Emerging insights into ferroptosis in cholangiocarcinoma (Review)

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Abstract. Cholangiocarcinoma (CCA) is a malignant tumor that arises within the biliary system, which exhibits a progressively increasing incidence and a poor patient prognosis. A thorough understanding of the molecular pathogenesis that drives the progression of CCA is essential for the development of effective molecular target therapeutic approaches. Ferroptosis is driven by excessive iron accumulation and catalysis, lipid peroxidation and the failure of antioxidant defense systems. Key molecular targets of iron metabolism, lipid metabolism and antioxidant defense systems involve molecules such as transferrin receptor, ACSL4 and GPX4, respectively. Inhibitors of ferroptosis include ferrostatin-1, liproxstatin-1, vitamin E and coenzyme Q10. By contrast, compounds such as erastin, RSL3 and FIN56 have been identified as inducers of ferroptosis. Ferroptosis serves a notable role in the onset and progression of CCA. CCA cells exhibit high sensitivity to ferroptosis and aberrant iron metabolism in these cells increases oxidative stress and iron accumulation. The induction of ferroptosis markedly reduces the ability of CCA cells to proliferate and migrate. Certain ferroptosis agonists, such as RSL3 and erastin, cause lipid peroxide build up and GPX4 inhibition to induce ferroptosis in CCA cells. Current serological markers, such as CA-199, have low specificity and cause difficulties in the diagnosis of CCA. However, novel techniques, such as non-invasive liquid biopsy and assays for oxidative stress markers and double-cortin-like kinase 1, could improve diagnostic accuracy. CCA is primarily treated with surgery and chemotherapy. A close association between the progression of CCA with ferroptosis mechanisms and related regulatory pathways has been demonstrated. Therefore, it could be suggested that multi-targeted therapeutic approaches, such as ferroptosis inducers, iron chelating agents and novel modulators such as YL-939, may improve treatment efficacy. Iron death-related genes, such as GPX4, that are highly expressed in CCA and are associated with a poor prognosis for patients may represent potential prognostic markers for CCA. The present review focused on molecular targets such as p53 and ACSL4, the process of targeted medications in combination with PDT in CCA and the pathways of lipid peroxidation, the Xc^- system and GSH-GPX4 in ferroptosis. The present review thus offered novel perspectives to improve the current understanding of CCA.

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1. Introduction

Cholangiocarcinoma (CCA) is a type of malignant tumor that originates from the epithelial cells of the biliary system. CCA ranks among the most common malignant tumors affecting the biliary system and is the second most prevalent primary liver tumor following hepatocellular carcinoma. In the US, ~23,000 individuals are diagnosed annually, while the condition is more prevalent in Asia, partly due to parasitic infections common in that region. In Australia, ~1,300 new cases are reported each year. The incidence rate of CCA in the US is ~9.4 per 100,000 individuals, with a mortality rate of ~6.6 per 100,000 (1). CCA can be categorized based on its location as intrahepatic CCA (iCCA) or extrahepatic CCA (eCCA; Fig. 1) (2,3). Numerous factors influence the onset of CCA. Prolonged inflammation in the biliary tract, associated with factors such as cholecystitis and bile duct stones, is deemed a significant risk factor for bile duct cancer. Parasitic infections, particularly liver fluke infections prevalent in northeastern Thailand (4), are also associated to the development of bile duct cancer. Additionally, environmental, genetic

and dietary factors closely intertwine with the occurrence of bile duct cancer (5).

Ferroptosis, a novel form of programmed cell death that is associated with intracellular iron ion accumulation and oxidative stress, manifests key features such as intracellular iron overload and subsequent lipid peroxidation (6). However, recent studies have shown that ferroptosis is associated with the development and progression of a variety of types of cancers, such as liver, triple-negative breast and non-small cell lung cancers (7-9). An association between ferroptosis and CCA has been previously reported (5). Therefore, an in-depth exploration of the role of ferroptosis in CCA is important to improve the current understanding of the developmental mechanisms of CCA, the development of new diagnostic and therapeutic strategies and the assessment of patient prognosis.

2. Concepts and characteristics of ferroptosis

Ferroptosis was initially conceptualized by Dixon *et al* (10) in 2012 as an iron-dependent form of regulated cell death instigated by an overload of lipid peroxides on cell membranes. This definition underscores the connection between ferroptosis and lipid peroxide overload, framing it as a regulated mode of cell death. In ferroptosis, the buildup of intracellular iron ions results in an escalation of intracellular oxidative stress, which ultimately triggers cell death (11). Ferroptosis can be distinguished from conventional apoptosis and necrosis through its distinct triggering mechanism and morphological features, as it represents a novel form of cell death regulated by specific iron metabolic pathways. Regarding morphological characteristics, apoptosis typically features cell shrinkage, nuclear chromatin condensation and the formation of apoptotic vesicles on the cell membrane (12). By contrast, a distinguishing feature of ferroptosis is the altered structure and function of mitochondria, as ferroptosis leads to impaired mitochondrial function. This is evident through observations of reduced or absent mitochondrial cristae, rupture of the outer mitochondrial membrane and increased mitochondrial membrane concentration (13,14). The main biochemical characteristics of ferroptosis are iron ion accumulation overload and increased oxidative stress (15,16).

3. Ferroptosis and inflammation

Inflammation represents the natural defense and repair response of an organism to various stimuli, such as infection or injury, and typically manifests as redness, swelling, heat, pain and dysfunction of local tissues (17). Abnormal inflammatory responses are closely associated with disorders in iron metabolism and imbalances in the redox system (18). Pro-inflammatory cytokines, including IL-6 and IL-1β, impact iron processing by hepatocytes, which influences iron storage and distribution throughout the body (19). The release of TNF-α and IFN-γ disrupts the redox system and increases intracellular oxidative stress (20). This state of oxidative stress affects the accumulation and utilization of intracellular iron ions, which potentially results in abnormal iron accumulation and triggers iron-related cell damage, including ferroptosis (21). NF-κB, a transcription factor with specific DNA-binding activity, is involved in classical pro-inflammatory signaling pathways [TNF-α, IL-1, IKK (the IKK complex is a key regulator in the NF-κB signaling pathway and consists of three subunits, IKKα, IKKβ and NEMO); NF-κB essential modulator, also known as IKKγ] that regulate inflammation and the immune system. Modulating the NF-κB signaling pathway can reduce inflammatory responses and injury, while also regulating iron metabolism pathways during inflammation (22). For instance, dimethyl fumarate (DMF) serves a role in reducing neuroinflammation and ferroptosis by regulating the NF-κB signaling pathway (23). It has been reported that DMF demonstrates efficacy in improving cognitive deficits caused by vessel occlusion in a rat model of chronic cerebral hypoperfusion (24). A previous study by Zhao *et al* (25) reported that artemisinin prevents ferroptosis-induced liver injury by inhibiting reactive oxygen species (ROS) and inflammation through the activation of the nuclear factor E2-related factor 2 (Nrf2)/heme oxygenase-1/glutathione peroxidase 4 (GPX4) pathway and downregulation of NF-κB.

The MAPK family, including ERK, JNK and p38 MAPK isoforms, serve crucial roles in intracellular signaling. When activated by external stimuli, such as the role of cytokines, oxidative stress and mechanical stress. These kinases trigger a series of enzymatic reactions, which lead to the expression of inflammation-related genes and inflammatory responses (26). Similar to NF-κB, the activation of the MAPK pathway is associated with the process of ferroptosis. It has been suggested that salvianolic acid B, a potent polyphenolic compound derived from *Salvia miltiorrhiza* (Danshen) known for its strong antioxidant, anti-inflammatory and cardioprotective properties, may attenuate ferroptosis and apoptosis during myocardial ischemia and reperfusion injury by inhibiting ROS production or modulating ROS levels, thereby inhibititing activation of the JNK/MAPK pathway (27). Furthermore, Toll-like receptor 4 (TLR4) is a crucial immune receptor that initiates the p38 MAPK pathway upon activation, which prompts the production of cytokines such as IL-1β, IL-6 and IL-18. This process may impact the expression levels of solute carrier family 7 member 11 (SLC7A11) and GPX4, both of which regulate redox homeostasis and antioxidant stress responses (28). A reduction in SLC7A11 and GPX4 expression levels may lead to neuroinflammation and ferroptosis (29). The aforementioned evidence suggests a notable association between inflammation and ferroptosis.

4. Ferroptosis and mitochondrial metabolism

Mitochondria serve as the energy-producing hubs within cells, generating adenosine triphosphate (ATP) through oxidative phosphorylation to supply energy to the cell (30). Beyond their role in energy production, mitochondria are crucial in regulating intracellular signaling pathways (31). Moreover, mitochondria are involved in numerous cell death pathways, including apoptosis, necrosis and ferroptosis (32). In the context of ferroptosis, intracellular iron ions catalyze the production of ROS that initiate lipid peroxidation and lead to cell death. Mitochondria act as the primary source of ROS (33). The electron transport chain within mitochondria, a component of oxidative phosphorylation, generates ROS, with numerous electrons binding to oxygen molecules to form superoxide anions. Moreover, diverse metabolic processes that occur within mitochondria, including lipid and amino acid metabolism, actively

