Extracellular vesicle-mediated ferroptosis plays a role in the disease

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Abstract

Ferroptosis is a newly discovered form of iron-dependent cell death caused by excessive lipid peroxidation. It is widely involved in a variety of pathological processes in the body and has been considered to be related to the development and treatment of various diseases. The study of extracellular vesicles has also advanced significantly over the past ten years, transitioning from a field of fundamental biological study to one with significant clinical implications. It is also presently actively investigating its potential use as a brand-new drug delivery system, combination therapy, etc. Research on ferroptosis mediated by extracellular vesicles has made progress in disease treatment. Given this, this review will focus on the association of extracellular vesicles with ferroptosis and the role that it mediates in the disease. Firstly, a description of ferroptosis’ primary mechanisms is provided. Secondly, the pathways associated with ferroptosis and extracellular vesicles are elaborated, with attention to the relevance of the corresponding mechanisms to disease and potential applications for therapeutic use. Finally, future expectations and challenges of targeting the extracellular vesicle-mediated ferroptosis pathway in disease treatment are discussed.

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Figure 1. **Main mechanisms of ferroptosis.**

The mechanism of ferroptosis is complex, which is described from two main aspects.

1. **Iron metabolism in ferroptosis.**
   a. Iron enters recipient cells through the Tf and Tfr system. Iron binds to Tf and distributes throughout the body, and then it is endocytosed into cells after forming complexes with Tf and Tfr.
   b. DMT1 transports Fe^{2+} into cells. This protein is the main transmembrane transporter of ferrous and other divalent metal ions into the cell.
   c. Iron is stored in lysosome-dependent ferritin. Ferritin temporarily stores unwanted iron in the cell in the inner cavity to protect the cell from harmful pro-oxidation. Ferritin generally releases iron in the autophagy-lysosomal pathway, a process by which ferritin is selectively targeted by NCOA4 to autophagy/lysosomal degradation and promotes cell ferroptosis.
   d. Changing the sensitivity of ferroptosis by regulating hepcidin-mediated iron transporters. FPN is a plasma laminar iron export protein located on the cell membrane. It transports iron from the intestine, spleen and liver cells to the blood, supplying iron to other tissues, organs and cells in the organism.

2. **Counterbalance between lipid peroxidation and antioxidant system in ferroptosis.**
   e. Lipid peroxidation promotes cell ferroptosis. In the process of ferroptosis, PUFA in lipids undergoes peroxidation, which causes the break of the lipid bilayer, affects the membrane function, and participates in the accumulation of toxic ROS that trigger ferroptosis. ACSL4 is one of the major enzymes involved in ceroid lipid metabolism, which is related to the biosynthesis and regulation of PUFA on the membrane, and can promote the binding of PUFA to phospholipids. AMPK-mediated phosphorylation of acetyl-CoA carboxylase promotes ferroptosis by inhibiting the production of reduced GSH and influences the process of ferroptosis by regulating PUFA content.

f. **Intracellular antioxidant system inhibits ferroptosis process.** GPX4 is a very important substance in the antioxidant defense system. GPX4 prevents ferroptosis by converting lipid hydroperoxide into non-toxic lipid alcohols. The presence of GSH and selenium is important for the expression and activity of GPX4 in ferroptosis. Xc-system is present in cells, which consists of two subunit transporters, SLC7A11 and SLC3A2. When a molecule of Cys2, the oxidized form of Cys, is introduced into the cell and a molecule of glutamate is released outside the cell, Cys2 can be oxidized to Cys once in the cell, thus promoting the synthesis of GSH.
Figure 2: EVs inhibit ferroptosis by reducing intracellular iron content.
Ferritin is the main intracellular iron storage protein. Ferritin can be secreted outside the cell through the MVB-exosome pathway. The MVBs formed in the cell encapsulate ferritin, carry iron out of the cell, and regulate the content of iron in the cell to inhibit the occurrence of ferroptosis. The increase of Prominin2 protein will promote the formation of ferritin-containing MVBs, so that more ferritin will be transported out of cells to inhibit ferroptosis. Expression of the EV-associated protein CD63 is regulated by iron through the IRP-IRE system.

Figure 3: Designing exosomes Er/RB@ExosCD47.
CD47, a widely expressed integrin-associated transmembrane protein, is a ligand for signal regulatory protein alpha (SIRPα), and CD47-SIRPα binding initiates the "don't eat me" signal that inhibits phagocytosis. Er is an inducer of ferroptosis and RoseBengal(RB) is a well-known photosensitizer that can form high ROS production in PDT. Based on this, a three-part exosome was designed: surface functionalization with CD47, membrane loading with Er, and nucleation with photosensitizer RB. The designed exosome protects it from macrophage clearance and triggers ferroptosis.
Designed exosome Drug therapy Combination therapy

This review focuses on the association of EVs with ferroptosis and the role of EV-mediated ferroptosis in disease. a Fenton reaction and excessive lipid peroxidation trigger ferroptosis. b Secretion of EVs containing ferritin by cells inhibits ferroptosis. c Secretion of EVs by other cells transports relevant miRNAs to the cell and inhibits ferroptosis in that cell. By exploring the link between ferroptosis and EVs, we help researchers to investigate possible therapeutic options.

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