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RESEARCH HIGHLIGHT

A new checkpoint against ferroptosis

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By taking different approaches, two research groups made the same discovery that a CoQ oxidoreductase (renamed as ferroptosis suppressor protein-1, or FSP1) can inhibit ferroptosis, a form of cell death driven by iron-dependent phospholipid peroxidation. As FSP1 is expressed in various cancers and can halt ferroptosis even in the absence of glutathione peroxidase 4 (GPX4), which was previously considered indispensable for ferroptosis surveillance, the potential of FSP1 as a new cancer therapeutic target should be explored.

In cells, peroxidation of polyunsaturated fatty acid (PUFA)-containing phospholipid (PL) occurs via metabolic reactions catalyzed by lipoxygenases (LOX) or through non-enzymatic reactions triggered by reactive oxygen species (ROS). These unique lipid species partake in various biological processes. However, if the phospholipid peroxides (PLOOH) are not kept in check, they can initiate an iron-dependent, auto-amplifying free radical chain reaction known as Fenton reaction, ultimately leading to ferroptotic cell death.[1]

A specific glutathione (GSH) peroxidase, GPX4, is the sole enzyme in mammalian cells responsible for the reduction of PLOOH. As such, GPX4 is considered essential for ferroptosis surveillance. This theory is supported by a plethora of evidence: (i) GPX4-knockout mice are embryonically lethal, likely due to ferroptosis; and (ii) inhibition of GPX4, either directly or indirectly through depletion of intracellular GSH, can trigger ferroptosis.[2] Importantly, recent studies have revealed that cancer cells with specific cellular properties (such as mesenchymal character[3]) or mutational signatures (such as multiple mutations in the CDH1-NF2-Hippo-YAP signaling pathway[4]) can be highly susceptible to GPX4 inhibition or deprivation of cellular cysteine and GSH. Moreover, immune checkpoint blockade might execute its anticancer function partially through induction of cancer cell ferroptosis in vivo, an event mediated by $CD8^+$ T cell-secreted IFNγ, which downregulates the expression of System Xc^- cystine importer in cancer cells.[5] All these findings posit ferroptosis induction as a promising cancer therapeutic strategy.

Would cells survive ferroptosis in the absence of GPX4? Presumably, by minimizing PLOOH production via inhibiting PLOOH-generating processes or enzymes would work. Indeed, that can explain why certain cancerous and noncancerous cells, under "desired" biological conditions, can live when GPX4 function is inhibited.[4] Experimentally, lipophilic radical trapping agents are used as "specific" ferroptosis inhibitors (the claim of "specificity" is not without controversy), and they can potently (often with nM potency) inhibit ferroptosis and restore cellular viability, even when the GPX4 gene is deleted.

But a more provocative question might be: can cells live and propagate at all in the absence of GPX4 under "normal" biological conditions? One can imagine that if there is a cellular enzyme that can catalyze the production of a lipophilic radical trapper with sufficient quantity (nM level), then the cell should survive the absence of GPX4 activity. And Bersuker et al.[6] and Doll et al.[7] reported that FSP1 is exactly such a lipophilic radical trapper that protects cells from ferroptosis even in the absence of GPX4.

But it is only in hindsight and with the "wisdom" of having seen all facts; both groups took an unbiased approach without first entertaining or relying on such a potential mechanism. Bersuker et al. used a CRISPR-Cas9 screening that resulted in the identification of FSP1 (previously known as AIFM2) as a suppressor of ferroptosis, since sgRNAs targeting the FSP1 gene were dramatically reduced in the RSL3-treated group compared with the control group, and $FSP1^{-/-}$ cancer cells are more sensitive to RSL3-induced cell death. Doll et al. transduced a cDNA library to identify genes that can complement GPX4 loss. Among the 14 clones resistant to ferroptosis, 7 of them express GPX4 and the rest express FSP1. Both teams found that FSP1 functions as a NADPH-dependent coenzyme Q (CoQ) oxidoreductase. The reduced form of CoQ, ubiquinol, can act as a radical-trapping antioxidant to prevent lipid peroxidation. Interestingly, both teams reported that myristoylation at the N-terminus of the FSP1 protein is important for its ferroptosis-inhibitory activity by targeting FSP1 to the plasma membrane, where it reduces CoQ, thus providing radical trapping activity to counter ferroptosis.

The identification of the FSP1/CoQ/NADPH pathway as a novel route for the suppression of phospholipid peroxidation and ferroptosis — complementary yet non-redundant with the GSH/GPX4 pathway — has important mechanistic and therapeutic implications. It also raises several intriguing questions. **Mechanistically**: (i) In addition to FSP1, is there any alternative reductase for CoQ? In mitochondria, CoQ receives electrons (i.e., being reduced) not only from Complex I (NADH-ubiquinone oxidoreductase) or Complex II (succinate-ubiquinone oxidoreductase) but also from several other dehydrogenases.[8] It might be possible that other reductases besides FSP1 could do the same job reducing CoQ and mitigating ferroptosis in a context-dependent manner. (ii) How does the concentration of cellular CoQ alter in ferroptosis? CoQ is composed of a benzoquinone ring head group, which is synthesized through a large protein complex localized in the inner mitochondrial membrane, and a polyisoprenoid chain that is synthesized through the mevalonate pathway. It is unknown how the CoQ synthesis pathway is regulated and how such regulation may control the sensitivity of the cell to ferroptosis. Moreover, multiple genes encoding CoQ biosynthetic proteins have been shown to have pathogenic variants causing human CoQ deficiency, resulting in a wide range of clinical manifestations.[9]

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It is thus worthwhile to test whether these manifestations are caused by ferroptosis due to CoQ deficiency and how genetic CoQ deficiency affects the sensitivity of the affected cells to ferroptosis. **Therapeutically**: (i) As FSP1 expression level varies in cancer cells, can it, in addition to those identified previously,[4] be used a biomarker for the prediction of cancer cell sensitivity to GPX4/system Xc^- inhibitors? (ii) Can the inhibition of FSP1 itself be explored as a ferroptosis-inducing therapy, either alone or in combination with the inhibition of GPX4/system Xc^-? The possible toxicity of GPX4 inhibitors might be strong, given that GPX4-knockout mice are embryonically lethal and GPX4 conditional knockout mice in neurons and myeloid cells or T cells have defects in motor neuron survival and immunity, respectively.[10,11] By contrast, FSP1-knockout mice are viable and develop normally without obvious phenotypic defects.[12] Therefore, compared to direct targeting of GPX4, targeting FSP1 might be a safer alternative for triggering ferroptosis in cancer. (iii) Obviously, future ferroptosis induction-based cancer therapies, including those targeting FSP1, might be used in combination with other modalities of cancer treatment, such as immune checkpoint blockade therapies.

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