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Progress in the Study of Iron Death Induced in Hepatocellular Carcinoma Cells by Traditional Chinese Medicine and Its Active Ingredients

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Abstract: ***The liver is the sixth most common site for primary cancers, but hepatocellular carcinoma is the third leading cause of cancer-related deaths worldwide. Iron death is a novel programmed cell death mechanism involved in hepatocellular carcinoma cell death through multiple pathways, and modulation of iron death is a potential therapeutic option for hepatocellular carcinoma. In this paper, we reviewed herbal compounds inducing iron death in hepatocellular carcinoma cells through multiple pathways. These include iron metabolism pathway, lipid metabolism pathway, System XC-/GPX4/GSH pathway, and other pathways. Chinese medicines provide new ideas and directions for the treatment of hepatocellular carcinoma in traditional Chinese medicine (TCM) due to their natural sources and less side effects.***

Keywords: Herbal medicine, Herbal compounds, Hepatocellular carcinoma, Iron death**.**

1. Introduction

Cancer is a public problem in all countries of the world and is a major cause of shortened life expectancy and mortality. The liver is the sixth most common site of primary cancer, but liver cancer is the third leading cause of cancer-related deaths worldwide. Generally, if diagnosed at an early stage, liver cancer can be treated with surgical resection and liver transplantation. However, in advanced stages, only sorafenib is currently approved by the FDA for advanced liver cancer [1]. Many therapies have been tested in clinical trials over the past decades, but most have not benefited patients with liver cancer. Some of the effective drugs also later failed to inhibit tumor growth due to the emergence of resistance mechanisms. Therefore, it is important to find new and better therapeutic strategies for liver cancer patients. Iron death can be used to eliminate hepatocellular carcinoma cells through multiple signaling pathways and gene expression and can overcome the challenge of multiple drug resistance [2]. With the in-depth study of iron death, traditional Chinese medicine and its active ingredients have shown great advantages in inducing iron death in hepatocellular carcinoma cells, which provides new ideas for the treatment of hepatocellular carcinoma [3, 4].

2. Iron Death

2.1 Overview of Iron Death

Iron death is a new mode of programmed cell death. In 2012, Dixon et al [5] named the Erastin-induced completely new mode of cell death with unique genetic features and biochemistry as iron death. When iron death occurs, cells have blistering cytoplasmic membranes, chromatin condensation, mitochondrial crumpling, volume reduction, increased membrane density, cristae reduction or disappearance, rupture of the outer mitochondrial membrane, and intact nuclear membranes, and all of these cytomorphological changes are distinguished from apoptosis and necrosis. It is usually accompanied by a complex network of genes, proteins and metabolism, which implies that it is associated with a variety of ontogenetic mechanisms, with imbalance of iron metabolism, lipid peroxidation and imbalance of the systemic Xc-/GSH/GPx4 axis being three of its main hallmarks [6].

2.2 Mechanisms of Iron Death

Iron has two different valence states and can undergo redox reactions in vivo. The iron-death-sensitive cellular transferrin receptor 1 (TFR1) reduces Fe3+ to Fe2+, which is stored in the presence of the divalent metal ion transporter protein 1 as an iron storage protein complex in an unstable intracellular iron pool. However, when excess free Fe2+ is present in the cell, ferritin is recognized by specific receptors for solubilization, releasing excess Fe2+, which, through the Fenton reaction, induces reactive oxygen species (ROS) production and increases susceptibility to cellular iron death [7].

Lipid peroxidation is an important driver of iron death [8], Polyunsaturated fatty acids (PUFA) contain readily extractable diallyl hydrogen atoms, making them sensitive to lipid peroxidation. Hydrogen of PUFA is acquired by hydroxyl groups to form carbon-centered lipid atom groups (L-), while O2 reacts rapidly with L- to produce lipid peroxidation atom groups (LOO-). Phosphatidylethanolamine (PE) containing arachidonic acid (AA) is a key membrane phospholipid in the onset of oxidatively driven iron death. Acyl coenzyme A synthase long-chain family member 4 (ACSL4) and Lys phosphatidylcholine acyltransferase 3 (LPCAT3) are involved in PE biosynthesis, activation of PUFA and formation of PUFA-PE. deletion of ACSL4 and LPCAT3 depletes the substrate for lipid peroxidation and increases the inhibition of iron death. Ultimately, PUFA-PE further promotes lipoxygenase (LOX)-catalyzed iron oxidation [9].

