COMMENTARY

LIF-ting ferroptosis to improve liver cancer therapy

Fan Yao a,b,**, Yang Zhao c, Li Ma c,d,*

a Hubei Hongshan Laboratory, College of Biomedicine and Health, College of Life Science and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, PR China
b Hubei Clinical Research Center for Precise Diagnosis and Treatment of Liver Cancer, Taihe Hospital, Huabei University of Medicine, Shiyan, Hubei 442000, PR China
c Department of Experimental Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA
d The University of Texas MD Anderson UTHealth Graduate School of Biomedical Sciences, Houston, TX 77030, USA

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The liver is the largest internal organ that has vital roles in many processes, including digestion, detoxification, immunity, metabolism, blood clotting, and iron storage. It is susceptible to many pathological conditions that result in liver diseases. Liver cancer, ranking sixth in cancer incidence, is the fourth most common cause of cancer-related death worldwide and is the second most lethal cancer after pancreatic cancer. The 5-year survival rate is 18%. Unfortunately, liver cancer barely responds to chemotherapy and radiotherapy. At present, surgical resection and transplantation are recommended for patients with early-stage liver cancer, but for patients with advanced liver cancer and poor liver function, systemic therapy is the only treatment option. Sorafenib is the first frontline drug approved by the Food and Drug Administration (FDA) for the treatment of hepatocellular carcinoma (HCC), which only prolongs the overall survival by approximately 3 months. Thus far, phase 3 clinical trials of most systemic therapies have not performed better than sorafenib in HCC. Recently, clinical trials of the combination of immune checkpoint inhibitors and anti-angiogenesis drugs have shown promising results in HCC, leading to FDA approval in 2020; however, this therapy only benefits a subset of HCC patients without predictive markers available.

Ferroptosis is a type of non-apoptotic cell death characterized by the accumulation of lipid hydroperoxides resulting from the iron-mediated Fenton reaction (Fig. 1). Although induction of ferroptosis impedes tumor growth and represents a potential therapeutic strategy, targeting ferroptosis for cancer therapy remains challenging. Sorafenib, a weak inducer of apoptosis, has been reported by many labs to trigger ferroptosis under certain conditions. On the other hand, one study showed that sorafenib failed to trigger ferroptosis in a panel of cancer cell lines. An important question is how to sensitize therapy-resistant tumors to ferroptosis effectively and safely. Recently, a study from our team revealed that loss of leukemia
inhibitory factor receptor (LIFR) in liver cancer activates NF-κB signaling to upregulate the iron-chelating cytokine lipocalin 2 (LCN2), which in turn promotes liver tumorigenesis and confers resistance to sorafenib and other ferroptosis-inducing agents. Notably, an LCN2-neutralizing antibody can enhance the ferroptosis-inducing and anti-cancer effects of sorafenib in HCC patient-derived xenograft (PDX) tumors with low LIFR expression and high LCN2 expression.5

Through The Cancer Genome Atlas data analysis, we found that the expression level of LIFR was commonly downregulated in HCC compared with normal liver tissues. In the N-nitrosodimethylamine (DEN)-induced mouse liver tumors, Lifr protein was also downregulated. To determine the role of LIFR in HCC, we generated conditional Lifr knockout mice (Lifr^{fl/fl};Alb-Cre) and found that hepatocyte-specific ablation of Lifr promoted both spontaneous and DEN-induced liver tumorigenesis. Importantly, liver-specific knockout (Lifr^{fl/fl};Alb-Cre) or inducible knockout (Lifr^{fl/fl};Cre-ERT2, with tamoxifen treatment) of Lifr substantially exacerbated, while overexpression of LIFR markedly suppressed oncogene-induced liver cancer in mice.5 Collectively, by constructing and characterizing multiple in vivo models, we discovered that LIFR is a liver tumor suppressor.

Subsequently, by analyzing data from the Cancer Therapeutic Response Portal, we found that LIFR expression in liver cancer cell lines significantly correlated with the sensitivity to erastin, which induces ferroptosis by inhibiting the cystine transporter SLC7A11. To determine the role of LIFR in ferroptosis, we treated LIFR-depleted and LIFR-overexpressing liver cell lines with erastin or other classic ferroptosis-inducing agents and found that LIFR is a positive regulator of ferroptosis. Interestingly, LIFR overexpression enhanced sorafenib-induced cell death, and this effect was reversed by the ferroptosis inhibitor liproxstatin-1. In oncogene-induced mouse HCC, knockout of Lifr resulted in resistance to sorafenib treatment, whereas overexpression of LIFR enhanced sorafenib-induced ferroptosis and therapeutic efficacy, which could be reversed by co-treatment with liproxstatin-1.5 Therefore, in vitro and in vivo data demonstrated that LIFR confers sensitivity to ferroptosis and sorafenib treatment on liver cancer cells.
From unbiased profiling, Lcn2 (lipocalin 2) stood out as a top upregulated gene in Lifr-knockout livers and was the only commonality between RNA-sequencing analysis and cytokine array analysis of factors regulated by Lifr. Subsequent mechanistic studies revealed that LIFR interacts with the phosphatase SHP1 to inhibit the K63-linked polyubiquitination of the ubiquitin ligase TRAF6, leading to inactivation of the NF-κB signaling pathway and repression of the NF-κB target gene LCN2, which encodes a secreted iron-sequestering cytokine (Fig. 1, left panel). Therefore, loss of LIFR in HCC results in upregulation of secreted LCN2, downregulation of iron levels in liver tumor cells, and insensitivity to ferroptosis-inducing drugs (Fig. 1, middle panel).

In preclinical studies, an LCN2-neutralizing antibody enhanced sorafenib’s ability to induce ferroptosis and eliminate cancer cells in PDX tumors with low LIFR and high LCN2 levels (Fig. 1, right panel), while in tumors with high LIFR expression and low LCN2 expression, sorafenib treatment alone triggered ferroptosis and exhibited substantial anti-tumor effect. The findings suggest that anti-LCN2 therapy should be further explored to target ferroptosis and improve liver cancer treatment. We envision that high LIFR expression and low LCN2 expression could be used to predict sorafenib responders, and that low LIFR expression and high LCN2 expression could be used to select liver cancer patients who will likely benefit from the combination therapy with sorafenib and the LCN2-neutralizing antibody. It should be noted that Lcn2-knockout mice have normal development, growth, and behavior under physiological conditions, and thus systemic LCN2 neutralization is expected to be safe to normal tissues.

Altogether, this study uncovered the role of an actionable LIFR–NF-κB–LCN2 axis in controlling liver tumorigenesis and drug-induced ferroptotic cell death, which could pave the way for improving liver cancer treatment by targeting an iron-sequestering pathway.

**Author contributions**

F.Y. drafted the main text. Y.Z. made the figure and drafted the figure legend. L.M. edited the paper.

**Conflict of interests**

The authors declare no conflict of interest.

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