The enemy within: cysteine metabolism helps pancreatic tumors escape ferroptosis

Preview for "Badgley et al. (2020) Cysteine depletion induces pancreatic tumor ferroptosis in mice. Science, 368, 85–89"

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Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease holding the fourth highest cancer-related mortality rate worldwide and the poorest 5-year survival rate (8%) (Siegel et al., 2018). PDAC occurs primarily among the elderly population, as well as those with metabolic syndromes and unhealthy lifestyle (Orth et al., 2019). In addition, genetic predispositions contribute to PDAC, particularly mutations in *KRAS* is present in more than 90% of PDAC cases. *KRAS* mutations in cancer cells play a role in elevating glucose, glutamine and fatty acids uptake in sustaining metabolic adaptations (Pupo et al., 2019). Among these adaptive regulations, this current study investigates the interplay between cysteine metabolism and ferroptosis aiming to inspire medicinal development for PDAC.

Unlike apoptosis, a caspase-dependent mechanism, ferroptosis is mediated by iron and lipid peroxidation resulting in lipid reactive oxygen species (ROS) accumulation and cell death. It was first noticed as part of a synthetic lethality screen for identifying antitumor agents in engineered human tumor cells (Dolma et al., 2003). Increased ferroptosis in cancer cells leads to ROS-accumulated stress that promotes cell death. To escape from this cell fate, cellular processes in cancer evolved several protective mechanisms, such as promoting glutathione (GSH) from its precursor cysteine, to circumvent stress-related death. To date, several studies reported the phospholipid hydroperoxide-reducing enzyme glutathione peroxidase 4 (GPX4) and ferroptosis suppressor protein 1 (FSP1) play a role in the suppression of ferroptosis-related cell death through thioredoxin system (Bersuker et al., 2019; Doll et al., 2019; Viswanathan et al., 2017). Targeted inhibition on either of these enzymes effectively overcame ferroptosis resistance; however, the underlying mechanisms and the precise roles of cysteine metabolism in regulating PDAC remain unclear.

In this study, the authors proposed that the cysteine metabolism is crucial to PDAC progression. To counteract ferroptosis-induced cell death, cancer cells utilize cysteine-derived metabolites in detoxifying ROS. By performing both *in vitro* and *in vivo* studies in PDAC cell lines and novel transgenic mouse models, the authors illustrated a comprehensive story of how impairment of the cystine-glutamate exchange transporters induced ferroptosis and inhibited PDAC proliferation. Their discovery opened up a new genre of therapeutic development to fight against PDAC.

First, the authors demonstrated the interaction between cysteine metabolism and ferroptosis in the context of pancreatic cancers using *in vitro* systems. They starved the human pancreatic cancer cell lines of cysteine and found that the cell viability is only around 20%. They also demonstrated that treating the pancreatic cancer cells with a cysteine transporter inhibitor, imidazole ketone erastin (IKE), mimics cysteine starvation and leads to cell death. The authors observed that these cells are likely dying through ferroptosis, and confirmed this by showing co-treatment with a ferroptosis inhibitor, ferrostatin-1, reduces cell death induced by cysteine depletion. Cysteine regulates ferroptosis primarily through the synthesis of GSH to enhance the detoxification of lipid ROS. The authors found that increasing GSH levels prevents ferroptosis in pancreatic cancer cells, but GSH loss is not sufficient to induce ferroptosis. They therefore traced ^{13}C-labeled cysteine to find additional cysteine-derived metabolites. In addition to GSH, cysteine is also converted to coenzyme A (CoA), which plays an important role in lipid metabolism. The authors then showed that inhibition of cysteine transport by administering IKE reduced the levels of CoA in pancreatic cancer cells and treating them with exogenous CoA is sufficient to suppress IKE-induced ferroptosis. Together, their results suggest that pancreatic cells escape ferroptosis by utilizing cysteine and its metabolic derivatives, CoA and GSH by upregulating metabolic programs that eliminate lipid ROS.

