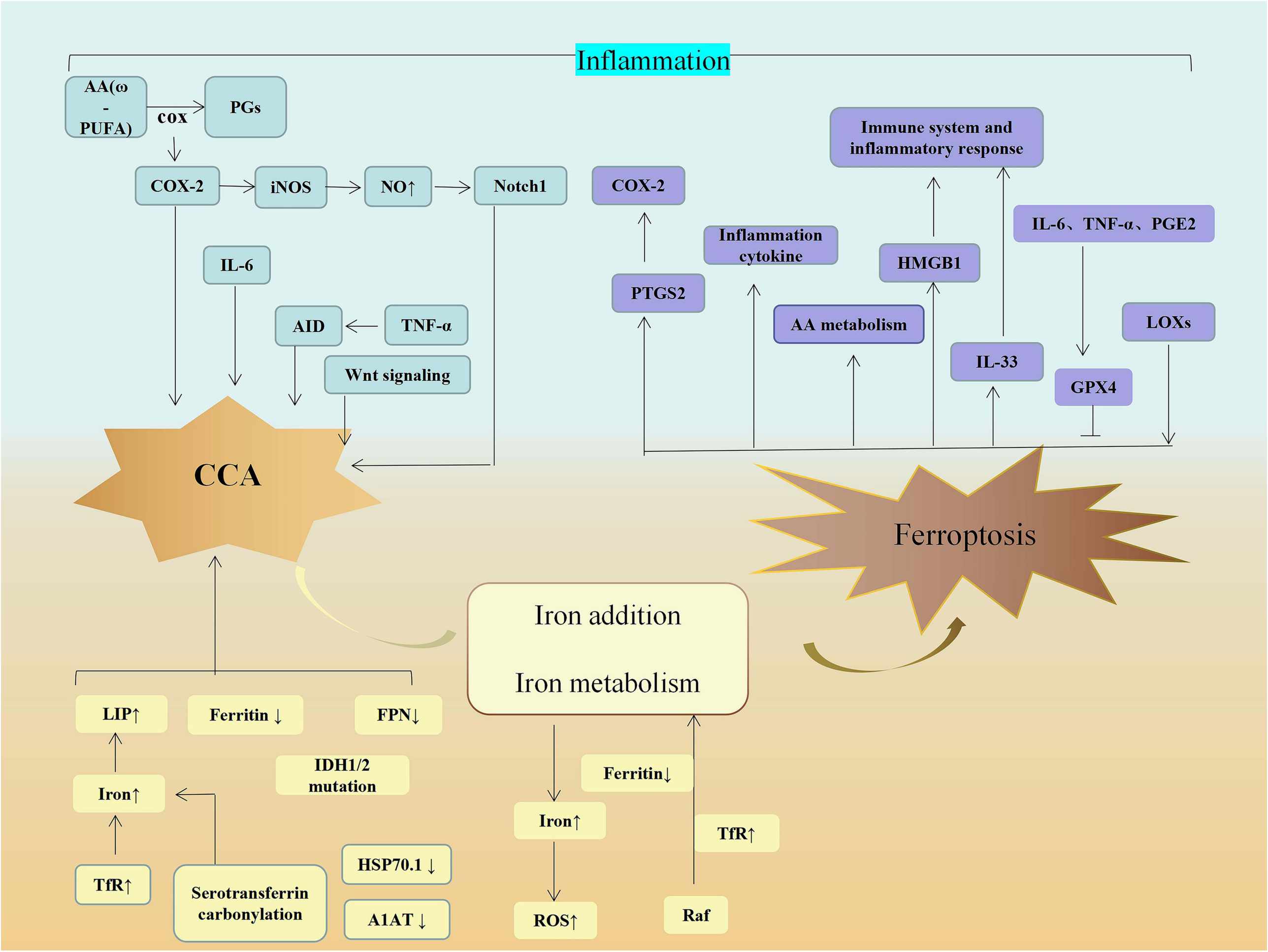

Figure 1 Cholangiocarcinoma and ferroptosis.

Abbreviations: AA, arachidonic acid; A1At, α1-antitrypsin; AID, activation-induced cytidine deaminase; CCA, cholangiocarcinoma; COX, cyclooxygenase; FPN, ferroportin; GPX4, glutathione peroxidase 4; HMGB1, high mobility group 1; HSP70.1, heat shock protein 1; IL, interleukin; iNOS, inducible nitric oxide synthase; LIP, labile iron pool; LOX, lipoxygenase; NO, nitric oxide; PG, prostaglandin; PTGS2, prostaglandin-endoperoxide synthase 2; PUFA, polyunsaturated fatty acids; ROS, reactive oxygen species; TfR-1, transferrin receptor 1; TNF-α, tumor necrosis factor α.

Ferroptosis and Therapeutic Target Potential

Recently, an increasing number of studies have focused on the role of ferroptosis in tumorigenesis and disease progression as a new and promising suppression mechanism.[79] It is shown that 3D spheres (SPH) formation efficiency reduced after iron chelator treatment, as well as there was a decrease in the levels of cancer stem cell markers and stem-like genes. However, iron exposure exhibited an opposite trend. Of special interest, many types of cancers have been discovered to be sensitive to ferroptosis inducers. Moreover, growing evidence suggests that ferroptosis, at least partly, contributes to the tumor-suppressive effects of several conventional therapies, including chemotherapy,[80] targeted therapy,[81] and immunotherapy.[6] As a result, inducing ferroptosis would provide more opportunities for tumor therapeutics. Notably, recent research revealed that some conventional therapies could induce ferroptosis, and ferroptosis-inducers -combination could facilitate the synergistic anti-tumor activities and further enhance the therapeutic efficacy accordingly. Likewise, inducing ferroptosis could also prevent and reverse, at least partly, acquired therapy resistance in some cancers.[22] This further supports the clinical application of ferroptosis-inducing combination therapeutic strategies.

In addition, a variety of nanomaterials have been exploited to induce ferroptosis locally or to strengthen the activity of ferroptosis inducers.[82] Nanoparticle-induced ferroptosis caused an elimination effect on all the neighboring cells, and ferroptosis resulting from intravenous nanoparticle administration suppressed xenograft tumor growth, supporting the hypothesis that ferroptosis has the potential to inhibit cancer progression.[83] Similarly, photodynamic therapy, (PDT), an important therapeutic approach, can be enhanced by ferroptosis through a potential oxygen supplement effect and ROS accumulation, providing evidence for a new therapeutic strategy of ferroptosis inducers combined with PDT.[84]

Conclusions and Future Perspectives

Taken together, the underlying regulation of ferroptosis in CCA development is poorly understood and requires further investigation. However, its clinical significance in the treatment of CCA has gradually emerged by combining ferroptosis-promoting therapy (ferroptosis inducers, pro-ferroptotic-drug-nanocarrier, etc.) with different methods to get synergistic anti-cancer effects and to prevent acquired resistance to some existing chemotherapy drugs. Therefore, it would be of great significance to explore the in-depth molecular mechanism of ferroptosis in CCA as it appears to be a promising therapeutic target. In addition, the screening and search for biomarkers involved in the detection and tracking of ferroptosis also poses a challenge, requiring future studies.

Author Contributions

All authors made a significant contribution to the work reported, including the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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