



COMMENTARY

LIF-ting ferroptosis to improve liver cancer therapy

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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.¹ The 5-year survival rate is 18%.² 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.³

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).⁴ 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 study⁵ from our team revealed that loss of leukemia

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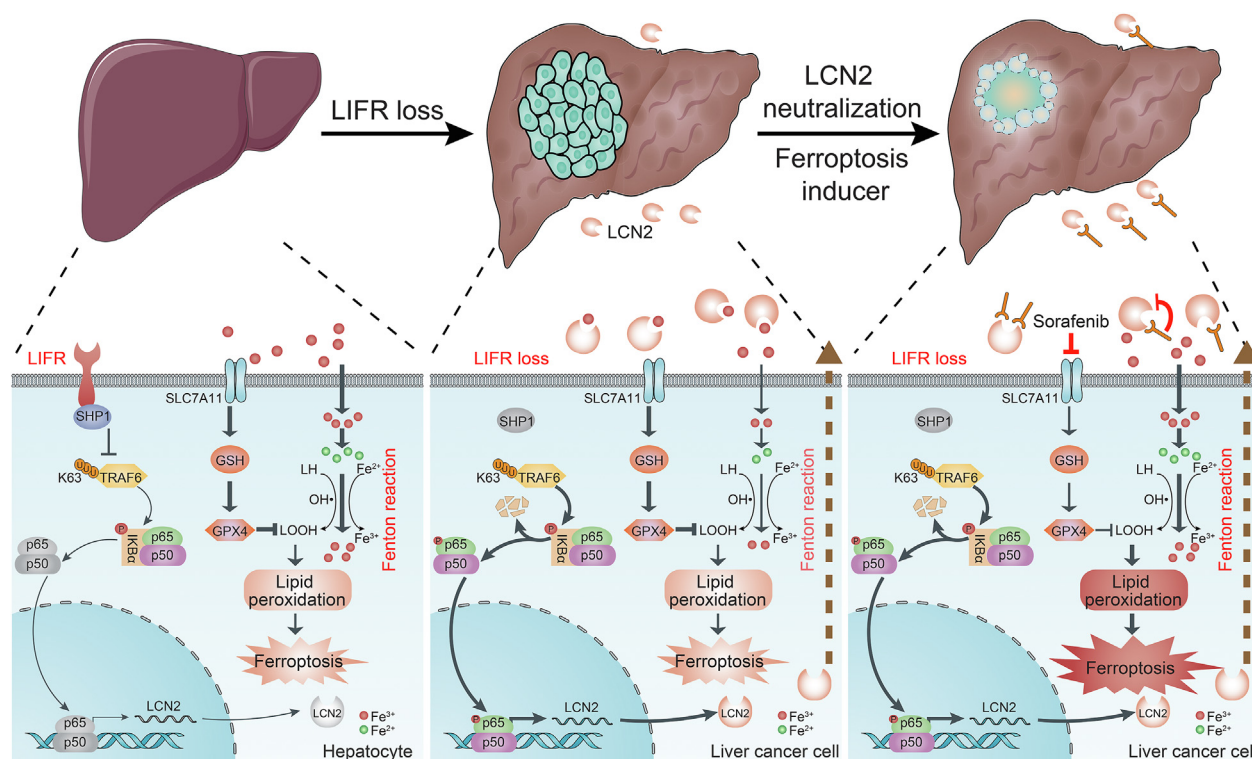


Figure 1 Model for the role of a targetable LIFR–NF- κ B–LCN2 axis in liver cancer development and therapy. Left panel: In normal hepatocytes, LIFR binds SHP1 to inhibit the K63-linked ubiquitination and activity of TRAF6, thereby keeping NF- κ B at bay. Middle panel: In liver cancer cells, LIFR expression is frequently lost or downregulated, which derepresses TRAF6, leading to activation of NF- κ B signaling and upregulation of the NF- κ B target LCN2, a secreted iron-sequestering protein. This lowers free ferrous iron (Fe^{2+}) levels in liver cancer cells and renders them insensitive to drug-induced ferroptosis, a non-apoptotic cell death process characterized by the iron-dependent accumulation of lipid hydroperoxides (LOOH). This process requires Fe^{2+} , which donates one electron in the Fenton reaction to produce the hydroxyl radical ($\text{OH}\cdot$). Right panel: In liver cancer cells treated with sorafenib plus the LCN2-neutralizing antibody, sorafenib inhibits SLC7A11-mediated cystine import, leading to depletion of glutathione (GSH) and inactivation of the glutathione peroxidase 4 (GPX4) that uses GSH to convert toxic lipid hydroperoxides (LOOH) to non-toxic lipid alcohols. In parallel, the LCN2-neutralizing antibody increases iron levels and facilitates the Fenton reaction, resulting in the overproduction of lipid hydroperoxides. Therefore, the combination treatment with sorafenib and anti-LCN2 promotes ferroptosis in liver cancer cells with low LIFR expression and high LCN2 expression.

inhibitory factor receptor (LIFR) in liver cancer activates NF- κ B 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-nitrosodiethylamine (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*.⁵ 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- κ B signaling pathway and repression of the NF- κ B 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).⁵

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.⁵ 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 study⁵ uncovered the role of an actionable LIFR–NF- κ B–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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References

1. Villanueva A. Hepatocellular carcinoma. *N Engl J Med*. 2019; 380(15):1450–1462.
2. Jemal A, Ward EM, Johnson CJ, et al. Annual report to the nation on the status of cancer, 1975-2014, featuring survival. *J Natl Cancer Inst*. 2017;109(9):dix030.
3. Llovet JM, Kelley RK, Villanueva A, et al. Hepatocellular carcinoma. *Nat Rev Dis Primers*. 2021;7(1):6.
4. Jiang X, Stockwell BR, Conrad M. Ferroptosis: mechanisms, biology and role in disease. *Nat Rev Mol Cell Biol*. 2021;22(4): 266–282.
5. Yao F, Deng Y, Zhao Y, et al. A targetable LIFR–NF- κ B–LCN2 axis controls liver tumorigenesis and vulnerability to ferroptosis. *Nat Commun*. 2021;12(1):7333.