Next, the authors generated an *in vivo* mouse model to test genetically the role of cysteine in pancreatic tumor progression. Slc7a11, a subunit of the cysteine antiporter, is frequently overexpressed in several human cancers. To test whether pancreatic tumors rely on cysteine transport *in vivo* to escape ferroptosis and to survive, they generated a novel mouse model: KPFSR mice ($Kras^{FSF.G12D/+}$; $Tp^{53R172H/+}$; $Pdx1FlpO^{tg/+}$; $Slc7a11^{Fl/Fl}$; $Rosa26^{CreERT2/+}$), which develops PDAC due to pancreatic Kras and germline p53 mutations and generates systemic deletion of Slc7a11 upon tamoxifen administration to examine the effects of cysteine transport on PDAC. They found that the median survival of the mice with Slc7a11 deletion (29 days) is double of the mice that received vehicle (15 days). Restoring cysteine levels by administering a cell-permeable analog of cysteine, N-acetyl-cysteine (NAC), in the water of Slc7a11 deletion mice suppresses their median survival back to baseline levels (17 days). They then observed the tumors in KPFSR mice and found several histological hallmarks of ferroptosis: destabilized plasma membrane, enlarged lipid droplets and aberrant mitochondria. They also performed high-throughput transcriptomic analysis of the KPFSR tumors and showed that the genes associated with ferroptosis are upregulated. Therefore, the KPFSR mouse model creates an ideal tool for studying ferroptosis *in vivo*.

Finally, the authors also demonstrated pharmacological ways to target cysteine metabolism and to induce ferroptosis in pancreatic tumors. They showed that treatment with cyteinase, an engineered enzyme drug that depleted cysteine and cystine, is sufficient to induce ferroptosis in mice with pancreatic tumors. They demonstrated that when treated with cyteinase, the pancreatic tumors in mice exhibited stabilizations or regressions, whereas vehicle-treated

controls never stabilized. Overall, this study demonstrates that pancreatic tumors rely on enhanced cysteine metabolism to escape ferroptosis *in vitro* and *in vivo,* and proposes cysteinase as a therapeutic intervention.

The highly proliferating nature of cancer cells and thus the demand of ample supply of nutrients for tumor growth alter the metabolic network during malignancy. This increased metabolic demand also puts the growing tumor at the risk of accumulating toxic by-products such as lipid reactive oxygen species and thus requires a more sophisticated detoxification system. The cysteine dependency seems to be a general mechanism for the survival of different cancer types (Viswanathan et al., 2017). One important question that was raised by this study is if the treatment effect of cyst(e)inase can be generalized across different cancer types or it is specific to pancreatic cancer carrying the oncogenic Kras mutation. The anti-tumor activity of P53 has been partially attributed to the induction of ferroptosis by inhibiting system Xc^- (Jiang et al., 2015). The KPC mouse model used in this study carries a p53 mutation that loses tumor suppressive function and gains oncogenic activity. From a precision medicine perspective, defining the response of different cancers to cysteine inhibition according to the p53 mutation status would help clarify if p53 mutation lowers the tumor sensitivity to cysteine inhibition.

Different from the role of mitochondria in apoptosis that releases cytochrome c to initiate the activation cascade of caspases, the function of mitochondria in ferroptosis remains to be explored. As it is observed in this study and many others, the morphology, size and integrity of the mitochondria change dramatically during ferroptosis (Lu et al., 2017). The first question regarding mitochondria is if these changes are universal to all ferroptosis or they are distinct within normal and cancerous tissues. Secondly, it would be interesting to explore what the function of these mitochondrial morphology changes is in regards to ferroptosis during normal development and tumorigenesis.

In this study, only four IKE sensitive PDAC cell lines were tested, meaning that the sensitivity to IKE or cyst(e)inase varies across cancer cell lines, which suggests the existence of additional factors governing resistance to cysteine depletion induced ferroptosis. A synthetic lethal CRISPR–Cas9 screen would help identify parallel mechanisms that control ferroptosis in addition to cysteine depletion and also antagonistic factors against cysteine depletion induced ferroptosis. Along the same line, CRISPR-Cas9 rescue screens in IKE or cyst(e)inase sensitive cancer cell lines will reveal more information downstream of cysteine depletion in addition to ferroptosis. These further studies will continue to deepen our understanding of the altered metabolic regulation in malignancy and the knowledge will be harnessed to expand the therapeutic potential of targeting the tumor specific metabolic pathways.

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