REVIEW

Implication of Ferroptosis in Cholangiocarcinoma: A Potential Future Target?

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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 cholangio-carcinogenesis 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

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

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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.
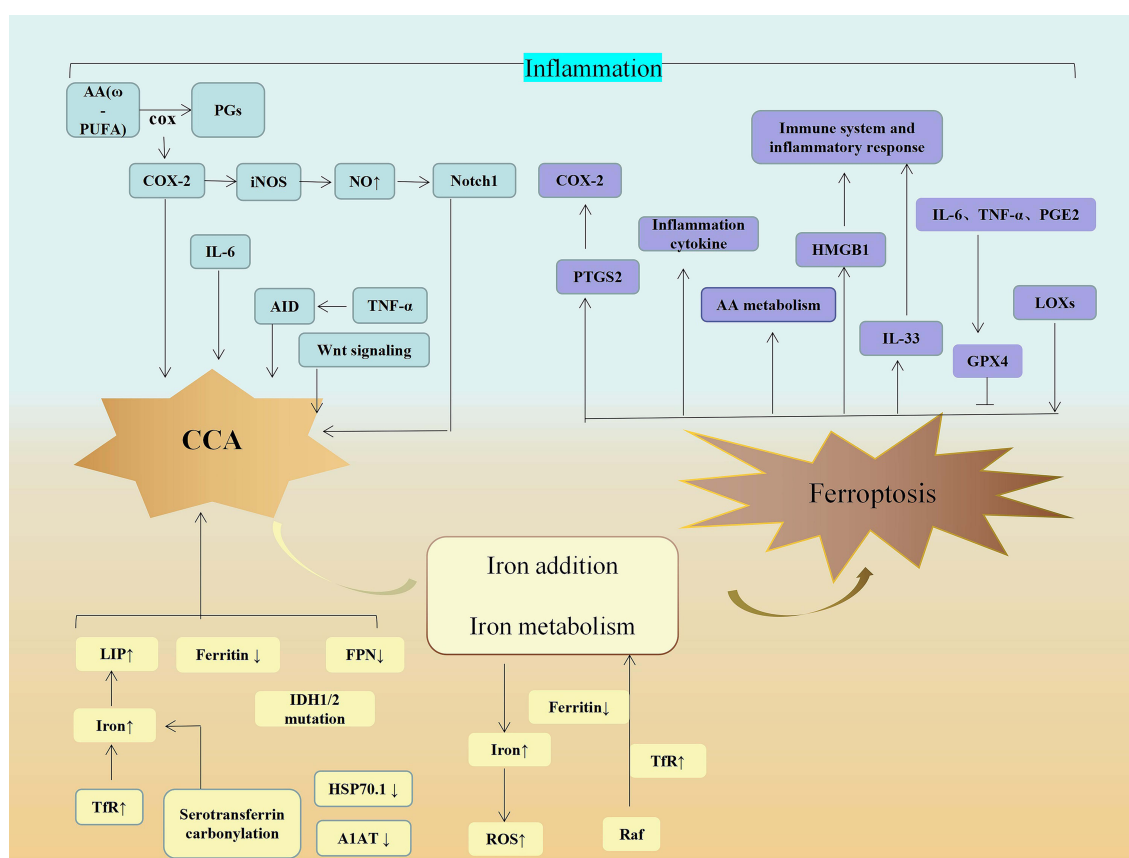
Figure 1 Cholangiocarcinoma and ferroptosis.
Abbreviations: AA, arachidonic acid; A1At, α1-antitrypsin; AID, activation-induced cytidine deaminase; CCA, cholangiocarcinoma; COX, cyclooxygenase; FPN, ferroportin; GPX4, glutathione peroxidase 4; HMGB1, high mobility group 1; HSP70.1, heat shock protein 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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References

