HHS Public Access

Author manuscript

Cell Chem Biol. Author manuscript; available in PMC 2019 December 30.

Published in final edited form as:

Cell Chem Biol. 2019 May 16; 26(5): 621–622. doi:10.1016/j.chembiol.2019.05.002.

Aiming at cancer *in vivo*: ferroptosis-inducer delivered by nanoparticles

Junmei Yi[1], **Alexander M. Minikes**[1,2], **Xuejun Jiang**[1,*]

[1]Cell Biology Program, Memorial Sloan Kettering Cancer Center, New York City, NY 10065, USA

[2]BCMB Allied Program, Weill Cornell Graduate School of Medical Sciences, New York City, NY 10065, USA

Abstract

Induction of ferroptosis has emerged as a potential cancer therapeutic approach. However, this possibility has not been formally validated due to a lack of pharmacological agents suitable for the safe and specific induction of ferroptosis *in vivo*. In this issue of *Cell Chemical Biology*, Zhang et al. (2019) demonstrate the anticancer efficacy and safety of the ferroptosis inducer imidazole ketone erastin (IKE) in a xenograft model by using a nanoparticle-based delivery system.

Ferroptosis is a regulated necrosis process driven by iron-dependent lipid peroxidation. The term “ferroptosis,” first coined by the Stockwell lab (Dixon et al., 2012), refers to the requirement of cellular labile iron for this specific cell death modality. The process centers around GPX4, a glutathione peroxidase with the ability to detoxify phospholipid peroxides. Inactivation of GPX4, either directly or indirectly, leads to rapid accumulation of phospholipid peroxides via iron-dependent Fenton chemistry, among other processes, culminated with ferroptotic cell death (Stockwell et al., 2017).

While the physiological function of ferroptosis remains obscure, its role in disease has been unambiguously established (Stockwell et al., 2017). Ferroptosis is involved in ischemic organ injury, including ischemic heart diseases, brain damage, and kidney failure. It is also implicated in neurodegenerative diseases. Importantly, ferroptosis appears to have a prominent role in tumor suppression. The tumor suppressors p53, BAP1, and fumarase have been identified as positive regulators of ferroptosis (Gao et al., 2019; Jiang et al., 2015; Zhang et al., 2018). Intriguingly, it has been demonstrated that certain cancer cells that are highly resistant to various treatments are more prone to ferroptotic cell death (Viswanathan et al., 2017). These therapy-resistant cells typically demonstrate mesenchymal properties, suggesting that ferroptosis may be a potent therapy for these aggressive, highly metastatic cells that usually do not respond to treatment. Taken together, therapeutic modulation of ferroptosis (both positively and negatively) might be effective for the treatment of multiple diseases, such as cancer and ischemic heart disease.

For a potential ferroptosis-inducing cancer therapy, GPX4 is an obvious molecular target. However, would GPX4 inhibitors selectively or preferentially induce ferroptosis in cancer

*Correspondence: Xuejun Jiang, jiangx@mskcc.org.

cells? There is mounting evidence challenging this possibility. For example, pharmacological and genetic inhibition of GPX4 leads to ferroptosis in a variety of cell types, both cancerous and noncancerous; and genetic deletion of GPX4 gene, the gatekeeper of ferroptosis, is embryonically lethal, likely due to ferroptosis (Friedmann Angeli et al., 2014). Therefore, unless a quantitative window that distinguishes the sensitivity of cancer cells versus that of normal tissues can be identified, adverse side effects associated with GPX4 inhibition will discourage GPX4-targeted cancer therapies.

Fortunately, in addition to the direct inhibition of GPX4, there are other mechanisms for ferroptosis induction. As a glutathione peroxidase, GPX4 requires the reducing agent glutathione to function, and depletion of glutathione in cells will indirectly inhibit GPX4 activity. The small molecule erastin, the first ferroptosis-inducing compound, is an inhibitor of the cystine-glutamate antiporter system x_c-. System x_c- inhibition leads to the depletion of cellular cysteine, a building block for glutathione synthesis, and thus glutathione. Therefore, a natural question is, would the system x_c- antiporter be a potential cancer therapeutic target, and would erastin be a lead compound? Although erastin (as well as most other previously used ferroptosis-inducing compounds that directly or indirectly inhibit GPX4) is not suitable for *in vivo* use due to undesired pharmacological properties, the system x_c- antiporter appears to be a promising molecular target, because (1) various types of cancer overexpress SLC7A11, an essential subunit of the system x_c- antiporter, and (2) genetic knockout of the SLC7A11 gene in mice does not lead to developmental lethality or any obvious defect (McCullagh and Featherstone, 2014), suggesting manageable side effects. Directly relevant to cancer, depletion of plasma cystine levels by administration of cyst(e)inase has been shown to lead to tumor suppression via ferroptosis, while leaving healthy tissue unharmed (Poursaitidis et al., 2017). This difference in ferroptosis sensitivity is likely due to the high demand of metabolism and thus reactive oxygen species production in tumors, leading to an increased dependency on the antioxidant properties of glutathione and GPX4; on the other hand, in normal tissues, because of lower levels of oxidative stress, mechanisms such as transsulfuration might be sufficient to provide cysteine and sustain cellular viability.