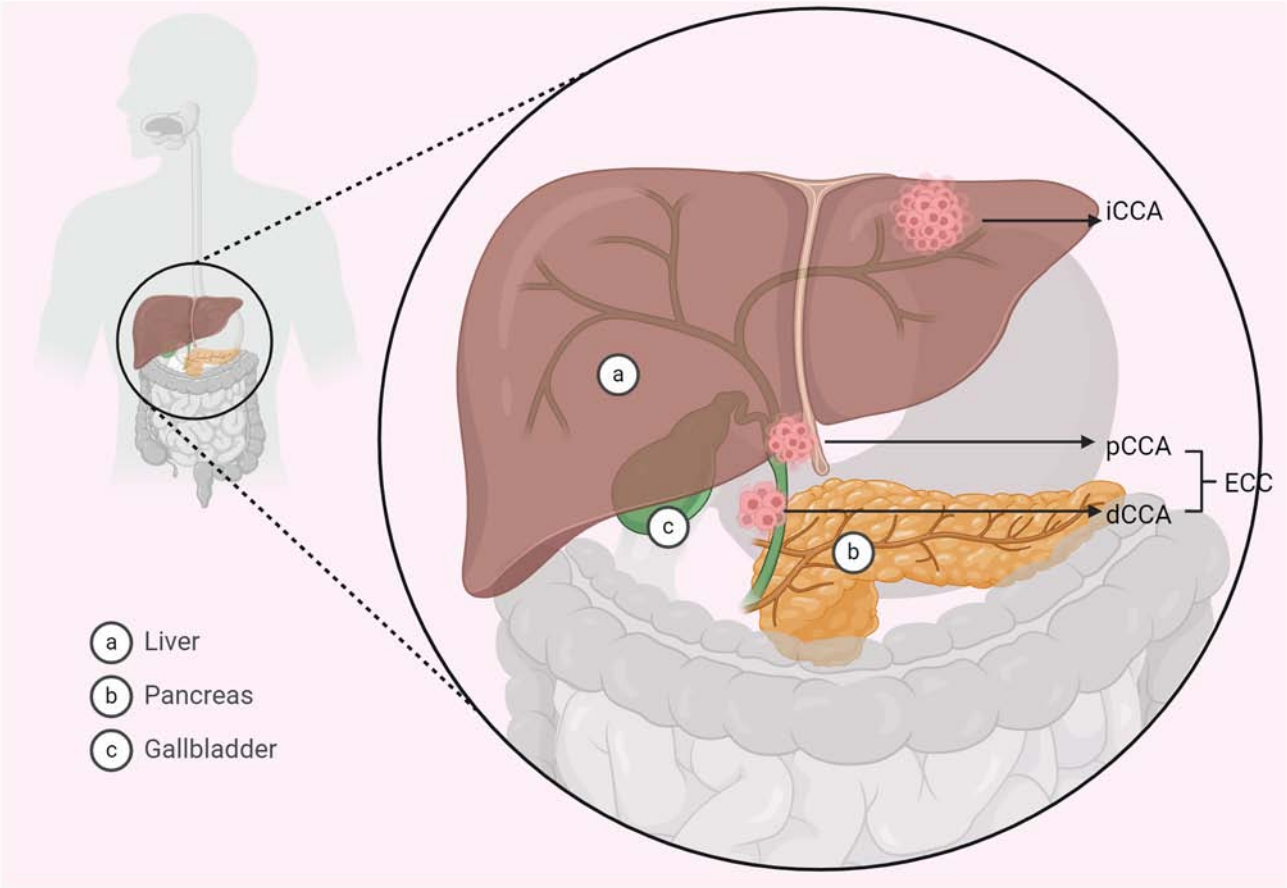

Figure 1. Classification of CCA. CCA can be categorized based on its intrahepatic (iCCA) or extrahepatic (eCCA) location. eCCA includes the perihilar and distal subtypes, pCCA and dCCA. respectively. iCCA primarily develops proximal to the secondary bile ducts within the liver parenchyma. pCCA is located between the secondary bile ducts and the junction of the cystic duct with the common bile duct, whereas dCCA is restricted to the common bile duct below the cystic duct junction. CCA, cholangiocarcinoma; iCCA, intrahepatic CCA; eCCA extrahepatic CCA; pCCA, perihilar CCA; dCCA, distal CCA.

participate in the production of ROS (34). The mitochondrial generation of ROS can potentially contribute to ferroptosis by fostering lipid peroxidation (35). In situations of intracellular ATP deficiency, the energy sensor AMP-activated protein kinase (AMPK) is activated. AMPK activation inhibits the activity of acetyl-coenzyme A carboxylase, which impacts the rate of fatty acid synthesis (36). Furthermore, AMPK activity influences the intracellular iron metabolism and ferroptosis by decreasing the uptake of iron ions by intestinal epithelial cells, as well as the storage and release of iron ions by the liver (37). By contrast, in conditions of abundant ATP, AMPK activation is less efficient, which leads to the activation of acetyl-coenzyme A carboxylase. This activation, in turn, promotes the synthesis of polyunsaturated fatty acid (PUFA) phospholipids and consequently supports the occurrence of ferroptosis (38). Nrf2 is a crucial transcription factor with roles in antioxidant and cytoprotective functions within cells. By regulating the expression of antioxidant defense systems, including antioxidant enzymes within mitochondria, Nrf2 serves a crucial role in ferroptosis (39). Nrf2 also influences mitochondrial energy metabolism and cell signaling, which impacts cell survival and antioxidant capacity (40). Given the association of ferroptosis with ROS, Nrf2 regulation of mitochondrial function has far-reaching effects on ferroptosis. Specifically, Nrf2 protects cells from damage by maintaining mitochondrial function and reducing ROS production, thereby reducing oxidative stress from ferritin deposition (41). In addition, exposure to exogenous hydrogen peroxide can activate iron response elements involved in the regulation of iron metabolism by binding to mRNA, which can inhibit the translation of ferritin and reduce ferritin synthesis, thus increasing the concentration of free iron in cells; in addition, it can promote the degradation of mRNA and further inhibit the expression of ferritin. The increase in free iron may lead to enhanced Fenton reaction and generation of more ROS, thus exacerbating intracellular oxidative stress (42).

5. Mechanisms related to ferroptosis

Iron metabolism. In healthy cells, iron is an essential trace element involved in a number of biological processes such as DNA synthesis, the respiratory chain and oxygen delivery (43). The body must control the uptake, storage and excretion of iron in order to maintain intracellular and body iron homeostasis (44). The human body contains iron in the form of ferric ions. When intracellular ferric ions are overloaded, harmful ROS and lipid peroxides, which are catalyzed by $Fe2^{+}$ via the Fenton reaction, can be produced, which potentially results in cell death. Thus, a major factor in cellular iron toxicity is the absorption, release, storage and transit of intracellular

iron ions (45). Several studies have reported that the demand for iron ions is typically higher in cancer cells compared with healthy cells, because cancer cells need more iron to support their rapid proliferation and metabolic activities (46). Therefore, regulating intracellular iron storage and release can have a direct impact on the survival and death of cancer cells (47,48). Cellular iron uptake occurs primarily through the transferrin (Tf) receptor (TfR)-mediated pathway. Tf binds Fe^{3+}, which then binds to TfR and enters the cell via receptor-mediated endocytosis. The acidic environment within the endocytosed vesicle dissociates Fe^{3+} from Tf, which is reduced to Fe^{2+} by a metal reductase, such as hexamethylene-tetramine reductase 1 and STEAP family member 1. Fe^{2+} is subsequently transported via the divalent metal transporter 1 into the cytoplasm of the cell (49,50). Dysregulation of iron metabolism can lead to excessive accumulation of intracellular Fe^{2+}. Fe^{2+} participates in the Fenton reaction, which generates high ROS levels, including hydroxyl radicals. These high ROS levels can trigger lipid peroxidation and damage the cellular membranes, which ultimately leads to ferroptosis (51). Deletion or dysfunction of the heavy chain of ferritin leads to aberrant accumulation of iron and increased sensitivity of cells to ferroptosis. Furthermore, the regulation of ferritin expression is overseen by the iron-responsive element and iron regulatory protein. These proteins govern the translation of ferritin and transferrin receptors and thereby exert finely tuned control over intracellular iron homeostasis (52).

ROS and lipid peroxidation. The mechanisms of ROS generation and lipid peroxidation are central features of ferroptosis. Examples of ROS include substances such as superoxide anion, hydrogen peroxide and hydroxyl radicals (53). These substances are products of redox reactions and are important cell signaling molecules. ROS can act as secondary messengers to regulate a variety of cell signaling pathways, including inflammation, apoptosis and cell proliferation. Oxidative stress refers to the excessive production of highly reactive molecules, such as ROS and reactive nitrogen species, in the body (54). When the body is subjected to various harmful stimuli (ultraviolet radiation, pollution, smoking, inflammatory reactions or infections), the oxidizing capacity exceeds the capacity of its own oxidant scavenging systems and the oxidative and antioxidant systems are imbalanced, thus leading to tissue pathology and damage (55). Iron ions are involved in a number of biological processes within the cell, but an excess of free iron ions can lead to the production of large amounts of free radicals, thus causing oxidative stress (56). Oxidative stress affects the metabolism and storage of iron ions, which leads to an abnormal accumulation of iron ions in the cells (57). Excessive intracellular ROS leads to lipid peroxidation, a key feature of ferroptosis. Phospholipids are a major component of cell membranes, of which phosphatidylethanolamines (PEs) are important. Since PE is rich in polyunsaturated fatty acids (PUFA), these unsaturated bonds are highly susceptible to attack by ROS, generating lipid peroxides (e.g., PUFA-PE-OOH). Arachidonic acid (AA) and its derivative adrenergic acid (ADA) are fatty acids that bind to PEs to form phospholipids (58). AA and ADA are key phospholipids in which oxidation occurs and are thought to be important contributors to iron-related cell death (10). Acyl coenzyme A synthase long-chain family member 4 (ACSL4) acylates AA to fatty acyl-coenzyme A, and lysophosphatidylcholine acyltransferase 3 (LPCAT3) catalyzes the acylation of ADA to membrane phospholipids. This process increases the oxidative sensitivity of membrane-sensitive fatty acids such as PUFA, which leads to the development of lipid peroxidation and further triggers ferroptosis (59). During ferroptosis, an abnormal accumulation of iron ions may lead to the production of lethal levels of lipid peroxides that can damage cell membrane integrity and cause cell death (60).

GSH-GPX4. GSH, a tripeptide composed of glutamate, cysteine and glycine, is an important antioxidant in cells. GSH exists mainly in the reduced form which protects cells from oxidative damage by donating electrons to neutralize ROS and other free radicals (61). The main function of GPX4 is to catalyze the glutathione GSH-dependent reduction of membrane lipid peroxides to their corresponding alcohols, thereby protecting cell membranes from oxidative damage (62). In addition, GPX4 is involved in the regulation of intracellular iron ion homeostasis, which is important for maintaining intracellular redox homeostasis and reducing oxidative stress (63). GPX4 utilizes GSH as a substrate to reduce lipid peroxides and prevent the expansion of lipid peroxidation reaction, which protects the integrity and function of the cell membrane. The depletion or inhibition of GSH results in inefficient lipid peroxide scavenging, leading to uncontrolled lipid peroxidation chain reaction and ultimately triggering the ferroptosis process (64,65). The Xc^- system, also known as the cysteine-glutamate reverse transporter system, is a transmembrane transporter protein complex consisting of two subunits: i) SLC7A11, which is responsible for cysteine uptake; and ii) SLC3A2, which aids in the function of SLC7A11. The activity of the Xc^-system has a direct effect on intracellular levels of GSH (50). An adequate supply of cysteine by the Xc^- system ensures continuous synthesis of GSH and maintenance of intracellular antioxidant capacity, thus indirectly enhancing the function of GPX4 and preventing the accumulation of lipid peroxides and ferroptosis (66). The expression levels and activity of the Xc^-system are regulated by a number of factors, including oxidative stress, intracellular glutamate levels and multiple signaling pathways, such as the Nrf2 pathway (53). Nrf2 can regulate the expression of antioxidant genes, including components of the Xc^-system and SLC7A11 (67).

