Is ferroptosis immunogenic? The devil is in the details!

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Ferroptosis has emerged as an iron-dependent type of regulated cell death in which excessive membrane peroxidation results into non-apoptotic, non-necroptotic demise. Ferroptosis is implicated in pathologies such as ischemia reperfusion injury, neurodegenerative disorders and cancer. It is molecularly distinct from other forms of regulated cell death including apoptosis, necroptosis and pyroptosis. Mechanistically, ferroptosis is characterized by the excessive oxidation of polyunsaturated fatty acid (PUFA) and the accumulation of cytotoxic lipid peroxides in response to increasing concentrations of free intracellular iron. The pharmacological inhibition of the selenoenzyme glutathione (GSH) peroxidase 4 (GPX4) or the amino acid antipporter system xc−mediated uptake of cystine further upstream in the same pathway can precipitate ferroptosis. Moreover, the concomitant inhibition of the oxidoreductase ferroptosis suppressor protein 1 (FSP1) synergizes with GPX4 inhibition in the induction of ferroptosis in a number of cancer types. The increased expression of acyl-CoA synthetase long-chain family member 4 (ACSL4) in cancerous cells catalyzes the incorporation of oleate and palmitate into phospholipids prone to lethal oxidation, thus sensitizing cells to ferroptosis, making it an interesting target for anti-neoplastic therapy.

In addition to the induction of cell death in cancer cells, ferroptosis can impact on systemic immunity by directly affecting innate and adaptive immune cells such as macrophages and cytotoxic T lymphocyte, respectively. Moreover, it has been suggested that ferroptosis can modulate the immunogenicity of cells through the release of danger associated molecular patterns (DAMPs) which in turn can be perceived by pattern recognition receptors (PRR) expressed on immune cells including dendritic cells (DC), such as advanced glycosylation end-product–specific receptor (AGER), Toll-like receptor 4 (TLR4), and cyclic guanosine monophosphate–adenosine monophosphate synthase (CGAS) all of which have been identified to integrate DAMP signaling emanating from ferroptotic cells. Thus, it has been reported that the proteoglycan decorin (DCN) is released from ferroptotic cells and then acts as an extracellular DAMP on the PRR AGER to trigger the production of immunostimulatory cytokines by macrophages. Moreover, early ferroptosis reportedly stimulates the release of immunogenic cell death (ICD)-associated danger associated molecular patterns (DAMPs) such as ATP and high mobility group box 1 (HMGB1), altogether facilitating T cell mediated adaptive immune responses. ICD can also occur in the context of necroptosis, especially when the lethal phase of the process is preceded by activation of the NF-kB pathway. In stark contrast, Wiernicki et al. recently described ferroptosis as a tolerogenic cell death modality that, despite the emission of ICD-associated DAMPs, decreases the phagocytic potential and maturation of DC and dampens antigen cross-presentation, thus impeding dendritic cell-mediated anti-tumor immunity.

The research on ferroptosis often focuses on the inhibition of the GSH pathway converging on GPX4 and its control by radical-trapping antioxidants. An ever-increasing number of pro-ferroptotic agents are being employed for scientific exploration, each of which burdened with a panoply of side effects that might blur the assessment of biological downstream effects other than the induction of cell death. Moreover, the order and magnitude of cellular stress and death routines probably affects the spatial and temporal appearance of ICD-relevant DAMPs and might also impact the manifestation of immunosuppressive signals such as oxidized phospholipids and cyclooxygenase 2 (COX2), which generates immunoinhibitory prostaglandin E2 (PGE2). At this point, research on the immunological consequences of ferroptosis used a heterogeneous panel of distinct cell types, ferroptosis inducers and inhibitors (Table 1). Thus, further investigation should aim at standardizing the experimental assessment of ferroptosis and its downstream immunological consequences including the potential molecular brakes that modulate the engagement of immune effectors.