System Xc- is a heterodimeric cell surface amino acid reverse transporter. Extracellular cystine will be translocated into the

cell and reduced to cysteine to synthesize glutathione (GSH). Glutathione is a cofactor for GPx4. If GSH levels are affected by system Xc-, ROS will accumulate and iron death will be initiated by GPx4 with reduced activity [10].

3. Induction of Iron Death in Hepatocellular Carcinoma Cells by Active Ingredients of Traditional Chinese Medicine

3.1 Trans-iron Metabolic Pathway

Sophoridine, a natural plant alkaloid extracted from bitter ginseng, has a variety of pharmacological functions, such as hypolipidemic, antiplatelet aggregation, anti-inflammatory, analgesic, anti-oxidative stress, and antifibrotic effects. ATF3 is a member of the ATF/CREB family of transcription factors, which is widely expressed in normal human tissues, including the liver, gastrointestinal tract, and endocrine tissues. Previous studies have shown that ATF3 can impede tumorigenesis and progression in hepatocellular carcinoma. Meanwhile, ATF3 is a key mediator of drug-induced iron death. Sophoridine derivative 6j can up-regulate ATF3 expression in hepatocellular carcinoma cells through ER stress, and knockdown of ATF3 can inhibit iron death induced by sophoridine derivative 6j by decreasing intracellular Fe content [11].

Artemisinin is a bioactive molecule derived from the Chinese medicinal plant Artemisia annua (Asteraceae), and its semi-synthetic derivatives have higher bioavailability and are potent antimalarial drugs. Its derivative, dihydroartemisinin (DAT), modulates cellular iron homeostasis regulated by the iron storage protein ferritin and the IRP-IRE signaling pathway [12], thereby affecting iron death. In mouse experiments exploring the critical role of peroxidation and iron in iron death, and the chemistry of artemisinin compounds as iron-reactive peroxides, researchers using in vivo tolerable doses of DAT found that DAT sensitized cells to cysteine- and GPX4-inhibition-induced iron death, and at the same time, DAT was associated with cytosolic free iron such that DAT-associated iron still had the same oxidative activity, but cannot be recognized by the IRP-IRE iron homeostasis regulatory mechanism. Consequently, cells respond by enhancing lysosome-mediated ferritin degradation and IRP-mediated ferritin translational inhibition, both of which lead to an increase in cellular free iron content to sensitize to iron death.

Strychnine, a weakly alkaline indole alkaloid extracted from strychnine seeds, is commonly used to relieve arthritis and traumatic pain. Strychnine has potent antitumor activity in various types of cancers, and it can promote extracellular ferrous ion transport into hepatocellular carcinoma cells by up-regulating the TFR, which then activates the AMPK-mTOR pathway to cause autophagic degradation of intracellular ferritin, and further increases the intracellular free Fe2+ concentration. Meanwhile, strychnine can inhibit the GSH-dependent antioxidant activity of hepatocellular carcinoma cells by inhibiting the activity of xCT and CAT, increase the intracellular ROS level and lipid peroxidation level, and induce iron death in hepatocellular carcinoma cells [13].

In summary, traditional Chinese medicines and their active ingredients can alter intracellular iron ion concentration through various pathways of action in order to affect intracellular iron homeostasis, thereby inducing iron death in hepatocytes.

3.2 Translipidic Metabolic Pathways

DAT can enhance the sensitivity to lipid ROS accumulation and GPX4 inhibition-induced iron death in cancer cells in a lysosome-dependent but autophagy-independent manner, e.g., by inhibiting the ubiquitin-proteasome pathway of PEBP1, increasing its protein level and binding it to 15-LO, which further promotes lipid peroxidation of hepatocellular carcinoma cell membranes and induces iron death, thus exerting the anti-hepatocellular carcinoma activity of DAT [14].