Other access

p53. p53 is an important tumor suppressor gene, and the p53 protein serves a key role in a number of physiological processes such as cell cycle regulation, DNA repair and cell death (68). Recent studies have shown that p53 also serves an important role in ferroptosis, mainly affecting the ferroptosis pathway through the regulation of several factors (SLC7A11, GPX4, FPN1) (69-72). p53 can reduce the expression levels of SLC7A11 by directly inhibiting its transcription, which reduces cysteine uptake and GSH synthesis, thereby impairing GPX4 activity and increasing cellular sensitivity to ferroptosis (73,74). Arachidonate 12-lipoxygenase, 12S type (ALOX12) is an enzyme involved in lipid metabolism, and its full name is '12-lipoxygenase'. It is primarily responsible for catalyzing the production of specific lipid metabolites

from polyunsaturated fatty acids such as arachidonic acid. p53 can promote lipid peroxidation by regulating ALOX12 expression. It has been shown that p53 can activate ALOX12 transcription under conditions of DNA damage or oxidative stress and increase the production of lipid peroxides, thereby promoting ferroptosis (75). p53 can enhance the sensitivity to ferroptosis by upregulating the expression levels of SAT1 and p53-induced nuclear protein 1 (TP53INP1). TP53INP1 is a stress-responsive protein regulated by the p53 protein and is involved in the regulation of apoptosis, proliferation, and stress response. TP53INP1 is considered a downstream effector of the p53 protein. When cells are stressed or injured, TP53INP1 enhances p53 activity, further driving ferroptosis. The upregulation of SAT1 can trigger an increase in intracellular oxidative stress and the accumulation of lipid peroxides, which further promote ferroptosis (76,77).

p62-Kelch-like ECH-associated protein 1 (Keap1)-Nrf2 pathway. p62, also known as sequestosome 1, is a multifunctional junction protein involved in a number of cellular processes including autophagy, signaling and protein degradation (78). Keap1 is a cytoplasmic protein that, under normal conditions, can bind to Nrf2 to inhibit its activity by promoting Nrf2 ubiquitination and degradation, thereby regulating the stability and activity of Nrf2 (79). p62 can bind to Keap1 through its Keap1-interacting region, which causes Nrf2 to be released from the Keap1 complex, preventing the inhibitory effect of Keap1 on Nrf2. Nrf2-activated antioxidant genes, such as GPX4, reduce intracellular lipid accumulation of peroxides and prevent ferroptosis (80). Nrf2 can also affect intracellular iron distribution and storage by regulating the expression of ferredoxin and iron transporter proteins, thus indirectly affecting the sensitivity to ferroptosis (81).

Ferroptosis suppressor protein 1 (FSP1)-coenzyme Q10 (CoQ10)-NAD(P)H pathway. FSP1 is a ferroptosis inhibitory protein, also known as apoptosis-inducing factor mitochondria associated 2. FSP1 functions independently of GPX4 and participates in ferroptosis pathways (82). CoQ10 is an antioxidant that dissolves in fat and is found in almost all body cells particularly the inner membrane of the mitochondria. FSP1 can reduce ferroptosis by decreasing CoQ10 expression levels and inhibiting the lipid peroxidation chain reaction (83). NAD(P)H is the phosphorylated form of NADH that is essential for cellular metabolism and antioxidant responses. By providing the electrons needed for FSP1 to reduce CoQ10 and by preserving the levels of CoQ10H2, NAD(P)H maintains antioxidant defense. CoQ10H2 is the reduced form of coenzyme Q10 (CoQ10), also known as ubiquinol. CoQ10H2 is a potent fat-soluble antioxidant that protects cell membranes from oxidative damage by directly neutralizing free radicals, especially by trapping and neutralizing lipid peroxides (84). The FSP1-CoQ10-NAD(P)H pathway protects the structure and function of cell membranes, while also directly scavenging free radicals and lipid peroxides to strengthen cellular antioxidant defenses (Fig. 2) (85).

6. Ferroptosis in CCA

Numerous cancer-related signaling pathways have been shown to control ferroptosis in cancer cells (47). In CCA cells, iron metabolism may be abnormal, which leads to iron accumulation and increased oxidative stress triggering ferroptosis (86). The abnormal iron accumulation in CCA cells can result from various factors, including heightened iron uptake, irregular expression of transporter proteins and anomalies in iron storage proteins (87). Ferroportin (FPN) is a pivotal protein responsible for regulating iron export. It serves a crucial role in the cell membrane as it facilitates the export of intracellular iron to maintain the balance of iron ions both inside and outside the cell (88). It was shown that the expression of FPN was significantly reduced in CCA (89). Bile duct stones, bile duct stenosis, and bile duct parasitic infections can cause biliary obstruction. Chronic bile duct obstruction can cause cholestasis, which in turn promotes chronic bile duct inflammation is an important factor that can cause CCA. ROS and reactive nitrogen species can damage biomolecules in the inflammatory milieu, such as DNA, proteins and lipids, and cause malfunction to create a cycle of oxidative stress imbalance that eventually promotes tumorigenesis and progression, including cholangiocarcinoma (90). Oxidative stress from inflammation can result in Fe^{3+} binding and TfR oxidation, which promotes the release and buildup of iron. Iron transporter protein function is inhibited by IL-6 activation in response to inflammatory or infectious stimuli through FPN-dependent pathways or alternative routes (91). Carbonylation of serum transferrin, heat shock protein 70 (HSP70) and α1-antitrypsin that occurs through inflammation may be crucial. Carbonylation alters the function of these key proteins, leading to a worsening of the inflammatory response, increased oxidative stress and deeper cellular damage (92). Carbonylation of serum transferrin improves the Fenton reaction, which leads to the accumulation of iron in intracellular accumulations and extracellular release in case of cell damage or death. When iron is released and free iron increases, the iron generates large amounts of ROS via the Fenton reaction, leading to oxidative stress. Carbonylation of a1-antitrypsin, a protease inhibitor, and HSP70, an antioxidant, causes their malfunction, GPX4 degradation and ferroptosis promotion. These factors contribute to the advancement of CCA and are associated with a poor prognosis (93). Therefore, it is important to understand the survival mechanism of ferroptosis in CCA cells to develop new therapeutic strategies that prevent the growth and spread of CCA cells (Fig. 3).

7. Diagnosis of ferroptosis in CCA

In its early stages, CCA typically presents with minimal symptoms, which poses a challenge for early diagnosis. A substantial proportion of patients with CCA receive a diagnosis only in the advanced stages and miss the optimal window for treatment (2). Although commonly utilized, the prevailing serologic diagnostic tool, CA-199, exhibits limitations in specificity and sensitivity (52). Notably, doublecortin-like kinase 1 (DCLK1) is a promising candidate for the diagnosis of bile duct cancers, such as CCA (94). Given the inconspicuous nature of CCA symptoms, early detection of CCA often relies on histological testing methods. Immunohistochemical staining is a frequently employed technique as it allows for the identification of characteristic alterations in ferroptosis within patient tissue sections. This is achieved by labeling iron-related proteins or molecules

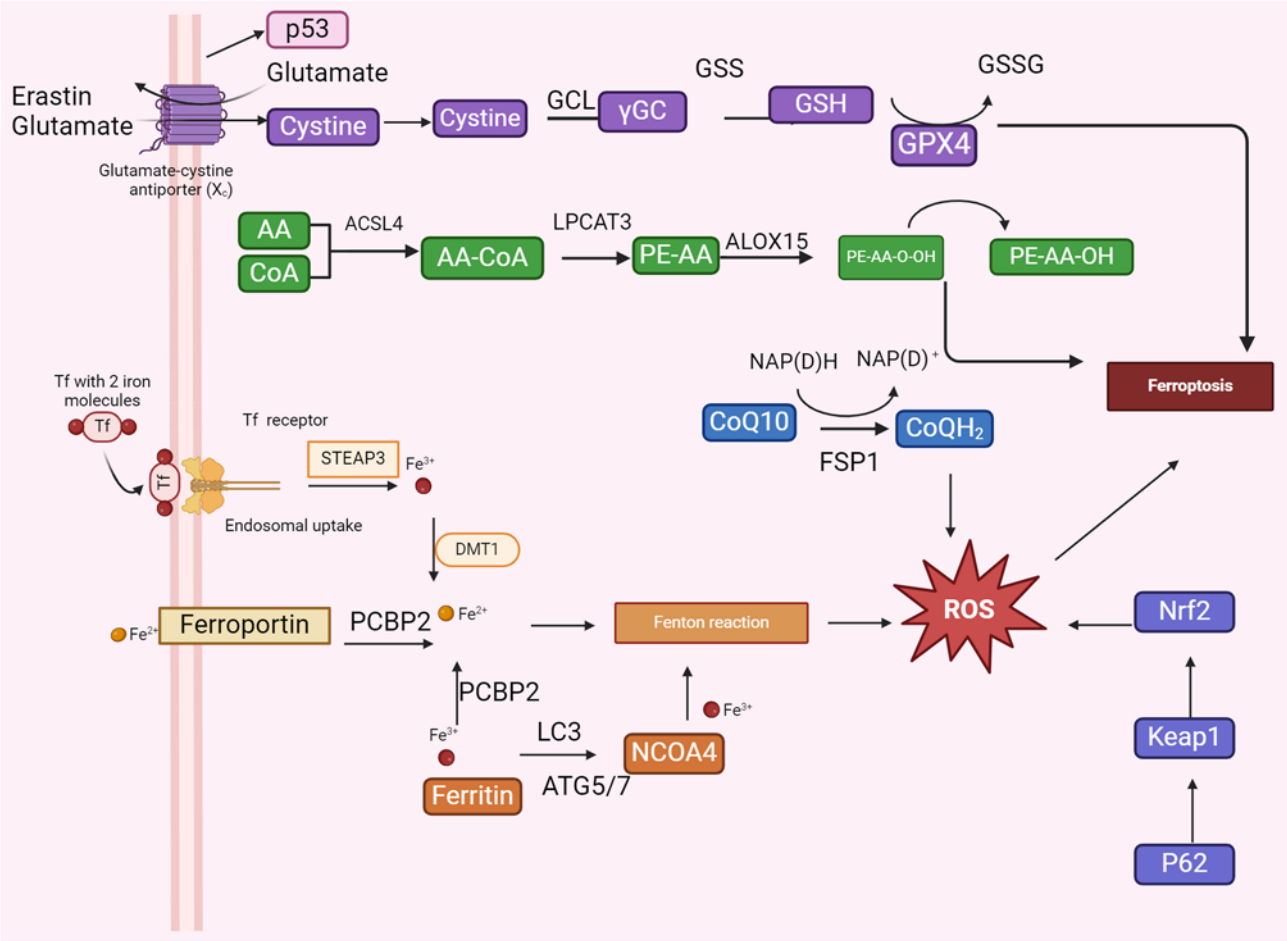

Figure 2. Regulatory mechanisms of ferroptosis. ROS, reactive oxygen species; GCL, glutamate-cysteine ligase; GSS, glutathione synthetase; GSH, glutathione; GPX4, glutathione peroxidase; GSSG, glutathione disulfide; ACSL4, acyl-CoA synthetase long chain family member 4; LPCAT3, lysophosphatidylcholine acyltransferase 3; AA, arachidonic acid; ALOX15, arachidonic acid 15-lipoxygenase; CoQ10, coenzyme Q10; FSP1, ferroptosis regulatory protein 1; STEAP3, six-transmembrane epithelial antigen of the prostate 3; DMT1, divalent metal-ion transporter-1; PCBP2, poly(rC)-binding protein 2; LC3, microtubule-associated protein 1A/1B-light chain 3; Keap1, Kelch-like ECH-associated protein 1; Nrf2, nuclear factor erythroid 2-related factor 2; Tf, transferrin; γGC, γ-glutamylcysteine; Erastin is an experimental drug used primarily in research on cancer treatment. It is a known inducer of ferroptosis.