1. Rimini M, Puzzoni M, Pedica F, et al. Cholangiocarcinoma: new perspectives for new horizons. *Expert Rev Gastroenterol Hepatol*. 2021;15 (12):1367–1383. doi:10.1080/17474124.2021.1991313
2. Banales JM, Cardinale V, Carpino G, et al. Expert consensus document: cholangiocarcinoma: current knowledge and future perspectives consensus statement from the European Network for the Study of Cholangiocarcinoma (ENS-CCA). *Nat Rev Gastroenterol Hepatol*. 2016;13(5):261–280. doi:10.1038/nrgastro.2016.51
3. Han JY, Ahn KS, Baek WK, et al. Usefulness of bile as a biomarker via ferroptosis and cysteine prenylation in cholangiocarcinoma; role of diagnosis and differentiation from benign biliary disease. *Surg Oncol*. 2020;34:174–181. doi:10.1016/j.suronc.2020.04.019
4. Li J, Cao F, Yin HL, et al. Ferroptosis: past, present and future. *Cell Death Dis*. 2020;11(2):88. doi:10.1038/s41419-020-2298-2

5. Yang M, Li X, Li H, Zhang X, Liu X, Song Y. Baicalein inhibits RLS3-induced ferroptosis in melanocytes. *Biochem Biophys Res Commun*. 2021;561:65–72. doi:10.1016/j.bbrc.2021.05.010

6. Wang W, Green M, Choi JE, et al. CD8+ T cells regulate tumour ferroptosis during cancer immunotherapy. *Nature*. 2019;569(7755):270–274. doi:10.1038/s41586-019-1170-y

7. Bragazzi MC, Ridola L, Safarikia S, et al. New insights into cholangiocarcinoma: multiple stems and related cell lineages of origin. *Ann Gastroenterol*. 2018;31(1):42–55. doi:10.20524/aog.2017.0209

8. Razumilava N, Gores GJ. Cholangiocarcinoma. *Lancet*. 2014;383(9935):2168–2179. doi:10.1016/S0140-6736(13)61903-0

9. Cardinale V, Carpino G, Reid L, Gaudio E, Alvaro D. Multiple cells of origin in cholangiocarcinoma underlie biological, epidemiological and clinical heterogeneity. *World J Gastrointest Oncol*. 2012;4(5):94–102. doi:10.4251/wjgo.v4.i5.94

10. Patel T. New insights into the molecular pathogenesis of intrahepatic cholangiocarcinoma. *J Gastroenterol*. 2014;49(2):165–172. doi:10.1007/s00535-013-0894-y

11. Peng JJ, Song WT, Yao F, et al. Involvement of regulated necrosis in blinding diseases: focus on necroptosis and ferroptosis. *Exp Eye Res*. 2020;191:107922. doi:10.1016/j.exer.2020.107922

12. Torti SV, Torti FM. Iron and cancer: more ore to be mined. *Nat Rev Cancer*. 2013;13(5):342–355. doi:10.1038/nrc3495

13. Stockwell BR, Friedmann Angeli JP, Bayir H, et al. Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. *Cell*. 2017;171(2):273–285. doi:10.1016/j.cell.2017.09.021

14. Dixon SJ, Lemberg KM, Lamprecht MR, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell*. 2012;149(5):1060–1072. doi:10.1016/j.cell.2012.03.042

15. Friedmann Angeli JP, Schneider M, Proneth B, et al. Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. *Nat Cell Biol*. 2014;16(12):1180–1191. doi:10.1038/ncb3064

16. Agmon E, Solon J, Bassereau P, Stockwell BR. Modeling the effects of lipid peroxidation during ferroptosis on membrane properties. *Sci Rep*. 2018;8(1):5155. doi:10.1038/s41598-018-23408-0

17. Su LJ, Zhang JH, Gomez H, et al. Reactive oxygen species-induced lipid peroxidation in apoptosis, autophagy, and ferroptosis. *Oxid Med Cell Longev*. 2019;2019:5080843. doi:10.1155/2019/5080843

18. Tang D, Chen X, Kang R, Kroemer G. Ferroptosis: molecular mechanisms and health implications. *Cell Res*. 2021;31(2):107–125. doi:10.1038/s41422-020-00441-1

19. Mou Y, Wang J, Wu J, et al. Ferroptosis, a new form of cell death: opportunities and challenges in cancer. *J Hematol Oncol*. 2019;12(1):34. doi:10.1186/s13045-019-0720-y