Resolving Blood Stasis and Dispelling Phlegm Formula, which is composed of Codonopsis pilosulae, Astragalus membranaceus, Poria cocos, Fagus sylvatica, Gynostemma gibbosum, Calamus calamus, Rhizoma ligustici chuanxiong, Salvia miltiorrhiza, and Yujin, etc., was found to have not only ameliorated the degree of oxidative damage in the liver cells of the mice in experiments of mice controlled by different concentrations of the formula for resolving blood stasis and dispelling phlegm by detecting the blood lipid levels and the levels of ROS and GSH in the liver tissues, but also made the antioxidant capacity enhanced and reduced the lipid peroxidation levels, induced iron death, and eliminated the further development of hepatocellular carcinoma [15].

Sempervirens, a perennial herb in the genus Scutellaria of the family Labiatae, has the functions of clearing heat and removing toxins, activating blood circulation, eliminating blood stasis, subduing swelling and relieving pain, and anticancer. Significantly lower mRNA and protein expression levels of anti-iron death in ROS metabolism-related genes GPX4 and SLC7A11 were detected in hepatocellular carcinoma mice treated with Hemicellulose, suggesting that Hemicellulose induces iron death in hepatocytes by promoting hepatocyte lipid ROS metabolism [16].

Chonglou is the collective name for the genus Chonglou in the family of Yerba Materiaceae, and Chonglou saponin II, one of the most important components of Chonglou, is a novel natural iron death inducer, which triggers iron death in hepatocellular carcinoma HepG3 cells containing NCOA4 and FTH1 by inducing ferritin autophagy, promoting the expression of the ACSL4 gene, inhibiting cellular activity, and lowering the level of intracellular lipid ROS [17].

Ginsenosides, the active ingredients extracted from ginseng, have been shown to have inhibitory effects on a variety of cancer cells. Liu Mingyue et al [18] found that ginsenosides not only increase the Fe2+ level and MDA content, reduce GSH content, affect the level of lipid peroxides, inhibit the expression of iron death GPX4 and SLC7A11 inhibitory factors in hepatocellular carcinoma cells, which can then achieve the effect of inhibiting hepatocellular carcinoma Huananxin is made from toadstools.

Hua Zuin is extracted from the dried skin of the toad family,

and has been clinically proven to exert anticancer effects by inhibiting cell proliferation, apoptosis and other pathways, and a variety of clinical preparations have been widely utilized in the treatment of clinical hepatocellular carcinoma [19]. A recent study [20] found that huazhouin can promote the expression of iron death in hepatocellular carcinoma cells by affecting lipid peroxidation, i.e., glutathione synthetase.

Lipid metabolism is an important component of iron death, and traditional Chinese medicines and their active substances promote lipid peroxidation of hepatocellular carcinoma cell membranes by affecting lipid ROS in hepatocytes to different degrees, thus inducing cellular iron death and realizing the inhibition of hepatocellular carcinoma cell development.

3.3 Trans-System XC- and GPX4/GSH Pathways

The Liver-Sparing, Stasis-Expelling and Detoxifying Formula consists of eight herbs: Chaihu, Paeonia lactiflora, Cumulus, Peach kernel, Semen ziziphi, Lobelia, Cichorium, and Rhizoma Swollen-jointed Fungus. In a controlled experiment with sorafenib, an effective drug for drug hepatocellular carcinoma, the Liver-Sparing, Stasis-Expelling and Detoxifying Formula up-regulated the level of P53, inhibited the expression of SLC7A11 and GPX4, and induced the iron death of hepatocellular carcinoma MHCC97H cells [21], and its high-concentration serum solution had the same effect as that of sorafenib.

Corosolic acid (CA) is a monomer extracted from kiwifruit root. A key gene, HERPUD1, for increased sensitivity to iron death in hepatocellular carcinoma cells by CA was screened by RNA-seq and TMT. HERPUD1 is an endoplasmic reticulum membrane protein that is normally expressed and highly induced during the unfolded protein response (UPR) and cellular stress. HERPUD1 plays an important role in the formation of the endoplasmic reticulum-associated degradation (ERAD) complex by the proteasome pathway in the regulation of cellular homeostasis and protein degradation. It was confirmed by in vivo and ex vivo experiments that CA could sensitize hepatocellular carcinoma cells to iron death. In addition, the mechanism by which CA could increase the iron death sensitivity of hepatocellular carcinoma cells and revealed that CA sensitized iron death by up-regulating homocysteine-induced ER protein with HERPUD1 in hepatocellular carcinoma cells. In cellular and mouse xenograft models, HERPUD1 decreased the ubiquitination of MDM2, a GSS-associated E3 ubiquitin ligase, which promotes the ubiquitination of GSS, thereby inhibiting GSH synthesis to increase iron death sensitivity [22].