associated with iron metabolism using specific antibodies (CK7, CK19, CK20, MUC5AC) (95). In the diagnostic evaluation of CCA, assessing protein expression levels related to iron metabolism, such as transferrin, iron carrier proteins and ferritin, is instrumental in gauging intracellular iron accumulation. To complement histological testing methods, imaging techniques offer additional avenues for diagnosis (96). Ultrasonography facilitates the examination of tumor morphology and hemodynamic characteristics in patients with CCA, while CT scans provide insights into changes in tissue density and morphological features of tumors (97). In the context of ferroptosis, CT scans may identify distinct features that distinguish ferroptosis from other forms of cell death, such as areas of low tissue density or uniform enhancement within the tumor (98). However, the direct detection of ferroptosis remains a challenge through CT imaging. In CCA, the progression of tumorigenesis correlates with the extent of ferroptosis-induced oxidative stress (99). Therefore, the degree of intracellular oxidative stress may be indirectly assessed by measuring oxidative stress markers, such as ROS levels and the activity of peroxidases, including superoxide dismutase, GSH peroxidase and catalase (92).

While histological examination is a pivotal tool for a definitive diagnosis of CCA, its utility is hampered by limited access to tissue specimens, as this often necessitates invasive procedures such as surgery or puncture. The inherent risks associated with these invasive methods, particularly in patients with compromised health, underscore the importance of alternative diagnostic approaches (100). Liquid biopsy, characterized by the extraction of exosomes from CCA cells, has potential to improve diagnostic accuracy and highlights the demand for precise non-invasive biomarkers for CCA (101). However, this technique has yet to find application in routine clinical practice (102). Therefore, serologic diagnostic methods could be further explored and improved to enhance the accurate diagnosis and treatment of CCA in the future.

8. Therapeutic strategies for ferroptosis in CCA

At present, the primary approach to treat patients with CCA remains rooted in surgical interventions, and the prognosis for patients ineligible for surgery is poor (103). Survival outcomes, however, show promise with the integration of chemotherapeutic modalities. The combination of gemcitabine and

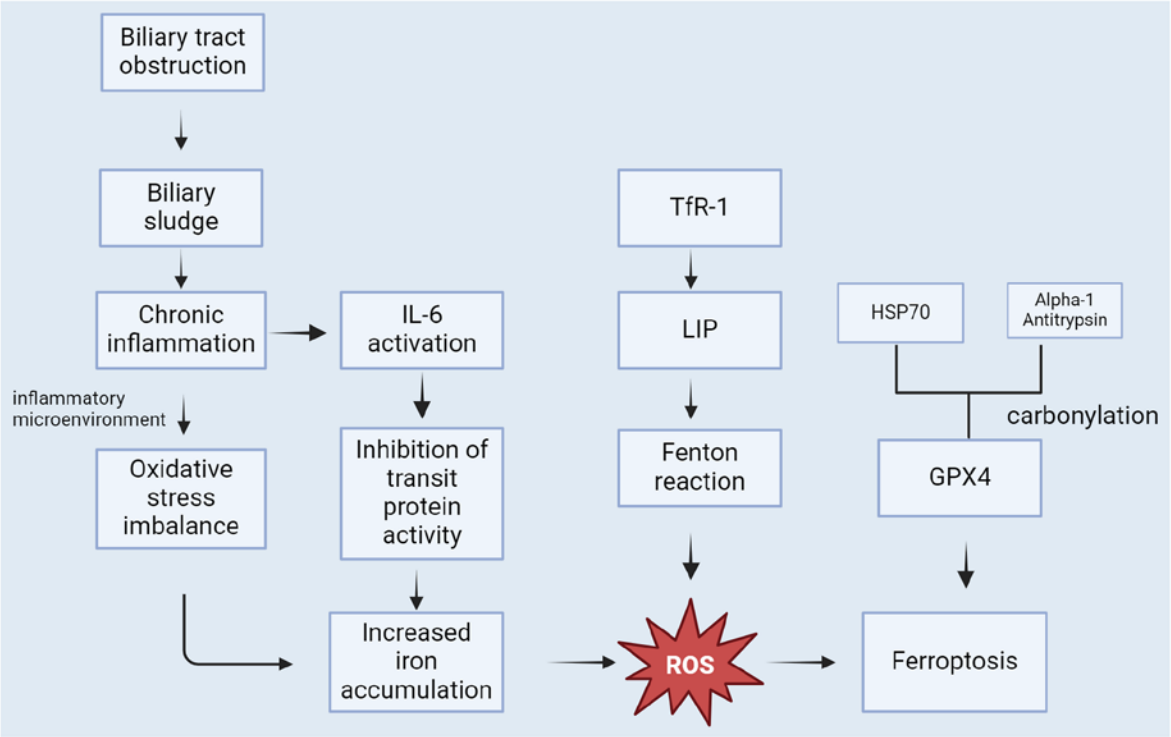

Figure 3. Mechanisms of ferroptosis in cholangiocarcinoma. TfR-1, transferrin receptor 1; LIP, labile iron pool; HSP70, heat shock protein 70; a1AT, a-1 antitrypsin; GPX4, glutathione peroxidase; ROS, reactive oxygen species.

cisplatin is currently suggested as the most effective first-line treatment option for CCA to improve patient prognosis (104). Recent studies have explored the efficacy of combining bevacizumab with Gemcitabine and cisplatin cytotoxic therapy, which demonstrated improved survival rates for patients with CCA (105,106). Based on the roles of ferroptotic mechanisms and pathways in cancer, it has been shown that the addition of ferroptosis-regulating drugs to the certain treatments may increase their efficacy (99). The negative regulator of ferroptosis, p53-induced glycolysis and apoptosis regulator (TIGAR), inhibits glycolysis by regulating the activity of phosphofructokinase-2 in the glycolytic pathway, thereby reducing intracellular ROS production (107). Studies have shown that combination treatment of cisplatin and low expression of TIGAR significantly induced ferroptosis (108). Several ferroptosis inducers, such as Ras selective lethal 3 (RSL3), sulfasalazine and erastin, have shown potential to induce ferroptosis in CCA cells *in vitro* and in animal models (109). The drug reduces intracellular antioxidant capacity by inhibiting system Xc^- and increases cellular sensitivity to ferroptosis (110). In the treatment of CCA, iron chelators may inhibit tumor growth and metastasis by regulating the balance of intracellular iron metabolism and reducing the toxic effects of iron ions. The use of chelators may improve the therapeutic efficacy compared with the use of platinum-based chemotherapeutic agents (111). In addition, the use of multi-targeted drugs, including those involving iron chelators and iron-chelator complexes, is a therapeutic direction to be considered (112). Application of ferroptosis antioxidants may help to attenuate tumor cell damage and death. Antioxidants may inhibit ferroptosis by scavenging excessive intracellular ROS and attenuating oxidative stress (113). Glutathione is an important antioxidant molecule that can help reduce other oxidized antioxidants and reduce cellular damage from oxidative stress (66). Moreover, Yang *et al* (114) have identified a novel ferroptosis inhibitor, YL-939, which is distinct from traditional iron chelators and antioxidants. YL-939, a non-classical ferroptosis inhibitor, has been reported to target prohibitin 2 (PHB2), which suggests binding of YL-939 to PHB2 promotes expression of the iron storage protein ferritin, which reduces iron content and thus susceptibility to ferroptosis. Despite the association of YL-939 with liver injury, this discovery opens new avenues for investigating the role of YL-939 in CCA (114).

It has been shown that aberrant p53 expression levels are typically closely associated with tumor development (115). p53, a well-established oncogene, is a critical regulator of apoptosis and cell cycle processes. In response to DNA damage or stresses such as oxidative stress, anticancer drugs and inflammatory responses, p53 proteins are activated and play important protective roles in cells by orchestrating survival or death pathways (116). p53 may indirectly increase intracellular oxidative stress and lipid peroxidation through the activation of SAT1 and regulation of the ALOX-15 pathways. These processes are implicated in regulating ferroptosis, contributing to cholangiocarcinogenesis (76). Through *in vitro* and *in vivo* experiments, low expression levels of FBXO31 have been demonstrated to promote ferroptosis by facilitating the ubiquitination of GPX4 and leading to protease degradation. This process increases the sensitivity of CCA stem cells to cisplatin, which exerts a tumor-suppressive effect (117).

