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Neutrophil ferroptotic death promotes autoimmune pathogenesis

Zijian Pan[1,2,3,4†], Nathchar Naowarojna[1,2,3†], Yuqi Wang[1,2,3], Mangze Hu[1,2,3] & Yilong Zou[1,2,3*]

[1]*School of Life Sciences, Westlake University, Hangzhou 310024, China;*
[2]*Westlake Institute for Advanced study, Hangzhou 310024, China;*
[3]*Westlake Four-Dimensional Dynamic Metabolomics (Meta4D) Lab, Westlake Laboratory of Life Sciences and Biomedicine, Hangzhou 310024, China;*
[4]*Fudan University, Shanghai 200433, China*

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Ferroptosis is an iron-dependent, non-apoptotic cell death program marked by aberrant accumulation of membrane-associated lipid radicals and hydroperoxides (Zou and Schreiber, 2020). Normal cells deploy multiple mechanisms, centered on the glutathione peroxidase 4 (GPX4) and ubiquinol axes, to prevent excessive spontaneous lipid peroxidation and ferroptosis. Failure to restrict ferroptosis in normal tissues underlies the pathogenesis of various diseases, including acute damages in the kidney, liver, heart and brain by ischemia/reperfusion, and chronic disorders represented by neurodegeneration (Zou and Schreiber, 2020). It remains poorly characterized as to how ferroptosis contributes to immune responses and autoimmune diseases, during which extensive pathogen clearance and cell death is frequently engaged.

Recently, Li et al. (2021) uncovered that the loss of neutrophils in systemic lupus erythematosus (SLE), a potentially fatal, chronic autoimmune disease, is largely mediated by ferroptotic cell death. SLE is elicited by aberrant immune responses to cellular particles containing nucleic acids. Other known pathological characteristics of SLE include accumulation of cell debris, elevated type I interferon (IFN) signaling, and autoantibody production in the circulation (Kaul et al., 2016). The loss of blood neutrophils, neutropenia, was recently recognized as a common feature of SLE (Kaplan, 2011); however, the mechanism that triggered neutrophil reduction was unclear. To investigate the dynamics of neutropenia in SLE disease progression, Li et al. first performed a clinical study by recruiting a large cohort of patients to confirm that neutrophil loss is shared among SLE patients but not in patients with other autoimmune diseases. The low viability of freshly-isolated neutrophils from SLE patients suggested that the reduced neutrophil counts observed in the clinics are likely caused by aberrant neutrophil death rather than by impaired neutrophil differentiation and maturation. The authors further showed that culturing with the sera from patients with active SLE was sufficient to induce neutrophil death *in vitro*. Cytokine profiling suggested that SLE sera enriched IFN-α, among three other secreted factors, and blocking type I IFN signaling rescued the SLE sera-induced neutrophil death (Li et al., 2021). Informed by prior studies, the authors also examined the role of autoantibodies, which are produced in excess among SLE patients but not covered in their cytokine arrays, in neutrophil viability assays *in vitro*, and found that SLE IgG and IFN-α both contribute to neutrophil death in the patients (Figure 1).

What are the cell death programs involved in neutrophil death? Although NETosis was proposed as a unique form of neutrophil death (Lood et al., 2016), morphological analysis suggested otherwise. Reminded by the resemblance between appearances of dying neutrophils and cancer cells undergoing ferroptosis, the authors characterized the contribution

†Contributed equally to this work
*Corresponding author (email: zouyilong@westlake.edu.cn)

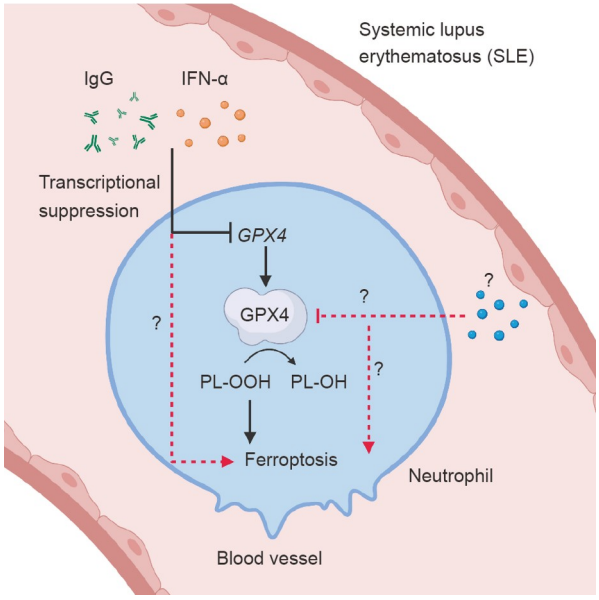

Figure 1 Neutrophil ferroptosis induced by IgG and IFN-α contributes to systemic lupus erythematosus (created with BioRender.com). GPX4, glutathione peroxidase 4; PL, polyunsaturated phospholipid.