Cryptotanshinone is a fat-soluble diterpene quinone isolated from the traditional Chinese medicine Salvia miltiorrhiza (Salvia miltiorrhiza root, family Labiatae) with bacteriostatic effects, and tanshinone acts on HepG2 cells to inactivate the cells, and in experiments on the effects of different iron death inhibitors on tanshinone, it was found that Fer-1 not only restored the activity of the inactivated HepG2, but also inhibited the cryptotanshinone-induced HepG2 cell ROS accumulation, and was able to restore the reduced GSH level and down-regulated expression of xCT and GPX4 caused by cryptotanshinone. It suggests that tanshinone is able to cause ROS accumulation in HepG2 cells by inhibiting GPX4 and xCT expression, leading to iron death in hepatocellular carcinoma cells [23].

Traditional Chinese medicine and its active ingredients achieve the reduction of GSH level or inhibition of GPX4 protein expression through multiple pathways, thus promoting iron death in hepatocellular carcinoma cells, with a view to providing new therapeutic ideas for the clinical treatment of hepatocellular carcinoma.

3.4 Other Induction Pathways

A novel sesquiterpene lactone component is the active ingredient from Perilla frutescens, whose compounds induce apoptosis by disrupting mitochondrial membrane potential depolarization and mitochondrial reactive oxygen species. It induces iron death as evidenced by mitochondrial disruption, lipid peroxidation and a significant increase in intracellular iron levels as well as a decrease in glutathione levels. Knockdown of NCOA4 attenuates cell death induced by pellagra compounds. This compound could control NCOA4 expression both transcriptionally and post-transcriptionally. Therefore, it is suggested that this sesquiterpene lactone component from Perilla frutescens enhances apoptosis through mitochondrial dysfunction and enhances iron death in hepatocellular carcinoma cells through NCOA4-mediated ferritin phagocytosis [24].

Based on bioinformatics and pharmacology studies, by analyzing Chinese medicines that can be used as iron death targets and their medication patterns, it was found that compounds such as quercetin, naringenin, sesquiterpene and lacinin are involved in iron death of hepatocellular carcinoma cells through the process of cellular oxidative metabolism and that most of the Chinese medicines inducing iron death to treat hepatocellular carcinoma are based on the application of cold, warm, flat, bitter, pungent and sweet [25].

Solid Spleen Anti-accumulation Formula contains ginsenosides, curcumin, curcumin, animal polypeptide toxins, glycoproteins and other anti-tumor active ingredients [9], and the elevated expression levels of iron death-related molecules p62, Keapl, and NRF2 were detected by protein imprinting, which proved that it induced iron death of hepatocellular carcinoma cells through the p62/Keap1/NRF2 pathway [26].

The Solid Spleen and Elimination of Accumulation Drink is an in-hospital preparation of Hunan Academy of Traditional Chinese Medicine, which consists of Astragalus, Ginseng, Poria, Chasteberry and Epimedium, etc. In experimental studies, it has been found that the Solid Spleen and Elimination of Accumulation Drink can play an anticancer role by affecting the balance of mitochondrial dynamics and inducing iron death and then inhibiting the survival rate and invasive ability of Hep G2 cells [27].

With the increasing research on iron death, iron death treatment for hepatocellular carcinoma is emerging in more forms with a view to proving that iron death has an important role in the treatment of hepatocellular carcinoma. Meanwhile, combining with motherland medicine not only magnifies the advantages of traditional medicine, but also provides a new direction for clinical treatment of cancer.

4. Summary and Outlook

Iron death is a novel programmed cell death mechanism involved in hepatocellular carcinoma cell death through multiple pathways, and regulation of iron death is a potential therapeutic option for hepatocellular carcinoma. Herbal compounds induced iron death in hepatocellular carcinoma cells by participating in multiple pathways of iron death. These include iron metabolism pathway, lipid metabolism pathway, System XC-/GPX4/GSH pathway, and other pathways. Due to its natural source and less side effects, TCM provides new ideas and directions for TCM treatment of hepatocellular carcinoma, and also lays a solid foundation for carrying forward the advantages of motherland medicine.