Of note, it is possible that the question whether ferroptosis elicits immunostimulatory or immunosuppressive effects is intrinsically erroneous. Indeed, it is a non sequitur to state that apoptosis would be immunologically neutral or suppressive, while necroptosis would be immunogenic. It has become clear that the immunological consequences of apoptosis and necroptosis are not only determined by the cell death modality but also − to a large extent − by the premortem stress responses. Thus, apoptosis is only immunogenic if it is preceded and accompanied by the induction of autophagy (for optimal release of the DAMP ATP), a peculiar form of endoplasmic reticulum stress (for exposure of the DAMP calreticulin at the cell surface), as well as a Type-1 interferon response. Similarly, necroptosis is only immunogenic if it is preceded by the premortem activation of the pro-inflammatory NF-kB pathway. Hence, it may be important to understand which stress pathways have been activated before cells ignite...
the ferroptotic cell death pathway and whether such stress pathways ultimately determine the immunological consequences of ferroptosis.

Acknowledgments

OK receives funding by the DGRS, DGR-ES, CNRS, Fondation pour la Recherche Médicale (FRM); a donation by Elior; European Research Area Network on Cardiovascular Diseases (ERA-CVD, MINOTAUR); Gustave Roussy Odyssea, the European Union Horizon 2020 Project Oncobiome; Fondation Carrefour; INCa; Inserm (HTE); Institut Universitaire de France; LeDucq Foundation; the LabEx Immuno-Oncology (ANR-18-IDEX-0001); the RHU Torino Lumière; the Seereave Foundation; the SIRIC Stratified Oncology Cell DNA Repair and Tumor Immune Elimination (SOCRATE) and the SIRIC Cancer Research and Personalized Medicine (CARPEM).

Data availability statement

All data that led to the conclusions in this manuscript have been referenced and all sources have been described.

Table 1. Preclinical exploration of ferroptosis.