Mechanistic pathways through which ferroptosis is regulated can also influence CCA progression. The regulatory network in CCA involves microRNA (miR)-3202 and GPX4, a protein pivotal in intracellular oxidative stress and iron metabolism. The interplay of JUND/linc00976 have been identified

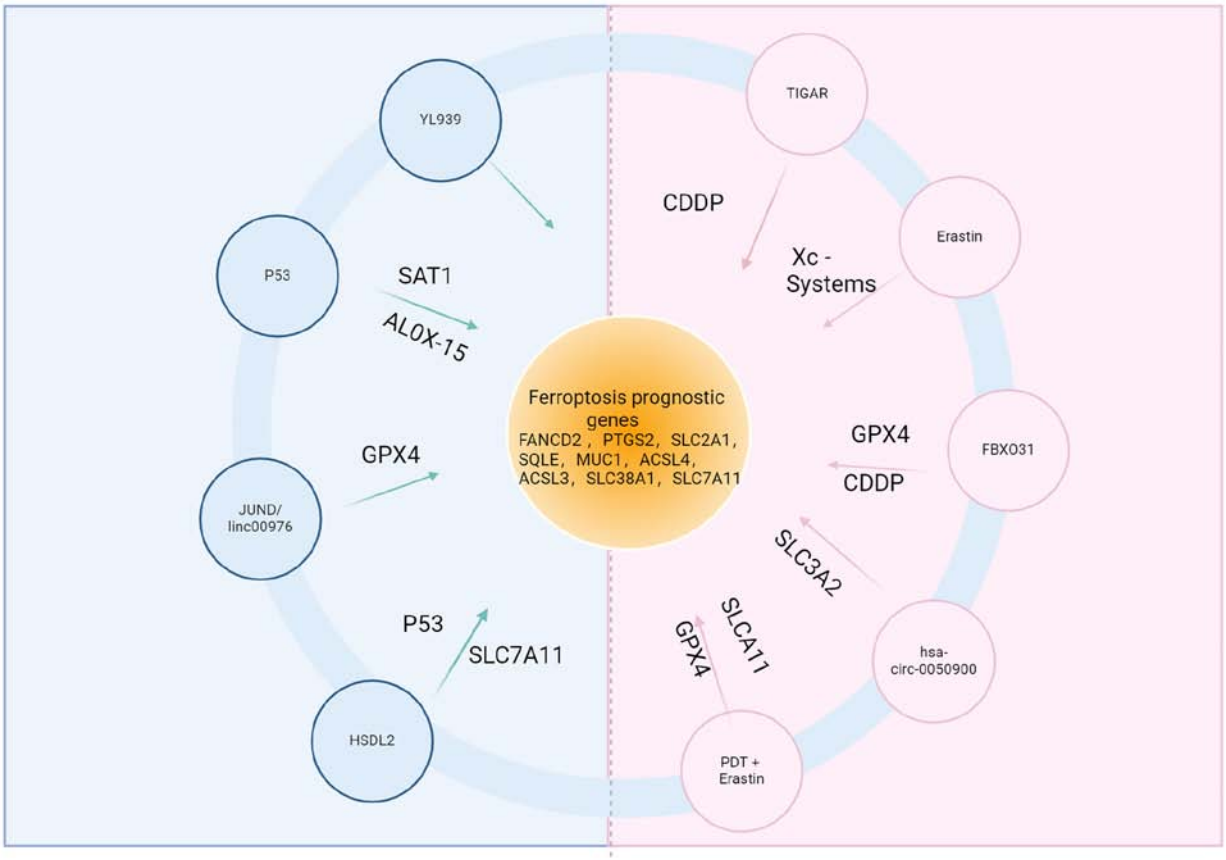

Figure 4. Relationship between treatments and prognosis of ferroptosis in CCA. The blue part indicates inhibition of ferroptosis and the pink part promotion of ferroptosis. The large circles are therapeutic targets and the yellow circles are prognostic genes for iron death in CCA. TIGAR, TP53-induced glycolysis and apoptosis regulator; CDDP, cisplatin; PDT, photodynamic therapy; ALOX15, arachidonic acid 15-lipoxygenase; HSDL2, hydroxysteroid dehydrogenase like 2; GPX4, glutathione peroxidase 4; Ub, ubiquitylated; ACSL4, acyl-CoA synthetase long chain family member 4; SLC7A11, solute carrier family 7 member 11; CCA, cholangiocarcinoma.

as a regulator of intracellular iron metabolism and modulates the ferroptosis process through the miR-3202/GPX4 pathway. This regulatory mechanism promotes the progression and metastasis of CCA (118). Immunohistochemistry was used to show that hydroxysteroid dehydrogenase-like 2 (HSDL2) expression levels in tissues was lower compared with those in matched neighboring non-tumor bile duct tissues. By decreasing malondialdehyde, ROS levels and inhibiting ferroptosis via the p53/SLC7A11 axis, HSDL2 knockdown was found to promote CCA progression. Therefore, HSDL2 may be a useful treatment target and prognosis indicator for CCA (119). A previous study demonstrated the potential of downregulating hsa_circ_0050900, identifying its role as a sponge that inhibits SLC3A2 expression while promoting the expression of hsa-miR-605-3p. This mechanism induces ferroptosis in CCA cells, which results in the inhibition of cell proliferation and migration (120). Notably, trematode infection stands among the risk factors for CCA (121). Photodynamic therapy (PDT) utilizes photosensitizers to generate ROS for the targeted eradication of CCA tumor cells (122,123). Recent research has demonstrated that by causing iron mortality in *in vitro* assays and tumor models, tofacitinib in conjunction with PDT could suppress CCA (124).

The integration of ferroptosis inducers in combination with PDT presents a dual-enhancement strategy for tumor therapy. Firstly, the substantial ROS production induced by PDT complements the necessary accumulation of lipid ROS for ferroptosis, which amplifies the therapeutic impact. Secondly, the ferroptosis inducer further augments the cytotoxic effect on tumor cells. By employing this combined strategy, a synergistic effect may emerge, which improves the overall efficacy against tumors (125). It has been shown that PDT inhibits cancer progression and induces ferroptosis and apoptosis by targeting the p53/GPX4/SLC7A11 signaling pathway in CCA (126). Although PDT has shown positive results in the treatment of CCA, the study is still in the early stages of clinical trials in mice (127). This combined therapeutic strategy may provide novel breakthroughs and research directions for the application of ferroptosis in tumor therapy (Fig. 4).

9. Relationship between ferroptosis and the prognosis of CCA

CCA, characterized by its high heterogeneity and aggressive nature within the bile duct, poses a formidable challenge in clinical management, marked by a lack of precise prognostic biomarkers and poor overall prognosis. In this challenging landscape, the emerging field of ferroptosis, a regulated cell death mechanism linked to cancer progression, offers a novel perspective and potential avenues for prognostic assessment in CCA. It has been demonstrated that patients with advanced CCA can live longer when using a combination of PDT, an

efficient anticancer treatment, and a novel multi-kinase inhibitor sofantinib (SUR) (128). A previous study measured the levels of ROS, lipid peroxides, malondialdehyde and glutathione to demonstrate that SUR combined with PDT can promote ferroptosis to suppress CCA cell proliferation. Therefore, it could be suggested that further research on combination therapeutic approaches that address ferroptosis could enhance patient prognosis (127,129). Furthermore, it was reported that circFOXP1-231aa, a recently identified protein, controls the stability of nuclear receptor coactivator 4; it is involved in iron homeostasis regulation and regulates intracellular iron release by mediating ferritin autophagy through de-ubiquitination modification, increase in ferroptosis in ICC cells (refers to cancer cells originating from the epithelium of the intrahepatic bile ducts, which constitute the major cell type of iCCA) and preventing ICC recurrence (130). Several ferroptosis-related genes have demonstrated prognostic implications not only in CCA but also in hepatocellular carcinoma and pancreatic cancer (131). Sae-Fung *et al* (99) conducted a comprehensive database analysis and identified several genes (FRG, FANCD2, PTGS2, SLC2A1, SQLE, ACO1 and GOT1) associated with ferroptosis. Notably, FANCD2, PTGS2, SLC2A1 and SQLE exhibit increased expression levels in CCA tumor-associated tissues, while ACO1 and GOT1 demonstrate reduced expression levels. These findings suggest a potential correlation with poor prognosis in patients with CCA (132,133). Additionally, five ferroptosis-related genes (MUC1, ACSL4, ACSL3, SLC38A1 and SLC7A11) not only hold promise for immunotherapeutic interventions in CCA but also exhibit prognostic relevance in the context of this malignancy (134). Among the highlighted ferroptosis-related genes, ACSL4 stands out as a crucial regulator of lipid metabolism and intracellular iron ion homeostasis. A significant correlation between increased ACSL4 expression levels in CCA and adverse patient prognosis has been reported, which suggests a potential role for ACSL4 as a prognostic marker for CCA (135). Another pivotal participant in the ferroptosis process is GPX4, and initial studies have suggested its candidacy as a prognostic marker for CCA, further contributing to the expanding understanding of the intricate molecular landscape associated with CCA (136,137). Although the results of these clinical studies provide a preliminary indication of the relationship between ferroptosis and the prognosis of CCA, further large-scale multicenter clinical studies are needed to validate and explore this relationship in depth.

10. Summary and outlook

Ferroptosis, as a distinct form of cell death deviating from traditional paradigms, has gained increasing prominence in clinical practice, particularly with the ongoing exploration of its mechanisms. Its pivotal role in a number of diseases, notably cancer, has generated significant interest due to the potential significance in the diagnosis, treatment and prognosis of CCA. There are still many unanswered concerns regarding the mechanism of ferroptosis and treatment approaches in CCA, which are currently in the exploratory stage. Future studies may concentrate on combinations of therapeutic strategies in order to further create and optimize strategies for treating ferroptosis. The safety, efficacy and prognosis improvement of therapeutic options related to ferroptosis may be assessed by large-scale clinical trials and prospective research, thereby offering a scientific foundation for clinical practice.

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Availability of data and materials

Not applicable.

Authors' contributions

XiZ, MZ, JH, XL and XuZ contributed to the idea and design of the article. Data collection and analysis were carried out by MZ, XL and JH. The first draft of the manuscript was written by XiZ. XiZ, MZ, JH, XL and XuZ commented on previous versions of the manuscript. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Rizvi S and Gores GJ: Pathogenesis, diagnosis, and management of cholangiocarcinoma. Gastroenterology 145: 1215-1229, 2013.
2. Cardinale V: Classifications and misclassification in cholangiocarcinoma. Liver Int 39: 260-262, 2019.
3. Brindley PJ, Bachini M, Ilyas SI, Khan SA, Loukas A, Sirica AE, The BT, Wongkham S and Gores GJ: Cholangiocarcinoma. Nat Rev Dis Primers 7: 65, 2021.
4. Sripa B, Suwannatrai AT, Sayasone S, Do DT, Khieu V and Yang Y: Current status of human liver fluke infections in the Greater Mekong Subregion. Acta Trop 224: 106133, 2021.
5. Qurashi M, Vithayathil M and Khan SA: Epidemiology of cholangiocarcinoma. Eur J Surg Oncol 9: 107064, 2023.
6. Kim R, Taylor D, Vonderheide RH and Gabrilovich DI: Ferroptosis of immune cells in the tumor microenvironment. Trends Pharmacol Sci 44: 542-552, 2023.
7. Xu Y, Xing Z, Suliman RAI, Liu Z and Tang F: Ferroptosis in liver cancer: A key role of post-translational modifications. Front Immunol 15: 1375589, 2024.
8. Zhou TJ, Zhang MM, Liu DM, Huang LL, Yu HQ, Wang Y, Xing L and Jiang HL: Glutathione depletion and dihydroorotate dehydrogenase inhibition actuated ferroptosis-augment to surmount triple-negative breast cancer. Biomaterials 305: 122447, 2024.