TCM has shown great potential in treating hepatocellular carcinoma by regulating iron death. However, further studies are needed before TCM compounds can be applied to the clinic. On the basis of the current research, the following problems remain to be solved: at present, the diagnosis of iron death is mainly based on the observation of mitochondrial morphology, iron content, ROS, MDA, 4-HNE SLC7A11, and GPX4 expression, whose mechanism has not yet been fully elucidated, and the diagnostic criteria have failed to be determined. The studies on iron death and hepatocellular carcinoma are relatively superficial, and most of them have elucidated the involvement of iron death in the pathologic process of hepatocellular carcinoma, but the deeper relationship has not been clarified. The exploration of herbal compounds that regulate iron death in hepatocellular carcinoma is limited. Current studies focus on basic research and lack the support of clinical trial data. Therefore, it is hoped that more scholars will pay attention to clinical trials in their studies to provide greater theoretical and data support for the clinical application of TCM-induced iron death.

In summary, iron death is an important mechanism involved in the pathogenesis of hepatocellular carcinoma, and herbal compounds have the potential to modulate iron death in the treatment of hepatocellular carcinoma. However, there are fewer studies on the mechanism of action of herbal compounds, hepatocellular carcinoma and iron death. Further studies are needed to provide high-quality evidence to translate the findings into clinical practice and provide new insights into strategies for treating hepatocellular carcinoma.

Fund Project

Exploring the mechanism of anti-malignant transformation of cirrhosis-hepatocellular carcinoma precancerous lesions by Yi-Spleen-Nourishing Liver Formula based on TLR/NF-κB miRNA regulatory network, No.2018KT-22.

References

[1] Aa A, Hm A, Fi A-J. Sorafenib[J]. Profiles of drug substances, excipients, and related methodology, Profiles Drug Subst Excip Relat Methodol, 2019, 44.

[2] Huang Y, Wang S, Ke A, et al. Ferroptosis and its interaction with tumor immune microenvironment in liver cancer[J]. Biochimica Et Biophysica Acta. Reviews on Cancer, 2023, 1878(1): 188848.

[3] Zhang Q, Zhao L, Xia P, et al. Progress in TCM Prevention and Treatment of Tumor Iron Death [J]. Clinical Journal of Traditional Chinese Medicine, 2021, 27(22):222-231.

[4] Zhang H, Zhang Y, Fu J, et al. Active Ingredients of Chinese Medicines Induce Ferroptosis in Tumor Cells: A Review [J]. Chinese Journal of Experimental Traditional Medical Formulae, 2023:1-13.

[5] Dixon S J, Lemberg K M, Lamprecht M R, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death[J]. Cell, 2012, 149(5): 1060–1072.

[6] Jiang X, Stockwell B R, Conrad M. Ferroptosis: mechanisms, biology and role in disease[J]. Nature Reviews. Molecular Cell Biology, 2021, 22(4): 266–282.

[7] Wu Q, Chen Z, Ding Y, et al. Protective effect of traditional Chinese medicine on non-alcoholic fatty liver disease and liver cancer by targeting ferroptosis[J]. Frontiers in Nutrition, 2022, 9: 1033129.

[8] Liang D, Minikes A M, Jiang X. Ferroptosis at the intersection of lipid metabolism and cellular signaling[J]. Molecular Cell, 2022, 82(12): 2215–2227.

[9] Kagan V E, Mao G, Qu F, et al. Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis[J]. Nature Chemical Biology, 2017, 13(1): 81–90.

[10] Liu J, Kang R, Tang D. Signaling pathways and defense mechanisms of ferroptosis[J]. The FEBS journal, 2022, 289(22): 7038–7050.

[11] Tian K, Wei J, Wang R, et al. Sophoridine derivative 6j inhibits liver cancer cell proliferation via ATF3 mediated ferroptosis[J]. Cell Death Discovery, 2023, 9(1): 296.