| Compartments/tissue | Experimental model | Immunological outcome | Ref. |
|---------------------|--------------------|-----------------------|------|
| Macrophages         | GPX4 depletion limits the STING1-mediated type I IFN antiviral immune response during HSV-1 infection in mice. | Abrogated immune response of GPX4-depleted macrophages to HSV-1 infection | 18 |
| T cells             | T cell-specific GPX4-deficient mice exhibits defective CD8+ T cell homeostasis. GPX4-deficient T fail to protect from lymphocytic choriomeningitis virus and Leishmania infection (in vivo). Accumulate lipid peroxides and undergo ferroptosis (ex vivo). | Lipid peroxidation induces ferroptosis in T cells and counteracts immunity to infection | 19 |
| T cells             | Genetic ablation of Cd36 in CD8+ T cells exhibit enhanced tumor eradication. Cd36 mediates uptake of fatty acids by tumor-infiltrating CD8+ T cells and induces ferroptosis as well as impaired antitumor effects. Blocking Cd36 or inhibiting ferroptosis with fer-1 restores antitumor activity. | CD36-mediated ferroptosis of CD8+ T cells reduces effector function. | 7 |
| Kidney              | TLR4-dependent NF-κB activation and subsequent production of proinflammatory cytokines and chemokines favors ferroptosis-related inflammation during rhodobomyolysis-associated kidney damage in mice. | Pro-inflammatory cytokines and chemokines are released from ferroptotic kidney cells in response to rhodobomylosis | 20 |
| Heart               | Fer-1 reduces cardiomyocyte cell death and blocks neutrophil recruitment following heart transplantation in mice. | Ferroptotic cell death and TLR4/Trif signaling initiate neutrophil recruitment after heart transplantation. | 21 |
| Cancer cells and MEFs| In vitro study employing erasite and RSL3 in HT1080, PANC1 and MEF cells together with BMDMs | AGER, not TLR4, is responsible for HMGB1-mediated TNF-α production in macrophages responding to RSL3-induced ferroptotic cells. Early ferroptotic cells were immunogenic in vivo, hence protecting mice against rechallenge with live tumor cells of the same kind. In the case of KP cells, this effect is lost upon antibody-mediated neutralization of DCN or AGER. | 8 |
| Cancer cells and tumor | Prophylactic tumor vaccination by subcutaneous injection of RSL-3-induced ferroptotic MCA205 fibrosarcoma cells or KP cells in immunocompetent mice. | Ferroptotic cell death and LB4/Trif signaling initiate neutrophil recruitment after heart transplantation. | 9 |
| Cancer cells and tumor | Induction of immunogenic ferroptosis by intramolecular motion-induced PTI together with RSL3 in 4T1 cancer cells and tumors in BALB/c mice which was inhibited by DFO. | Tumor infiltration with RSL3 and PTI | 22 |
| Cancer cells and tumor | TRAMP-C1 treated in vitro with MF-triggered HCV-mediated Fenton reaction triggered release of ATP and HMGB1 and CALR exposure during ferroptosis-like cell death. TRAMP-C1 implanted in C57BL/6 mice treated with MF upon intratumor injection of HCV showed DC activation in lymph nodes and CTL infiltration into the tumor. | ATP, HMGB1 and CALR emission and anticancer immune response to ferroptosis induction by MF-triggered HCV-mediated fenton reaction in vitro and in vivo. | 23 |
| Cancer cells and tumor | High-iron diets or GPx4 depletion promotes 8-OHG release and thus activates the TMEM173/STING-dependent DNA sensor pathway in macrophages in mice, which results in M2 macrophage infiltration and activation in Kras-driven PDAC. Administration of liproxstatin-1, clodronate-mediated macrophage depletion, or pharmacological and genetic inhibition of the 8-OHG-TMEM173 pathway suppresses Kras-driven pancreatic tumorigenesis in mice. | Inflammatory responses to ferroptotic PDAC cells by immunosuppressive M2 macrophages. | 24 |
| Cancer cells and tumor | AGER facilitates the uptake by macrophages of mutant KRAS120D protein from exosomes, released by H2O2-induced ferroptotic PANc1 cells in an autophagy-dependent manner resulting in M2 macrophage polarization and tumor growth that is inhibited by Fer-1 in vitro and in vivo (when xenografted with human PBMC into NOD SCID mice). | KRAS120D protein from ferroptotic PDAC resulted in inflammation-related M2 macrophage polarization and immunosuppression | 25 |
| Cancer cells         | MCA205 fibrosarcoma cells dying from ferroptosis triggered by ML162, RSL-3, erastin or inducible GPX4 knockdown impede dendritic cell-mediated anti-tumor immunity in vitro and in a prophylactic tumor vaccination model in mice. | Tolerogenic effect of ferroptotic cancer cells | 11 |

Abbreviations: bone marrow derived macrophages BMDM, advanced glycosylation end-product-specific receptor AGER, decorin, DCN, ferrostatin-1, Fer-1, hybrid core-shell vesicles, HCV/S, herpes simplex virus 1, HSV-1, interferon, IFN; mouse embryonic fibroblasts, MEF; magnetic field, MF; peripheral blood mononuclear cells, PBMC; pancreatic ductal adenocarcinoma, PDAC; photo-hyperthermia, PHT; tumor necrosis factor alpha, TNF-α
Disclosure statement

OK is a cofounder of Samsara Therapeutics. GK has been holding research contracts with Daiichi Sankyo, Eleor, Kaleido, Lytx Pharma, PharmaMar, Oasuna Therapeutics, Samsara Therapeutics, Sanofi, Soto, Tollys, Vascage and Vasculox/Tioma. GK has been consulting for Reithera. GK is on the Board of Directors of the Bristol Myers Squibb Foundation France. GK is a scientific co-founder of everImmune, Oasuna Therapeutics, Samsara Therapeutics and Therafast Bio. GK is the inventor of patents covering therapeutic targeting of aging, cancer, cystic fibrosis and metabolic disorders.

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