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AUTHOR'S VIEWS

Ferroptosis resistance mediated by exosomal release of iron

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ABSTRACT

Understanding how cells resist ferroptosis is necessary for exploiting this iron-dependent mode of cell death for the treatment of cancer and other diseases. We discovered that cells resist ferroptosis by enabling a PROMININ2-dependent iron export pathway involving multivesicular body/exosome trafficking of iron out of the cell, diminishing the intracellular iron needed for ferroptosis.

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Commentary

Ferroptosis is a regulated form of non-apoptotic cell death characterized by the iron-dependent accumulation of lethal lipid reactive oxygen species (ROS). Ferroptosis can be initiated by a number of stimuli that disrupt intracellular glutathione-mediated antioxidant systems or directly overload the cell with iron.[1] For example, it is possible to induce ferroptosis by depriving cells of the thiol-containing amino acid cystine (the disulfide of cysteine), a key glutathione precursor, or by directly inhibiting the reduced glutathione (GSH)-dependent phospholipid hydroperoxidase glutathione peroxidase 4 (GPX4).[2,3] GPX4 catalyzes the reduction of reactive lipid hydroperoxides to non-reactive lipid alcohols. This reaction is essential to prevent a buildup of lipid hydroperoxides, which can participate in Fenton chemistry reactions when free iron is present that lead to the generation of toxic lipid alkoxy radicals and subsequently other reactive lipid breakdown products.[3] The execution of ferroptosis, therefore, relies on the combined presence of oxidizable lipids (e.g. polyunsaturated phospholipids) and free iron which, in the absence of sufficient GPX4-mediated lipid peroxide detoxification, result in oxidative destruction of the plasma membrane and other internal organelle membranes.[4]

Our interest in ferroptosis stems from the observations that cancer cells are susceptible to this mode of cell death and that it can be exploited as a novel therapy for cancer. More specifically, compelling evidence indicates that more aggressive and drug resistant tumor cells are dependent upon GPX4 for their survival.[2,5,6] Drugs that inhibit GPX4 such as RSL3 have the potential to be an effective therapy for such tumors. Our own previous work has shown that physiological stress such as detachment of carcinoma cells from the extracellular matrix (ECM) can increase ROS and is sufficient to trigger ferroptosis if GPX4 activity is inhibited. This latter observation is significant because circulating tumor cells are detached from the ECM and, consequently, prime targets for the therapeutic induction of ferroptosis.

The potential for exploiting ferroptosis as a cancer therapy is hindered, however, by the fact that many cancer cells have the ability to resist GPX4 inhibition or physiological stresses such as ECM detachment. Our goal in this study was to decipher mechanisms that contribute to this resistance. Our approach involved using RNA-Seq to identify genes whose expression was induced by pro-ferroptotic stimuli including GPX4 inhibition and ECM detachment. This analysis revealed that these stimuli induce the expression of PROMININ2, a pentaspanin protein implicated in regulation of lipid dynamics.[7] Although the literature on PROMININ2 is scant, we were intrigued by its association with lipid dynamics because rapid changes in lipid chemistry are the root cause of ferroptotic cell death. Our initial experiments established a causal role for PROMININ2 in the ability of cells to resist ferroptosis triggered by either GPX4 inhibition or ECM detachment. The challenge was to elucidate the mechanism involved given that so little was known about PROMININ2. A major breakthrough in this direction was our discovery that PROMININ2 is localized specifically in multivesicular bodies (MVBs) and that it actually stimulates their formation in response to ferroptotic stress. Interestingly, the increase in MVB formation is rapid (~ 2hrs) after either GPX4 inhibition or ECM detachment. Given that MVBs can fuse with the plasma membrane and release intraluminal vesicles (ILVs) as exosomes[8], our subsequent experiments established that PROMININ2 stimulates the rapid release of exosomes from cells and that this process is essential for ferroptosis resistance.