9. Deng J, Lin X, Qin J, Li Q, Zhang Y, Zhang Q, Ji C, Shen S, Li Y, Zhang B and Lin N: SPTBN2 suppresses ferroptosis in NSCLC cells by facilitating SLC7A11 membrane trafficking and localization. Redox Biol 70: 103039, 2024.
10. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, Patel DN, Bauer AJ, Cantley AM, Yang WS, *et al*: Ferroptosis: An iron-dependent form of nonapoptotic cell death. Cell 149: 1060-1072, 2012.
11. Nakamura T, Naguro I and Ichijo H: Iron homeostasis and iron-regulated ROS in cell death, senescence and human diseases. Biochim Biophys Acta Gen Subj 1863: 1398-1409, 2019.
12. D'Arcy MS: Cell death: A review of the major forms of apoptosis, necrosis and autophagy. Cell Biol Int 43: 582-592, 2019.
13. Fu C, Cao N, Zeng S, Zhu W, Fu X, Liu W and Fan S: Role of mitochondria in the regulation of ferroptosis and disease. Front Med (Lausanne) 10: 1301822, 2023.
14. Otasevic V, Vucetic M, Grigorov I, Martinovic V and Stancic A: Ferroptosis in different pathological contexts seen through the eyes of mitochondria. Oxid Med Cell Longev 2021: 5537330, 2021.
15. Xie LH, Fefelova N, Pamarthi SH and Gwathmey JK: Molecular mechanisms of ferroptosis and relevance to cardiovascular disease. Cells 11: 2726, 2022.
16. Liu J, Kang R and Tang D: Signaling pathways and defense mechanisms of ferroptosis. FEBS J 289: 7038-7050, 2022.
17. Liu T, Zhang L, Joo D and Sun SC: NF-κB signaling in inflammation. Signal Transduct Target Ther 2: 17023, 2017.
18. Ahmad A and Ahsan H: Biomarkers of inflammation and oxidative stress in ophthalmic disorders. J Immunoassay Immunochem 41: 257-271, 2020.
19. Yang W, Wang Y, Zhang C, Huang Y, Yu J, Shi L, Zhang P, Yin Y, Li R and Tao K: Maresin1 protect against ferroptosis-induced liver injury through ROS inhibition and Nrf2/HO-1/GPX4 activation. Front Pharmacol 13: 865689, 2022.
20. Tanase DM, Gosav EM, Anton MI, Floria M, Isac PN, Hurjui LL, Tarniceriu CC, Costea CF, Ciocoiu M and Rezus C: Oxidative stress and NRF2/KEAP1/ARE pathway in diabetic kidney disease (DKD): New perspectives. Biomolecules 12: 1227, 2022.
21. Ueda N and Takasawa K: Impact of inflammation on ferritin, hepcidin and the management of iron deficiency anemia in chronic kidney disease. Nutrients 10: 1173, 2018.
22. Chen Y, Fang ZM, Yi X, Wei X and Jiang DS: The interaction between ferroptosis and inflammatory signaling pathways. Cell Death Dis 14: 205, 2023.
23. Schmitt A, Xu W, Bucher P, Grimm M, Konantz M, Horn H, Zapukhlyak M, Berning P, Brändle M, Jarboui MA, *et al*: Dimethyl fumarate induces ferroptosis and impairs NF-κ/STAT3 signaling in DLBCL. Blood 138: 871-884, 2021.
24. Yan N, Xu Z, Qu C and Zhang J: Dimethyl fumarate improves cognitive deficits in chronic cerebral hypoperfusion rats by alleviating inflammation, oxidative stress, and ferroptosis via NRF2/ARE/NF-κB signal pathway. Int Immunopharmacol 98: 107844, 2021.
25. Zhao C, Xiao C, Feng S and Bai J: Artemisitene alters LPS-induced oxidative stress, inflammation and ferroptosis in liver through Nrf2/HO-1 and NF-kB pathway. Front Pharmacol 14: 1177542, 2023.
26. Yeung YT, Aziz F, Guerrero-Castilla A and Arguelles S: Signaling pathways in inflammation and anti-inflammatory therapies. Curr Pharm Des 24: 1449-1484, 2018.
27. Xu X, Mao C, Zhang C, Zhang M, Gong J and Wang X: Salvianolic Acid B inhibits ferroptosis and apoptosis during myocardial ischemia/reperfusion injury via decreasing the ubiquitin-proteasome degradation of GPX4 and the ROS-JNK/MAPK pathways. Molecules 28: 4117, 2023.
28. Lu JS, Wang JH, Han K and Li N: Nicorandil regulates ferroptosis and mitigates septic cardiomyopathy via TLR4/SLC7A11 signaling pathway. Inflammation 47: 975-988, 2024.
29. Zhu K, Zhu X, Sun S, Yang W, Liu S, Tang Z, Zhang R, Li J, Shen T and Hei M: Inhibition of TLR4 prevents hippocampal hypoxic-ischemic injury by regulating ferroptosis in neonatal rats. Exp Neurol 345: 113828, 2021.
30. Cardoso-Pires C and Vieira HLA: Carbon monoxide and mitochondria: Cell energy and fate control. Biochim Biophys Acta Mol Basis Dis 1870: 167446, 2024.
31. Du L, Guo C, Zeng S, Yu K, Liu M and Li Y: Sirt6 overexpression relieves ferroptosis and delays the progression of diabetic nephropathy via Nrf2/GPX4 pathway. Ren Fail 46: 2377785, 2024.
32. Sun K, Zhi Y, Ren W, Li S, Zhou X, Gao L and Zhi K: The mitochondrial regulation in ferroptosis signaling pathway and its potential strategies for cancer. Biomed Pharmacother 169: 115892, 2023.
33. Murphy MP: How mitochondria produce reactive oxygen species. Biochem J 417: 1-13, 2009.
34. Su D, Ding C, Wang R, Qiu J, Liu Y, Tao J, Luo W, Weng G, Yang G and Zhang T: E3 ubiquitin ligase RBCK1 confers ferroptosis resistance in pancreatic cancer by facilitating MFN2 degradation. Free Radic Biol Med 221: 136-154, 2024.
35. Zheng J and Conrad M: The metabolic underpinnings of ferroptosis. Cell Metab 32: 920-937, 2020.
36. Xiang Q, Wen J, Zhou Z, Dai Q, Huang Y, Yang N, Guo J, Zhang J, Ren F, Zhou X, *et al*: Effect of hydroxy-α-sanshool on lipid metabolism in liver and hepatocytes based on AMPK signaling pathway. Phytomedicine 132: 155849, 2024.
37. Copur S, Yildiz AB, Covic A and Kanbay M: Is there any robust evidence showing that SGLT2 inhibitor predisposes to cancer? Eur J Clin Invest 54: e14131, 2024.
38. Gao M, Yi J, Zhu J, Minikes AM, Monian P, Thompson CB and Jiang X: Role of mitochondria in ferroptosis. Mol Cell 73: 354-363 e353, 2019.
39. Qi Y, Zheng J, Zi Y, Song W, Chen X, Cao S, Zhou Q, Fu H and Hu X: Loureirin C improves mitochondrial function by promoting NRF2 nuclear translocation to attenuate oxidative damage caused by renal ischemia-reperfusion injury. Int Immunopharmacol 138: 112596, 2024.
40. Zhang T, Jing M, Fei L, Zhang Z, Yi P, Sun Y and Wang Y: Tetramethylpyrazine nitrone delays the aging process of C. elegans by improving mitochondrial function through the AMPK/mTORC1 signaling pathway. Biochem Biophys Res Commun 723: 150220, 2024.
41. Lane DJR, Metselaar B, Greenough M, Bush AI and Ayton SJ: Ferroptosis and NRF2: An emerging battlefield in the neurodegeneration of Alzheimer's disease. Essays Biochem 65: 925-940, 2021.
42. Zhang JY, Meng X, Zhu XL, Peng SR, Li HB, Mo HZ and Hu LB: Thymol induces fenton-reaction-dependent ferroptosis in vibrio parahemolyticus. J Agric Food Chem 72: 14337-14348, 2024.
43. Torti SV and Torti FM: Iron and cancer: More ore to be mined. Nat Rev Cancer 13: 342-355, 2013.
44. Zhang C: Essential functions of iron-requiring proteins in DNA replication, repair and cell cycle control. Protein Cell 5: 750-760, 2014.
45. Koppenol WH and Hider RH: Iron and redox cycling. Do's and don'ts. Free Radic Biol Med 133: 3-10, 2019.
46. Xu L, Liu Y, Chen X, Zhong H and Wang Y: Ferroptosis in life: To be or not to be. Biomed Pharmacother 159: 114241, 2023.
47. Hassannia B, Vandenabeele P and Berghe TV: Targeting ferroptosis to iron out cancer. Cancer Cell 35: 830-849, 2019.
48. Fang X, Ardehali H, Min J and Wang F: The molecular and metabolic landscape of iron and ferroptosis in cardiovascular disease. Nat Rev Cardiol 20: 7-23, 2023.
49. Truman-Rosentsvit M, Berenbaum D, Spektor L, Cohen LA, Belizowsky-Moshe S, Lifshitz L, Ma J, Li W, Kesselman E, Abutbul-Ionita I, *et al*: Ferritin is secreted via 2 distinct nonclassical vesicular pathways. Blood 131: 342-352, 2018.
50. Rochette L, Dogon G, Rigal E, Zeller M, Cottin Y and Vergely C: Lipid peroxidation and iron metabolism: Two corner stones in the homeostasis control of ferroptosis. Int J Mol Sci 24: 449, 2022.
51. Chen X, Yu C, Kang R and Tang D: Iron metabolism in ferroptosis. Front Cell Dev Biol 8: 590226, 2020.
52. Yu X, Guo Q, Zhang H, Wang X, Han Y and Yang Z: Hypoxia-inducible factor-1alpha can reverse the Adriamycin resistance of breast cancer adjuvant chemotherapy by upregulating transferrin receptor and activating ferroptosis. FASEB J 38: e23876, 2024.
53. Sies H: Oxidative stress: A concept in redox biology and medicine. Redox Biol 4: 180-183, 2015.
54. Chen S, Li Q, Shi H, Li F, Duan Y and Guo Q: New insights into the role of mitochondrial dynamics in oxidative stress-induced diseases. Biomed Pharmacother 178: 117084, 2024.
55. Jomova K, Raptova R, Alomar SY, Alwasel SH, Nepovimova E, Kuca K and Valko M: Reactive oxygen species, toxicity, oxidative stress, and antioxidants: Chronic diseases and aging. Arch Toxicol 97: 2499-2574, 2023.
56. Galaris D, Barbouti A and Pantopoulos K: Iron homeostasis and oxidative stress: An intimate relationship. Biochim Biophys Acta Mol Cell Res 1866: 118535, 2019.
57. Carocci A, Catalano A, Sinicropi MS and Genchi G: Oxidative stress and neurodegeneration: The involvement of iron. Biometals 31: 715-735, 2018.