[12] Chen G-Q, Benthani F A, Wu J, et al. Artemisinin compounds sensitize cancer cells to ferroptosis by regulating iron homeostasis[J]. Cell Death and Differentiation, 2020, 27(1): 242–254.

[13] You G. Role and mechanism of AMPK-mediated autophagy in strychnine-induced iron death in hepatocellular carcinoma [D]. Jilin University, 2023.

[14] Su Y, Zhao D, Jin C, et al. Dihydroartemisinin Induces Ferroptosis in HCC by Promoting the Formation of PEBP1/15-LO[J]. Oxidative Medicine and Cellular Longevity, 2021, 2021: 3456725.

[15] Qiu X, Jia L, Song N, et al. Study on Effect and Mechanism of Huayu Qutan Fomula on Mice with High-Fat and Liver Cancer Based on Ferroptosis Related Protein [J]. Chinese Archives of Traditional Chinese Medicine, 2021, 39(9):137-141.

[16] Li Y, Zhang J, Zhang K, et al. Scutellaria barbata Inhibits Hepatocellular Carcinoma Tumorigenicity by Inducing Ferroptosis of Hepatocellular Carcinoma Cells[J]. Frontiers in Oncology, 2022, 12: 693395.

[17] Lin P-L, Tang H-H, Wu S-Y, et al. Saponin Formosanin C-induced Ferritinophagy and Ferroptosis in Human Hepatocellular Carcinoma Cells[J]. Antioxidants (Basel, Switzerland), 2020, 9(8): 682.

[18] Liu M, Cai Y, Chen Hao, et al. Study on the mechanism of iron death induced by ginsenoside CK in human hepatocellular carcinoma cells[J]. Lishizhen Medicine and Materia Medica Research, 2024, 35(03):554-559.

[19] Ren S, Fan Y, Guo D, et al. Systematic evaluation and meta-analysis of the efficacy and safety of cinobufagin injection combined with western medicine in the

treatment of liver cancer[J]. Journal of Hainan Medical University, 2023, 29(6):452-462.
[20] Wu Q, Yang J, Chen Q, et al. Study on the mechanism of cinobufotalin in inducing ferroptosis of hepatoma cells by inhibiting the expression [J]. Journal of Pharmaceutical Research, 2024, 43(3):214-219235.
[21] Cai X, Yang R, Wang Z, et al. Mechanism of Inducing Ferroptosis in Hepatocellular Carcinoma Cells by Shugan Quyu Jiedu Prescription Based on p53/SLC7A11/GPX4 Pathway [J]. Chinese Journal of Experimental Traditional Medical Formulae, 2023:1-11.
[22] Peng Y, Li N, Tang F, et al. Corosolic acid sensitizes ferroptosis by upregulating HERPUD1 in liver cancer cells[J]. Cell Death Discovery, 2022, 8(1): 376.
[23] Liu J, Tong L, Luo Y, et al. Cryptotanshinone May Induce Ferroptosis of Human Liver Cancer HepG2 Cells [J]. Acta Academiae Medicinae Sinicae, 2021, 43(3):366-370.
[24] Zhu Z-H, Xu X-T, Shen C-J, et al. A novel sesquiterpene lactone fraction from Eupatorium chinense L. suppresses hepatocellular carcinoma growth by triggering ferritinophagy and mitochondrial damage[J]. Phytomedicine: International Journal of Phytotherapy and Phytopharmacology, 2023, 112: 154671.
[25] Yao Y, Liu Y, Yang Y, et al. Regularity of traditional Chinese medicine regulating ferroptosis in intervention liver cancer based on bioinformatics. [J]. Journal of Guangxi University (Natural Science Edition), 2022, 47(1):235-244.
[26] Li K. Exploration of the mechanism of action of solidifying and eliminating accumulation formula to intervene iron death in human hepatocellular carcinoma HepG2 cells based on the p62/Keap1/NRF2 pathway [D]. Hunan University of Chinese Medicine, 2022.
[27] Jian H, Li K, Zeng P, et al. Effect of Shipi Xiaoji Beverage-containing Serum on Mitochondrial Dynamics Imbalance and Ferroptosis of Liver Cancer HepG2 Cells [J]. Journal of Traditional Chinese Medicine, 2024, 65(6):609-617.