To interrogate the targetability of system x_c^- *in vivo*, Stockwell and colleagues developed imidazole ketone erastin (IKE), a derivative of erastin, with increased potency (about 100-fold over erastin), solubility (erastin and its earlier version of analogs all have very poor hydrophilicity), and stability, making it an attractive molecule for *in vivo* preclinical study (Larraufie et al., 2015). In this issue of Cell Chemical Biology, Zhang et al characterized the potential of IKE as an *in vivo* ferroptosis-inducing cancer therapeutic agent, either as a single agent or in combination with chemotherapy. In this study, they showed that IKE exhibited antitumor activity in a diffuse large B cell lymphoma (DLBCL) xenograft model, suggesting that IKE could potentially be a therapeutic regimen for DLBCL. To further improve the safety and efficacy of IKE, they utilized nanocarriers, leading to more effective and selective delivery of IKE to tumor tissues. This nano-formulation inhibited tumor growth and showed less toxicity (based on its effect on animal weight loss) compared to free IKE.

At this point, it is worthwhile to revisit this case: does there exist a therapeutic window for GPX4 inhibition, which is the most straightforward way to induce ferroptosis, at least for

some specific cases of cancer? One potential case is mesenchymal cancer cells, which have been shown to be highly susceptible to GPX4 inhibition (Viswanathan et al., 2017). It is possible that low dose GPX4 inhibition might have therapeutic effect on these otherwise hard-to-treat cancers, hopefully with tolerable damage on normal tissues. In addition, given that cellular metabolism is often reprogrammed in cancer cells and that cellular metabolism plays a pivotal role in ferroptosis, it is likely that certain types of cancer with 'desired' genetic alterations might be under strong oxidative stress due to altered metabolism, thus even a modest inhibition of GPX4 can tilt the balance and trigger ferroptosis in cancer cells but not in normal tissues. For example, glutamine-addicted cancer cells might be more sensitive to ferroptosis induction because glutamine is essential for cysteine deprivation-induced ferroptosis by fueling the mitochondrial TCA cycle (Gao et al., 2019). Additionally, lipid metabolism and biogenesis are highly active in cancer cells to maintain homeostasis of cellular membranes and satisfy nutrient requirements for rapid proliferation. As the accumulation of lipid peroxides triggers ferroptosis, lipid metabolism is tightly intertwined with ferroptosis. Studies showed that polyunsaturated fatty acids (PUFAs) and PUFA-containing phospholipids are substrates for peroxidation during ferroptosis, and arachidonyl and adrenoyl phosphatidylethanolamines (PEs) are the preferred substrates for ferroptosis (Kagan et al., 2017); inhibition of the lipid metabolism-related genes acyl-coenzyme A (CoA) synthetase long-chain family member 4 (*ACSL4*) and lysophosphatidylcholine acyltransferase 3 (*LPCAT3*) mitigates ferroptosis (Doll et al, 2017). Furthermore, the mevalonate pathway, which is involved in synthesis of CoQ10, cholesterol, and GPX4 (via selenocystyl-tRNA regulation), has been implicated in ferroptosis, and FIN56, a ferroptosis-inducing compound, activates squalene synthase, depleting CoQ10 and simultaneously depleting GPX4, leading to ferroptosis (Shimada et al., 2016). As such, perturbating lipid metabolism in combination with GPX4 inhibitors, or treating cancers harboring specific lipid metabolic profiles with GPX4 inhibitors, may be an option to induce ferroptosis selectively in cancer cells.

Relevant to lipid metabolism, Zhang et al. demonstrate that IKE treatment can cause a profound change of the lipid profile in DLBCL cells and a DLBCL xenograft mouse model. There are significant changes of various lipid species and lipid metabolism-related genes upon IKE treatment. Lipid species including phospholipids, triacylglycerides (TAGs), diacylglycer-ides (DAGs), monoacylglycerides (MAGs), and free fatty acids are decreased dramatically. Lipid metabolic genes including the lipid *de novo* biosynthetic enzymes acetyl-CoA carboxylase 1 (ACC1) and elongation of very long chain fatty acids protein 7 (ELOVL7); lipid-remodeling enzymes adipose triglyceride lipase (ATGL), secretory phospholipase A2f (sPLA2f), lysophosphatidyl ethanolamine acyltransferase 1 (LPEAT1), and LPCAT4; and lipid peroxidation enzymes lipoxygenases 12 and 15 (ALOX12 and ALOX15) are all upregulated. These changes of cellular lipid metabolism may help shed light on how lipid peroxidation kills cells, which is still an outstanding question in the field. Additionally, the genes identified in this study will provide new potential targets for developing ferroptosis inducers or developing combination therapies with ferroptosis inducers. Further, the molecular basis of how IKE, presumably via depleting cellular cysteine, leads to systematic changes in lipid metabolism, warrants further investigation.