58. Boldyreva LV, Morozova MV, Saydakova SS and Kozhevnikova EN: Fat of the gut: Epithelial phospholipids in inflammatory bowel diseases. Int J Mol Sci 22: 11682, 2021.
59. Feng H and Stockwell BR: Unsolved mysteries: How does lipid peroxidation cause ferroptosis? PLoS Biol 16: e2006203, 2018.
60. Liang D, Minikes AM and Jiang X: Ferroptosis at the intersection of lipid metabolism and cellular signaling. Mol Cell 82: 2215-2227, 2022.
61. Li FJ, Long HZ, Zhou ZW, Luo HY, Xu SG and Gao LC: System X(c) (-)/GSH/GPX4 axis: An important antioxidant system for the ferroptosis in drug-resistant solid tumor therapy. Front Pharmacol 13: 910292, 2022.
62. Hussain S, Gupta G, Shahwan M, Bansal P, Kaur H, Deorari M, Pant K, Ali H, Singh SK, Allam VS, *et al*: Non-coding RNA: A key regulator in the Glutathione-GPX4 pathway of ferroptosis. Noncoding RNA Res 9: 1222-1234, 2024.
63. Xie Y, Kang R, Klionsky DJ and Tang D: GPX4 in cell death, autophagy, and disease. Autophagy 19: 2621-2638, 2023.
64. Liu Y, Wan Y, Jiang Y, Zhang L and Cheng W: GPX4: The hub of lipid oxidation, ferroptosis, disease and treatment. Biochim Biophys Acta Rev Cancer 1878: 188890, 2023.
65. Liu Y, Lu S, Wu LL, Yang L, Yang L and Wang J: The diversified role of mitochondria in ferroptosis in cancer. Cell Death Dis 14: 519, 2023.
66. Jiang X, Stockwell BR and Conrad M: Ferroptosis: Mechanisms, biology and role in disease. Nat Rev Mol Cell Biol 22: 266-282, 2021.
67. Dodson M, Castro-Portuguez R and Zhang DD: NRF2 plays a critical role in mitigating lipid peroxidation and ferroptosis. Redox Biol 23: 101107, 2019.
68. Liu Y, Su Z, Tavana O and Gu W: Understanding the complexity of p53 in a new era of tumor suppression. Cancer Cell 42: 946-967, 2024.
69. Shin D, Lee J and Roh JL: Pioneering the future of cancer therapy: Deciphering the p53-ferroptosis nexus for precision medicine. Cancer Lett 585: 216645, 2024.
70. Hu H, Zhang G, Tian M, Yin Y, Bao Y, Guan X, Ding C and Yu S: Brucella rough RB51 infection activates P53-Slc7a11-Gpx4/GSH pathway to induce ferroptosis to attenuate the intracellular survival on macrophages. Vet Microbiol 298: 110224, 2024.
71. Liao W, Zhang R, Chen G, Zhu X, Wu W, Chen Z, Jiang C, Lin Z, Ma L and Yu H: Berberine synergises with ferroptosis inducer sensitizing NSCLC to ferroptosis in p53-dependent SLC7A11-GPX4 pathway. Biomed Pharmacother 176: 116832, 2024.
72. Fu R, You Y, Wang Y, Wang J, Lu Y, Gao R, Pang M, Yang P and Wang H: Sanggenol L induces ferroptosis in non-small cell lung cancer cells via regulating the miR-26a-1-3p/MDM2/p53 signaling pathway. Biochem Pharmacol 226: 116345, 2024.
73. Zeng C, Xiong D, Zhang K and Yao J: Shank-associated RH domain interactor signaling in tumorigenesis. Oncol Lett 20: 2579-2586, 2020.
74. Zeng C, Lin J, Zhang K, Ou H, Shen K, Liu Q, Wei Z, Dong X, Zeng X, Zeng L, *et al*: SHARPIN promotes cell proliferation of cholangiocarcinoma and inhibits ferroptosis via p53/SLC7A11/GPX4 signaling. Cancer Sci 113: 3766-3775, 2022.
75. Chu B, Kon N, Chen D, Li T, Liu T, Jiang L, Song S, Tavana O and Gu W: ALOX12 is required for p53-mediated tumour suppression through a distinct ferroptosis pathway. Nat Cell Biol 21: 579-591, 2019.
76. Ou Y, Wang SJ, Li D, Chu B and Gu W: Activation of SAT1 engages polyamine metabolism with p53-mediated ferroptotic responses. Proc Natl Acad Sci USA 113: E6806-E6812, 2016.
77. Lu B, Christensen IT, Yu T, Wang C, Yan Q and Wang X: SUMOylation evoked by oxidative stress reduced lens epithelial cell antioxidant functions by increasing the stability and transcription of TP53INP1 in age-related cataracts. Oxid Med Cell Longev 2019: 7898069, 2019.
78. Wang J, Yi H, Li J, Yang Y, Sun G, Xue Y and He L: P62-autophagic pathway degrades SLC7A11 to regulate ferroptosis in doxorubicin-induced cardiotoxicity. Life Sci 356: 122981, 2024.
79. Lin Z, Wu C, Song D, Zhu C, Wu B, Wang J and Xue Y: Sarmentosin alleviates doxorubicin-induced cardiotoxicity and ferroptosis via the p62-Keap1-Nrf2 pathway. Redox Rep 29: 2392329, 2024.
80. Zhao Y, Lu J, Mao A, Zhang R and Guan S: Autophagy inhibition plays a protective role in ferroptosis induced by alcohol via the p62-Keap1-Nrf2 Pathway. J Agric Food Chem 69: 9671-9683, 2021.
81. Zhou Y, Yang Y, Yi L, Pan M, Tang W and Duan H: Propofol mitigates sepsis-induced brain injury by inhibiting ferroptosis via activation of the Nrf2/HO-1axis. Neurochem Res 49: 2131-2147, 2024.
82. Liu N, Wu WL, Wan XR, Wang J, Huang JN, Jiang YY, Sheng YC, Wu JC, Liang ZQ, Qin ZH and Wang Y: Regulation of FSP1 myristoylation by NADPH: A novel mechanism for ferroptosis inhibition. Redox Biol 73: 103176, 2024.
83. Dai E, Zhang W, Cong D, Kang R, Wang J and Tang D: AIFM2 blocks ferroptosis independent of ubiquinol metabolism. Biochem Biophys Res Commun 523: 966-971, 2020.
84. Guo J, Chen L and Ma M: Ginsenoside Rg1 suppresses ferroptosis of renal tubular epithelial cells in sepsis-induced acute kidney injury via the FSP1-CoQ(10)- NAD(P)H pathway. Curr Med Chem 31: 2119-2132, 2024.
85. Li D and Li Y: The interaction between ferroptosis and lipid metabolism in cancer. Signal Transduct Target Ther 5: 108, 2020.
86. Torti SV and Torti FM: Iron and cancer: 2020 vision. Cancer Res 80: 5435-5448, 2020.
87. Toshida K, Itoh S, Iseda N, Izumi T, Bekki Y, Yoshiya S, Toshima T, Iwasaki T, Oda Y and Yoshizumi T: The association of transferrin receptor with prognosis and biologic role in intrahepatic cholangiocarcinoma. Ann Surg Oncol 23: 10.1245/s10434-024-16065-3, 2024.
88. Vogt AS, Arsiwala T, Mohsen M, Vogel M, Manolova V and Bachmann MF: On iron metabolism and its regulation. Int J Mol Sci 22: 4591, 2021.
89. Guo W, Zhang S, Chen Y, Zhang D, Yuan L, Cong H and Liu S: An important role of the hepcidin-ferroportin signaling in affecting tumor growth and metastasis. Acta Biochim Biophys Sin (Shanghai) 47: 703-715, 2015.
90. Raggi C, Gammella E, Correnti M, Buratti P, Forti E, Andersen JB, Alpini G, Glaser S, Alvaro D, Invernizzi P, *et al*: Dysregulation of iron metabolism in cholangiocarcinoma stem-like cells. Sci Rep 7: 17667, 2017.
91. Kong L, Liu Y, Li J, Wang Y, Ji P, Shi Q, Han M, Xu H, Li W and Li W: Ginsenoside Rg1 alleviates chronic inflammation-induced neuronal ferroptosis and cognitive impairments via regulation of AIM2-Nrf2 signaling pathway. J Ethnopharmacol 330: 118205, 2024.
92. Caligiuri A, Becatti M, Porro N, Borghi S, Marra F, Pastore M, Taddei N, Fiorillo C and Gentilini A: Oxidative stress and redox-dependent pathways in cholangiocarcinoma. Antioxidants (Basel) 13: 28, 2023.
93. Thanan R, Oikawa S, Yongvanit P, Hiraku Y, Ma N, Pinlaor S, Pairojkul C, Wongkham C, Sripa B, Khuntikeo N, *et al*: Inflammation-induced protein carbonylation contributes to poor prognosis for cholangiocarcinoma. Free Radic Biol Med 52: 1465-1472, 2012.
94. Nevi L, Di Matteo S, Carpino G, Zizzari IG, Samira S, Ambrosino V, Costantini D, Overi D, Giancotti A, Monti M, *et al*: DCLK1, a putative stem cell marker in human cholangiocarcinoma. Hepatology 73: 144-159, 2021.
95. Takahashi Y, Dungubat E, Kusano H, Ganbat D, Tomita Y, Odgerel S and Fukusato T: Application of immunohistochemistry in the pathological diagnosis of liver tumors. Int J Mol Sci 22: 5780, 2021.
96. Voigtländer T, Metzger J, Husi H, Kirstein MM, Pejchinovski M, Latosinska A, Frantzi M, Mullen W, Book T, Mischak H and Manns MP: Bile and urine peptide marker profiles: Access keys to molecular pathways and biological processes in cholangiocarcinoma. J Biomed Sci 27: 13, 2020.
97. Xin H, Zhang Y, Lai Q, Liao N, Zhang J, Liu Y, Chen Z, He P, He J, Liu J, *et al*: Automatic origin prediction of liver metastases via hierarchical artificial-intelligence system trained on multiphasic CT data: A retrospective, multicentre study. EClinicalMedicine 69: 102464, 2024.
98. Yang Q, Cai Q, Wen H, Mao Y, Ban X, Rong D and Zhang R: The CT and MRI features of primary intrahepatic lymphoepithelioma-like cholangiocarcinoma. AJR Am J Roentgenol 216: 393-402, 2021.
99. Sae-Fung A, Mutirangura A and Jitkaew S: Identification and validation of a novel ferroptosis -related gene signature for prognosis and potential therapeutic target prediction in cholangiocarcinoma. Front Immunol 13: 1051273, 2022.
100. Bao F, Liu J, Chen H, Miao L, Xu Z and Zhang G: Diagnosis biomarkers of cholangiocarcinoma in human bile: An evidence-based study. Cancers (Basel) 14: 3921, 2022.
101. Lapitz A, Azkargorta M, Milkiewicz P, Olaizola P, Zhuravleva E, Grimsrud MM, Schramm C, Arbelaiz A, O'Rourke CJ, La Casta A, *et al*: Liquid biopsy-based protein biomarkers for risk prediction, early diagnosis, and prognostication of cholangiocarcinoma. J Hepatol 79: 93-108, 2023.