Our finding that PROMININ2 stimulated the formation of MVBs and the release of exosomes was interesting but it didn't explain how this pathway promoted ferroptosis resistance. We hypothesized that this resistance mechanism involved the exosome-mediated export of a factor that would otherwise promote ferroptosis. Given that the iron storage protein ferritin can be secreted from cells in exosomes,[9] we reasoned that MVB/exosome-mediated ferritin export inhibits ferroptosis. Indeed, we demonstrated that ferritin co-localizes with PROMININ2 in MVBs and that both

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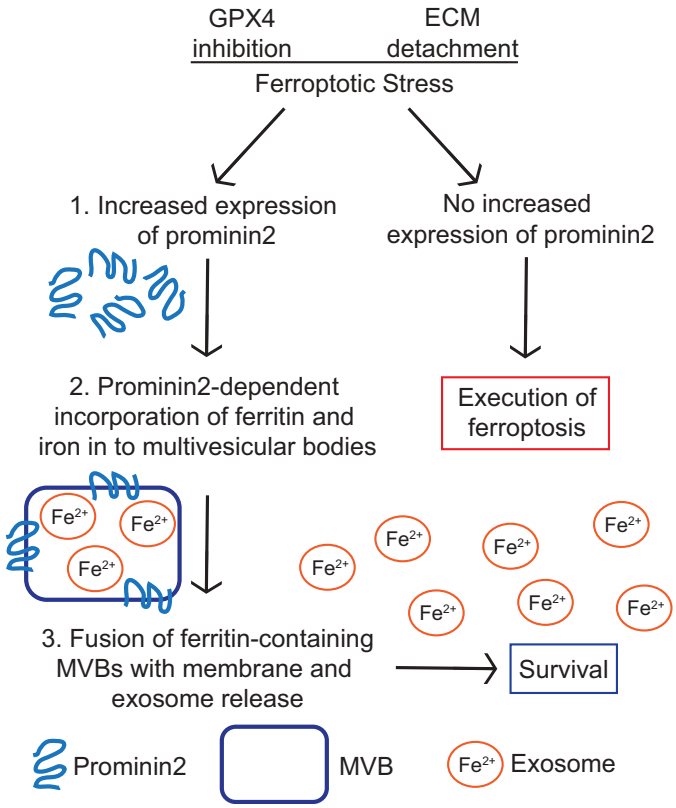

Figure 1. Schematic of PROMININ2-mediated evasion of ferroptosis. During ferroptotic stress, carcinoma cells increase expression of PROMININ2 to evade cell death. PROMININ2 promotes the formation of multi–vesicular bodies (MVBs) containing iron-laden ferritin nanocages. These MVBs are trafficked to the cell surface where the iron is exported to the extracelluar space in exosomes.

ferritin and iron are present in exosomes secreted from cells in response to ferroptotic stress. We also observed that PROMININ2 prevents an increase in the intracellular iron concentration in response to ferroptotic stress, which is consistent with the finding that ferritin-bound iron is secreted from cells in exosomes.

In summary, we demonstrated in this study that cells can resist the onset of ferroptosis by dynamically upregulating an unprecedented iron export pathway involving MVB/exosome trafficking of ferritin and iron out of the cell (Figure 1). This process limits the intracellular accumulation of free iron and inhibits the onset of ferroptosis. By contrast, inactivation of this program, either normally or following chemical or genetic inhibition of key steps of this process, substantially increases ferroptosis sensitivity. We believe that our work has significant implications for strategies aimed at inducing ferroptosis as a therapeutic strategy in cancer. As mentioned above, inhibiting GPX4 activity can selectively kill certain tumor cells via ferroptosis.[2,6] Given that cells can acquire resistance to GPX4 inhibition by inducing PROMININ2, strategies that simultaneously block PROMININ2 expression or function may enhance sensitivity to GPX4 inhibitors. Indeed, our analysis of the Broad Institute Cancer Therapeutics Response Portal resource revealed that high levels of PROMININ2 expression are significantly correlated with resistance to the GPX4 inhibitor ML210 across hundreds of cancer cells lines. These data were substantiated by the observation that PROMININ2 expression is correlated with poor clinical outcomes in several cancers.[10]

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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