Such mechanistic study might reveal novel interplay of cellular redox homeostasis with lipid metabolism, and, importantly, the biological function of such interplay.

Collectively, although ultimate efficacy and safety of a cancer therapeutic approach can only be established by clinical trials, the demonstration of IKE (and nano-IKE) as the first safe, specific, and effective ferroptosis inducer for *in vivo* use in Zhang et al. is a critical proof-of-principle evidence validating the therapeutic promise of targeting the system x_c^- antiporter to induce cancer cell ferroptosis. This study has established an invaluable and long-awaited pharmacological tool for the *in vivo* study of ferroptosis, as well as a platform for the development of new ferroptosis inducers for clinical use. This exciting work will prove to be an important step forward for the field of ferroptosis and its clinical exploration.

Acknowledgements

This work is supported by NIH R01CA204232 (to XJ), a Geoffrey Beene Cancer Research fund (to XJ), a Functional Genomic Initiative fund (to XJ), and NIH T32 fellowship 5T32GM008539-23 (to AMM). This work is also supported by NCI cancer center core grant P30CA008748 to Memorial Sloan Kettering Cancer Center.

References

Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, Patel DN, Bauer AJ, Cantley AM, Yang WS, et al. (2012). Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell 149, 1060–1072. [PubMed: 22632970]

Friedmann Angeli JP, Schneider M, Proneth B, Tyurina YY, Tyurin VA, Hammond VJ, Herbach N, Aichler M, Walch A, Eggenhofer E, et al. (2014). Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. Nature cell biology 16, 1180–1191. [PubMed: 25402683]

Gao M, Yi J, Zhu J, Minikes AM, Monian P, Thompson CB, and Jiang X (2019). Role of Mitochondria in Ferroptosis. Molecular cell 73, 354–363 e353. [PubMed: 30581146]

Jiang L, Kon N, Li T, Wang SJ, Su T, Hibshoosh H, Baer R, and Gu W (2015). Ferroptosis as a p53-mediated activity during tumour suppression. Nature 520, 57–62. [PubMed: 25799988]

Kagan VE, Mao G, Qu F, Angeli JP, Doll S, Croix CS, Dar HH, Liu B, Tyurin VA, Ritov VB, et al. (2017). Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. Nature chemical biology 13, 81–90. [PubMed: 27842066]

Larraufie MH, Yang WS, Jiang E, Thomas AG, Slusher BS, and Stockwell BR (2015). Incorporation of metabolically stable ketones into a small molecule probe to increase potency and water solubility. Bioorganic & medicinal chemistry letters 25, 4787–4792. [PubMed: 26231156]

McCullagh EA, and Featherstone DE (2014). Behavioral characterization of system xc- mutant mice. Behavioural brain research 265, 1–11. [PubMed: 24548853]

Poursaitidis I, Wang X, Crighton T, Labuschagne C, Mason D, Cramer SL, Triplett K, Roy R, Pardo OE, Seckl MJ, et al. (2017). Oncogene-Selective Sensitivity to Synchronous Cell Death following Modulation of the Amino Acid Nutrient Cystine. Cell reports 18, 2547–2556. [PubMed: 28297659]

Shimada K, Skouta R, Kaplan A, Yang WS, Hayano M, Dixon SJ, Brown LM, Valenzuela CA, Wolpaw AJ, and Stockwell BR (2016). Global survey of cell death mechanisms reveals metabolic regulation of ferroptosis. Nature chemical biology 12, 497–503. [PubMed: 27159577]

Stockwell BR, Friedmann Angeli JP, Bayir H, Bush AI, Conrad M, Dixon SJ, Fulda S, Gascon S, Hatzios SK, Kagan VE, et al. (2017). Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. Cell 171, 273–285. [PubMed: 28985560]

Viswanathan VS, Ryan MJ, Dhruv HD, Gill S, Eichhoff OM, Seashore-Ludlow B, Kaffenberger SD, Eaton JK, Shimada K, Aguirre AJ, et al. (2017). Dependency of a therapy-resistant state of cancer cells on a lipid peroxidase pathway. Nature 547, 453–457. [PubMed: 28678785]

Zhang Y, Shi J, Liu X, Feng L, Gong Z, Koppula P, Sirohi K, Li X, Wei Y, Lee H, et al. (2018). BAP1 links metabolic regulation of ferroptosis to tumour suppression. Nature cell biology 20, 1181–1192. [PubMed: 30202049]

Zhang Y, Tan H, Daniels JD, Zandkarimi F, Liu H, Brown LM, Uchida K, O'Connor OA, and Stockwell BR (2019). Imidazole Ketone Erastin Induces Ferroptosis and Slows Tumor Growth in a Mouse Lymphoma Model. Cell Chemical Biology.