102. El-Deiry WS, Goldberg RM, Lenz HJ, Shields AF, Gibney GT, Tan AR, Brown J, Eisenberg B, Heath EI, Phuphanich S, *et al*: The current state of molecular testing in the treatment of patients with solid tumors, 2019. CA Cancer J Clin 69: 305-343, 2019.
103. Scott A, Wong P and Melstrom LG: Surgery and hepatic artery infusion therapy for intrahepatic cholangiocarcinoma. Surgery 174: 113-115, 2023.
104. Benson AB III, D'Angelica MI, Abrams TA, Are C, Bloomston PM, Chang DT, Clary BM, Covey AM, Ensminger WD, Iyer R, *et al*: Hepatobiliary cancers, version 2.2014. J Natl Compr Canc Netw 12: 1152-1182, 2014.
105. Moris D, Palta M, Kim C, Allen PJ, Morse MA and Lidsky ME: Advances in the treatment of intrahepatic cholangiocarcinoma: An overview of the current and future therapeutic landscape for clinicians. CA Cancer J Clin 73: 198-222, 2023.
106. Saito N, Hatanaka T, Nakano S, Hazama Y, Yoshida S, Hachisu Y, Tanaka Y, Yoshinaga T, Kashiwabara K, Kubo N, *et al*: A case of unresectable combined hepatocellular and cholangiocarcinoma treated with atezolizumab plus bevacizumab. Clin Case Rep 10: e6129, 2022.
107. Simon-Molas H, Sanchez-de-Diego C, Navarro-Sabate A, Castaño E, Ventura F, Bartrons R and Manzano A: The expression of TP53-induced glycolysis and apoptosis regulator (TIGAR) can be controlled by the antioxidant orchestrator NRF2 in human carcinoma cells. Int J Mol Sci 23: 1905, 2022.
108. Toshida K, Itoh S, Iseda N, Izumi T, Yoshiya S, Toshima T, Ninomiya M, Iwasaki T, Oda Y and Yoshizumi T: Impact of TP53-induced glycolysis and apoptosis regulator on malignant activity and resistance to ferroptosis in intrahepatic cholangiocarcinoma. Cancer Sci 115: 170-183, 2024.
109. Shi Y, Gong M, Deng Z, Liu H, Chang Y, Yang Z and Cai L: Tirapazamine suppress osteosarcoma cells in part through SLC7A11 mediated ferroptosis. Biochem Biophys Res Commun 567: 118-124, 2021.
110. Cao JY and Dixon SJ: Mechanisms of ferroptosis. Cell Mol Life Sci 73: 2195-2209, 2016.
111. Johnstone TC, Suntharalingam K and Lippard SJ: The next generation of platinum drugs: Targeted Pt(II) agents, nanoparticle delivery, and Pt(IV) prodrugs. Chem Rev 116: 3436-3486, 2016.
112. Kontoghiorghes GJ: New iron metabolic pathways and chelation targeting strategies affecting the treatment of all types and stages of cancer. Int J Mol Sci 23: 13990, 2022.
113. Chen X, Li J, Kang R, Klionsky DJ and Tang D: Ferroptosis: Machinery and regulation. Autophagy 17: 2054-2081, 2021.
114. Yang W, Mu B, You J, Tian C, Bin H, Xu Z, Zhang L, Ma R, Wu M, Zhang G, *et al*: Non-classical ferroptosis inhibition by a small molecule targeting PHB2. Nat Commun 13: 7473, 2022.
115. Zhang C, Liu J, Xu D, Zhang T, Hu W and Feng Z: Gain-of-function mutant p53 in cancer progression and therapy. J Mol Cell Biol 12: 674-687, 2020.
116. Hanson RL and Batchelor E: Coordination of MAPK and p53 dynamics in the cellular responses to DNA damage and oxidative stress. Mol Syst Biol 18: e11401, 2022.
117. Zhang C, Liu X, Jin S, Chen Y and Guo R: Ferroptosis in cancer therapy: A novel approach to reversing drug resistance. Mol Cancer 21: 47, 2022.
118. Lei S, Cao W, Zeng Z, Zhang Z, Jin B, Tian Q, Wu Y, Zhang T, Li D, Hu C, *et al*: JUND/linc00976 promotes cholangiocarcinoma progression and metastasis, inhibits ferroptosis by regulating the miR-3202/GPX4 axis. Cell Death Dis 13: 967, 2022.
119. Ma S, Ma Y, Qi F, Lei J, Chen F, Sun W, Wang D, Zhou S, Liu Z, Lu Z and Zhang D: HSDL2 knockdown promotes the progression of cholangiocarcinoma by inhibiting ferroptosis through the P53/SLC7A11 axis. World J Surg Oncol 21: 293, 2023.
120. Shi X, Yang J, Wang M, Xia L, Zhang L and Qiao S: Hsa_circ_0050900 affects ferroptosis in intrahepatic cholangiocarcinoma cells by targeting hsa-miR-605-3p to regulate SLC3A2. Oncol Lett 27: 2, 2024.
121. Kaida T, Beppu T, Hayashi H, Imai K, Yamamura K, Okabe H, Matsumura K, Yoshii D, Komohara Y, Akahoshi S, *et al*: Inflammatory liver tumor caused by fasciola hepatica mimicking intrahepatic cholangiocarcinoma. Anticancer Res 40: 2795-2800, 2020.
122. Agostinis P, Berg K, Cengel KA, Foster TH, Girotti AW, Gollnick SO, Hahn SM, Hamblin MR, Juzeniene A, Kessel D, *et al*: Photodynamic therapy of cancer: An update. CA Cancer J Clin 61: 250-281, 2011.
123. Zhang ZJ, Huang YP, Li XX, Liu ZT, Liu K, Deng XF, Xiong L, Zou H and Wen Y: A novel ferroptosis-related 4-gene prognostic signature for cholangiocarcinoma and photodynamic therapy. Front Oncol 11: 747445, 2021.
124. Gonzalez-Carmona MA, Bolch M, Strassburg CP and Weismuller TJ: Editorial: Shining a light on cholangiocarcinoma-a new dawn for photodynamic therapy? Authors' reply. Aliment Pharmacol Ther 49: 953-954, 2019.
125. Du J, Wan Z, Wang C, Lu F, Wei M, Wang D and Hao Q: Designer exosomes for targeted and efficient ferroptosis induction in cancer via chemo-photodynamic therapy. Theranostics 11: 8185-8196, 2021.
126. Yan X, Li Z, Chen H, Yang F, Tian Q and Zhang Y: Photodynamic therapy inhibits cancer progression and induces ferroptosis and apoptosis by targeting P53/GPX4/SLC7A11 signaling pathways in cholangiocarcinoma. Photodiagnosis Photodyn Ther 47: 104104, 2024.
127. Huang YP, Wang YX, Zhou H, Liu ZT, Zhang ZJ, Xiong L, Zou H and Wen Y: Surufatinib combined with photodynamic therapy induces ferroptosis to inhibit cholangiocarcinoma in vitro and in tumor models. Front Pharmacol 15: 1288255, 2024.
128. Ingle J and Basu S: Mitochondria targeted AIE probes for cancer phototherapy. ACS Omega 8: 8925-8935, 2023.
129. Kojima Y, Tanaka M, Sasaki M, Ozeki K, Shimura T, Kubota E and Kataoka H: Induction of ferroptosis by photodynamic therapy and enhancement of antitumor effect with ferroptosis inducers. J Gastroenterol 59: 81-94, 2024.
130. Wang P, Hu Z, Yu S, Su S, Wu R, Chen C, Ye Y, Wang H, Ye X, Zhou Z, *et al*: A novel protein encoded by circFOXP1 enhances ferroptosis and inhibits tumor recurrence in intrahepatic cholangiocarcinoma. Cancer Lett 598: 217092, 2024.
131. Zhang Z, Akuetteh PD, Lin L, Wu Y, Li Y, Huang W, Ni H, Lv H and Zhang Q: Development and validation of a ferroptosis-related model for three digestive tract tumors based on a pan-cancer analysis. Epigenomics 13: 1497-1514, 2021.
132. Sae-Fung A, Mutirangura A and Jitkaew S: Identification and validation of a novel ferroptosis-related gene signature for prognosis and potential therapeutic target prediction in cholangiocarcinoma. Front Immunol 13: 1051273, 2022.
133. Yao X, Chen B, Wang M, Zhang S, He B, Shi Z, Deng T, Bao W, Wang Y, Chen G and Bo Z: Exploration and validation of a novel ferroptosis-related gene signature predicting the prognosis of intrahepatic cholangiocarcinoma. Acta Biochim Biophys Sin (Shanghai) 54: 1376-1385, 2022.
134. Wang Z, Zhang Y, Chen Y, Liu S, Li C and Li X: Identification of a ferroptosis-related gene signature for predicting the prognosis of cholangiocarcinoma. Expert Rev Gastroenterol Hepatol 16: 181-191, 2022.
135. Liu S, Fan S, Wang Y, Chen R, Wang Z, Zhang Y, Jiang W, Chen Y, Xu X, Yu Y, *et al*: ACSL4 serves as a novel prognostic biomarker correlated with immune infiltration in Cholangiocarcinoma. BMC Cancer 23: 444, 2023.
136. Hori Y, Yoh T, Nishino H, Okura K, Kurimoto M, Takamatsu Y, Satoh M, Nishio T, Koyama Y, Ishii T, *et al*: Ferroptosis-related gene glutathione peroxidase 4 promotes reprogramming of glucose metabolism via Akt-mTOR axis in intrahepatic cholangiocarcinoma. Carcinogenesis 45: 119-130, 2023.
137. Xu L, Gao X, Xing J and Guo Z: Identification of a necroptosis-related gene signature as a novel prognostic biomarker of cholangiocarcinoma. Front Immunol 14: 1118816, 2023.