20. Wang X, Chen Y, Yang X, et al. Activation of ALOX12 by a multi-organelle-orienting photosensitizer drives ACSL4-independent cell ferroptosis. *Cell Death Dis*. 2022;13(12):1040. doi:10.1038/s41419-022-05462-9

21. Chen X, Kang R, Kroemer G, Tang D. Broadening horizons: the role of ferroptosis in cancer. *Nat Rev Clin Oncol*. 2021;18(5):280–296. doi:10.1038/s41571-020-00462-0

22. Lei G, Zhuang L, Gan B. Targeting ferroptosis as a vulnerability in cancer. *Nat Rev Cancer*. 2022;22(7):381–396. doi:10.1038/s41568-022-00459-0

23. Zabron A, Edwards RJ, Khan SA. The challenge of cholangiocarcinoma: dissecting the molecular mechanisms of an insidious cancer. *Dis Model Mech*. 2013;6(2):281–292. doi:10.1242/dmm.010561

24. Çolakoğlu M, Tunçer S, Banerjee S. Emerging cellular functions of the lipid metabolizing enzyme 15-Lipoxygenase-1. *Cell Prolif*. 2018;51(5): e12472. doi:10.1111/cpr.12472

25. Rae SA, Davidson EM, Smith MJ. Leukotriene B4, an inflammatory mediator in gout. *Lancet*. 1982;2(8308):1122–1124. doi:10.1016/s0140-6736(82)92785-4

26. Marrogi AJ, Travis WD, Welsh JA, et al. Nitric oxide synthase, cyclooxygenase 2, and vascular endothelial growth factor in the angiogenesis of non-small cell lung carcinoma. *Clin Cancer Res*. 2000;6(12):4739–4744.

27. Kuwano T, Nakao S, Yamamoto H, et al. Cyclooxygenase 2 is a key enzyme for inflammatory cytokine-induced angiogenesis. *FASEB J*. 2004;18 (2):300–310. doi:10.1096/fj.03-0473com

28. Zhang Z, Lai GH, Sirica AE. Celecoxib-induced apoptosis in rat cholangiocarcinoma cells mediated by Akt inactivation and Bax translocation. *Hepatology*. 2004;39(4):1028–1037. doi:10.1002/hep.20143

29. Han C, Leng J, Demetris AJ, Wu T. Cyclooxygenase-2 promotes human cholangiocarcinoma growth: evidence for cyclooxygenase-2-independent mechanism in celecoxib-mediated induction of p21WAF1/cip1 and p27Kip1 and cell cycle arrest. *Cancer Res*. 2004;64(4):1369–1376. doi:10.1158/0008-5472.can-03-1086

30. Jaiswal M, LaRusso NF, Shapiro RA, Billiar TR, Gores GJ. Nitric oxide-mediated inhibition of DNA repair potentiates oxidative DNA damage in cholangiocytes. *Gastroenterology*. 2001;120(1):190–199. doi:10.1053/gast.2001.20875

31. Ishimura N, Bronk SF, Gores GJ. Inducible nitric oxide synthase up-regulates Notch-1 in mouse cholangiocytes: implications for carcinogenesis. *Gastroenterology*. 2005;128(5):1354–1368. doi:10.1053/j.gastro.2005.01.055

32. Wu WR, Zhang R, Shi XD, et al. Notch1 is overexpressed in human intrahepatic cholangiocarcinoma and is associated with its proliferation, invasiveness and sensitivity to 5-fluorouracil in vitro. *Oncol Rep*. 2014;31(6):2515–2524. doi:10.3892/or.2014.3123

33. Yoon HA, Noh MH, Kim BG, et al. Clinicopathological significance of altered Notch signaling in extrahepatic cholangiocarcinoma and gallbladder carcinoma. *World J Gastroenterol*. 2011;17(35):4023–4030. doi:10.3748/wjg.v17.i35.4023

34. Sugawara H, Yasoshima M, Katayanagi K, et al. Relationship between interleukin-6 and proliferation and differentiation in cholangiocarcinoma. *Histopathology*. 1998;33(2):145–153. doi:10.1046/j.1365-2559.1998.00445.x