of lipid peroxidation and ferroptosis in neutrophil impairment. Ferroptosis is experimentally defined by accumulation of lipophilic reactive oxygen species, iron-dependence, and the rescue of cell death via lipid radical scavengers including liproxstatin-1. Indeed, neutrophil death induced by SLE serum exhibited all three features (Li et al., 2021). Furthermore, neutrophil ferroptosis is specifically triggered by SLE IgG and IFN-α (Figure 1). Notably, ferroptotic features were not observed in other blood cell types including lymphocytes and monocytes, suggesting that ferroptosis induction by SLE serum is unique to neutrophils. Remarkably, using lupus-prone mouse models (MRL/*lpr* and NZB/W F1 mice), the authors demonstrated that liproxstatin-1 was more potent than NETosis inhibitor Cl-amidine in reversing various lupus-associated phenotypes and mitigating disease progression, pointing towards a therapeutic avenue in targeting ferroptosis for SLE treatment.

A key question towards comprehensive understanding of mechanisms underlying neutrophil ferroptosis in SLE is that how autoantibodies and IFN-α trigger aberrant accumulation of lipid peroxidation and subsequent cell death. By comparing the gene expression profiles of neutrophils from healthy donors and SLE patients, the authors found that expression of GPX4, the only cellular enzyme for reducing lipid hydroperoxides to nontoxic lipid alcohols, is strongly reduced in SLE neutrophils (Li et al., 2021) (Figure 1). The reduction of GPX4 expression was further confirmed in lupus-prone mouse models and cultured SLE neutrophils, and decreased expression of GPX4 sensitizes neutrophils to ferroptosis. The authors further demonstrated that the suppression of *GPX4* expression is initially triggered by SLE autoantibodies and IFN-α, and executed by the intracellular calcium/calmodulin-dependent protein kinase IV (CaMKIV)/cAMP responsive element modulator α (CREMα) transcriptional repression pathway. Importantly, neutrophil-specific, heterozygous *Gpx4*-depletion in a $Gpx4^{fl/wt}$ LysMCre^{+} mouse model recapitulated various lupus-like manifestations, supporting the predominant role of neutrophil ferroptosis in SLE disease progression.

Together, Li et al. demonstrated a compelling case in which ferroptotic death of an important immune cell type, neutrophils, contribute significantly to the development of systemic autoimmune disorders, and suggest a novel therapeutic strategy for controlling lupus-related diseases like SLE. There are several knowledge gaps that remain to be fully addressed (Figure 1): (i) how autoantibodies rather than circulating IgG in general selectively downregulate *GPX4* mRNA expression level? (ii) Considering that there are multiple ferroptosis-relevant genes besides *GPX4* that are differentially expressed in SLE patient-derived neutrophils, what are the other mechanisms by which IgG and IFN-α modulate neutrophil ferroptosis? (iii) Are there other circulating and metabolic factors other than autoantibodies and IFN-α in SLE patients that sensitize neutrophils to ferroptosis? (iv) Specifically, how neutrophil ferroptosis promotes SLE autoimmune disease progression?

Moreover, this study inspires a few open questions in the realm of ferroptosis and immunity that warrant future exploration: (i) are there any other immune cell types that are sensitive to ferroptosis under pathological conditions in addition to neutrophils, tumor-infiltrating CD8^{+} T cells (Ma et al., 2021), and follicular helper T cells (Yao et al., 2021)? Do ferroptosis-sensitive immune cell types share any common genetic, epigenetic, and metabolic features? (ii) How do immune cells protect themselves from the propagation of lipid peroxidation when ferroptosis is elicited in pathogens, neoplastic cells, or other immune cells? How is ferroptosis regulated differently when it is induced in the systemic circulation versus in a solid tissue environment? (iii) Given that ferroptosis is also recognized as immunogenic cell death by uncontrolled release of various cellular contents and debris, how will ferroptosis of various cell types modulate the immune homeostasis and responses in an organism? Altogether, unfolding the mechanisms regulating cell death in diverse immune responses promises to be an exciting area of research.

Compliance and ethics *The author(s) declare that they have no conflict of interest. Yilong Zou is a consultant of Keen Therapeutics.*

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