35. Isomoto H, Mott JL, Kobayashi S, et al. Sustained IL-6/STAT-3 signaling in cholangiocarcinoma cells due to SOCS-3 epigenetic silencing. *Gastroenterology*. 2007;132(1):384–396. doi:10.1053/j.gastro.2006.10.037

36. Komori J, Marusawa H, Machimoto T, et al. Activation-induced cytidine deaminase links bile duct inflammation to human cholangiocarcinoma. *Hepatology*. 2008;47(3):888–896. doi:10.1002/hep.22125

37. Boulter L, Guest RV, Kendall TJ, et al. WNT signaling drives cholangiocarcinoma growth and can be pharmacologically inhibited. *J Clin Invest*. 2015;125(3):1269–1285. doi:10.1172/JCI76452

38. Monga SP. β-catenin signaling and roles in liver homeostasis, injury, and tumorigenesis. *Gastroenterology*. 2015;148(7):1294–1310. doi:10.1053/j.gastro.2015.02.056

39. Fernández-Barrena MG, Perugorria MJ, Banales JM. Novel lncRNA T-UCR as a potential downstream driver of the Wnt/β-catenin pathway in hepatobiliary carcinogenesis. *Gut*. 2017;66(7):1177–1178. doi:10.1136/gutjnl-2016-312899

40. Yang WS, SriRamaratnam R, Welsch ME, et al. Regulation of ferroptotic cancer cell death by GPX4. *Cell*. 2014;156(1–2):317–331. doi:10.1016/j. cell.2013.12.010

41. Folco G, Murphy RC. Eicosanoid transcellular biosynthesis: from cell-cell interactions to in vivo tissue responses. *Pharmacol Rev*. 2006;58 (3):375–388. doi:10.1124/pr.58.3.8

42. Kim EH, Wong SW, Martinez J. Programmed necrosis and disease: we interrupt your regular programming to bring you necroinflammation. *Cell Death Differ*. 2019;26(1):25–40. doi:10.1038/s41418-018-0179-3

43. Yu Y, Xie Y, Cao L, et al. The ferroptosis inducer erastin enhances sensitivity of acute myeloid leukemia cells to chemotherapeutic agents. *Mol Cell Oncol*. 2015;2(4):e1054549. doi:10.1080/23723556.2015.1054549

44. Wen Q, Liu J, Kang R, Zhou B, Tang D. The release and activity of HMGB1 in ferroptosis. *Biochem Biophys Res Commun*. 2019;510(2):278–283. doi:10.1016/j.bbrc.2019.01.090

45. Splichal I, Donovan SM, Jenistova V, et al. High mobility group box 1 and TLR4 signaling pathway in gnotobiotic piglets colonized/infected with L. amylovorus, L. mucosae, E coli Nissle 1917 and S. Typhimurium. *Int J Mol Sci*. 2019;20(24):6294. doi:10.3390/ijms20246294

46. Son GH, Kim Y, Lee JJ, et al. MicroRNA-548 regulates high mobility group box 1 expression in patients with preterm birth and chorioamnionitis. *Sci Rep*. 2019;9(1):19746. doi:10.1038/s41598-019-56327-9

47. Kim S, Keku TO, Martin C, et al. Circulating levels of inflammatory cytokines and risk of colorectal adenomas. *Cancer Res*. 2008;68(1):323–328. doi:10.1158/0008-5472.CAN-07-2924

48. Proneth B, Conrad M. Ferroptosis and necroinflammation, a yet poorly explored link. *Cell Death Differ*. 2019;26(1):14–24. doi:10.1038/s41418-018-0173-9

49. Hentze MW, Muckenthaler MU, Galy B, Camaschella C. Two to tango: regulation of mammalian iron metabolism. *Cell*. 2010;142(1):24–38. doi:10.1016/j.cell.2010.06.028

50. Daniels TR, Delgado T, Helguera G, Penichet ML. The transferrin receptor part II: targeted delivery of therapeutic agents into cancer cells. *Clin Immunol*. 2006;121(2):159–176. doi:10.1016/j.clim.2006.06.006

51. Bian Z, Hann HW, Ye Z, et al. Ferritin level prospectively predicts hepatocarcinogenesis in patients with chronic hepatitis B virus infection. *Oncol Lett*. 2018;16(3):3499–3508. doi:10.3892/ol.2018.9099

52. Song A, Eo W, Kim S, Shim B, Lee S. Significance of serum ferritin as a prognostic factor in advanced hepatobiliary cancer patients treated with Korean medicine: a retrospective cohort study. *BMC Complement Altern Med*. 2018;18(1):176. doi:10.1186/s12906-018-2240-7

53. Lipper CH, Karmi O, Sohn YS, et al. Structure of the human monomeric NEET protein MiNT and its role in regulating iron and reactive oxygen species in cancer cells. *Proc Natl Acad Sci U S A*. 2018;115(2):272–277. doi:10.1073/pnas.1715842115

54. Mittler R, Darash-Yahana M, Sohn YS, et al. NEET proteins: a new link between iron metabolism, reactive oxygen species, and cancer. *Antioxid Redox Signal*. 2019;30(8):1083–1095. doi:10.1089/ars.2018.7502

55. Gu Z, Wang H, Xia J, et al. Decreased ferroportin promotes myeloma cell growth and osteoclast differentiation. *Cancer Res*. 2015;75 (11):2211–2221. doi:10.1158/0008-5472.CAN-14-3804

56. Xue D, Zhou CX, Shi YB, Lu H, He XZ. Erratum: decreased expression of ferroportin in prostate cancer. *Oncol Lett*. 2021;21(4):257. doi:10.3892/ ol.2021.12518

57. Guo W, Zhang S, Chen Y, et al. An important role of the hepcidin-ferroportin signaling in affecting tumor growth and metastasis. *Acta Biochim Biophys Sin*. 2015;47(9):703–715. doi:10.1093/abbs/gmv063

58. Shan Z, Wei Z, Shaikh ZA. Suppression of ferroportin expression by cadmium stimulates proliferation, EMT, and migration in triple-negative breast cancer cells. *Toxicol Appl Pharmacol*. 2018;356:36–43. doi:10.1016/j.taap.2018.07.017

59. Bystrom LM, Guzman ML, Rivella S. Iron and reactive oxygen species: friends or foes of cancer cells? *Antioxid Redox Signal*. 2014;20 (12):1917–1924. doi:10.1089/ars.2012.5014

60. Fonseca-Nunes A, Jakszyn P, Agudo A. Iron and cancer risk—a systematic review and meta-analysis of the epidemiological evidence. *Cancer Epidemiol Biomarkers Prev*. 2014;23(1):12–31. doi:10.1158/1055-9965.EPI-13-0733

61. Manz DH, Blanchette NL, Paul BT, Torti FM, Torti SV. Iron and cancer: recent insights. *Ann N Y Acad Sci*. 2016;1368(1):149–161. doi:10.1111/ nyas.13008

62. Marques O, da Silva BM, Porto G, Lopes C. Iron homeostasis in breast cancer. *Cancer Lett*. 2014;347(1):1–14. doi:10.1016/j.canlet.2014.01.029

63. Torti SV, Torti FM. Ironing out cancer. *Cancer Res*. 2011;71(5):1511–1514. doi:10.1158/0008-5472.CAN-10-3614

64. Recalcati S, Gammella E, Buratti P, Cairo G. Molecular regulation of cellular iron balance. *IUBMB Life*. 2017;69(6):389–398. doi:10.1002/ iub.1628

65. Biemond P, van Eijk HG, Swaak AJ, Koster JF. Iron mobilization from ferritin by superoxide derived from stimulated polymorphonuclear leukocytes. Possible mechanism in inflammation diseases. *J Clin Invest*. 1984;73(6):1576–1579. doi:10.1172/JCI111364

66. Yang WS, Stockwell BR. Synthetic lethal screening identifies compounds activating iron-dependent, nonapoptotic cell death in oncogenic-RAS-harboring cancer cells. *Chem Biol*. 2008;15(3):234–245. doi:10.1016/j.chembiol.2008.02.010

67. Hassannia B, Vandenabeele P, Vanden Berghe T. Targeting ferroptosis to iron out cancer. *Cancer Cell*. 2019;35(6):830–849. doi:10.1016/j. ccell.2019.04.002

68. Kwok JC, Richardson DR. The iron metabolism of neoplastic cells: alterations that facilitate proliferation? *Crit Rev Oncol Hematol*. 2002;42 (1):65–78. doi:10.1016/s1040-8428(01)00213-x

69. Yagoda N, von Rechenberg M, Zaganjor E, et al. RAS-RAF-MEK-dependent oxidative cell death involving voltage-dependent anion channels. *Nature*. 2007;447(7146):864–868. doi:10.1038/nature05859

70. Torti SV, Torti FM. Winning the war with iron. *Nat Nanotechnol*. 2019;14(6):499–500. doi:10.1038/s41565-019-0419-9

71. Gao M, Monian P, Quadri N, Ramasamy R, Jiang X. Glutaminolysis and transferrin regulate ferroptosis. *Mol Cell*. 2015;59(2):298–308. doi:10.1016/j.molcel.2015.06.011

72. Bogdan AR, Miyazawa M, Hashimoto K, Tsuji Y. Regulators of iron homeostasis: new players in metabolism, cell death, and disease. *Trends Biochem Sci*. 2016;41(3):274–286. doi:10.1016/j.tibs.2015.11.012

73. Kalinowski DS, Stefani C, Toyokuni S, et al. Redox cycling metals: pedaling their roles in metabolism and their use in the development of novel therapeutics. *Biochim Biophys Acta*. 2016;1863(4):727–748. doi:10.1016/j.bbamcr.2016.01.026

74. Lui GY, Kovacevic Z, Richardson V, Merlot AM, Kalinowski DS, Richardson DR. Targeting cancer by binding iron: dissecting cellular signaling pathways. *Oncotarget*. 2015;6(22):18748–18779. doi:10.18632/oncotarget.4349

75. Merlot AM, Kalinowski DS, Richardson DR. Novel chelators for cancer treatment: where are we now? *Antioxid Redox Signal*. 2013;18 (8):973–1006. doi:10.1089/ars.2012.4540

76. Ma B, Meng H, Tian Y, et al. Distinct clinical and prognostic implication of IDH1/2 mutation and other most frequent mutations in large duct and small duct subtypes of intrahepatic cholangiocarcinoma. *BMC Cancer*. 2020;20(1):318. doi:10.1186/s12885-020-06804-6

77. Jamnongkan W, Thanan R, Techasen A, et al. Upregulation of transferrin receptor-1 induces cholangiocarcinoma progression via induction of labile iron pool. *Tumour Biol*. 2017;39(7):1010428317717655. doi:10.1177/1010428317717655

78. Thanan R, Oikawa S, Yongvanit P, et al. Inflammation-induced protein carbonylation contributes to poor prognosis for cholangiocarcinoma. *Free Radic Biol Med*. 2012;52(8):1465–1472. doi:10.1016/j.freeradbiomed.2012.01.018

79. Yu H, Guo P, Xie X, Wang Y, Chen G. Ferroptosis, a new form of cell death, and its relationships with tumourous diseases. *J Cell Mol Med*. 2017;21(4):648–657. doi:10.1111/jcmm.13008

80. Guo J, Xu B, Han Q, et al. Ferroptosis: a novel anti-tumor action for cisplatin. *Cancer Res Treat*. 2018;50(2):445–460. doi:10.4143/crt.2016.572

81. Sun X, Ou Z, Chen R, et al. Activation of the p62-Keap1-NRF2 pathway protects against ferroptosis in hepatocellular carcinoma cells. *Hepatology*. 2016;63(1):173–184. doi:10.1002/hep.28251

82. Liang C, Zhang X, Yang M, Dong X. Recent progress in ferroptosis inducers for cancer therapy. *Adv Mater*. 2019;31(51):e1904197. doi:10.1002/adma.201904197

83. Riegman M, Bradbury MS, Overholtzer M. Population dynamics in cell death: mechanisms of propagation. *Trends Cancer*. 2019;5(9):558–568. doi:10.1016/j.trecan.2019.07.008

84. Zhu T, Shi L, Yu C, et al. Ferroptosis promotes photodynamic therapy: supramolecular photosensitizer-inducer nanodrug for enhanced cancer treatment. *Theranostics*. 2019;9(11):3293–3307. doi:10.7150/thno.32867