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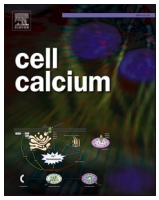

Commentary

PEG out through the pores with the help of ESCRTIII

Alejandro Marmolejo-Garza [a,b], Amalia M. Dolga [a,*]

[a] Faculty of Science and Engineering, Department of Molecular Pharmacology, Groningen Research Institute of Pharmacy (GRIP), University of Groningen, 9713 AV Groningen, The Netherlands

[b] Department of Biomedical Sciences of Cells & Systems, Section Molecular Neurobiology, Faculty of Medical Sciences, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

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ABSTRACT

Ferroptosis is a form of programmed cell death with particular hallmarks, such as oxidative stress, increased calcium fluxes, and altered cellular morphology. In ferroptosis, the disruption of plasma membrane is the step that culminates into cell death. By inducing ferroptosis with Erastin-1 and RSL3 in various human cellular models, Pedrera et al. tracked the behaviour of several hallmarks of ferroptosis and demonstrated that lipid peroxidation precedes cytosolic calcium rise and plasma membrane breakdown, which is dependent on nanopore formation. Ferroptotic cell death is inhibited by osmotically active protectants of proper size that can prevent water flux through nanopores.

1. Main

Regulated necrosis involves different cell death pathways such as necroptosis, pyroptosis, or ferroptosis, all of which have been detected in age-related diseases. The term ferroptosis was coined in 2012 as a novel type of cell death [1] characterized by iron accumulation and lipid peroxidation during the cell death process. Ferroptosis differs from classical forms of cell death in features regarding morphology, biochemistry, pharmacological and genetic regulators/inducers. Ferroptosis can be initiated upon: (a) pharmacological inhibition of the system Xc- with erastin and glutamate, thereby reducing cellular levels of glutathion synthase (GSH); (b) Ras-selective lethal small molecule 3 (RSL3), which directly inhibits glutathione peroxidase 4 (GPX4). The ferroptotic cascade culminates in membrane damage and permeabilization. Previous studies have suggested that ferroptosis may have the ability to have paracrine effects and to cause necroinflammation due to an increased cell membrane permeability [2].

In 2020, the group of Dr. García-Sáez profiled the events that lead to the permeabilization of the plasma membrane during ferroptosis [3]. The authors employed a clever approach using live-cell imaging and flow cytometry experiments to measure cytosolic Ca^{2+}, cell rounding, PI and lipid peroxidation in NIH-3T3, HT-1080 and Mda-157 cells. They concluded that in ferroptosis, lipid peroxidation precedes the increase in cytosolic Ca^{2+} and plasma membrane permeabilization by comparing the lag times between each process. Ca^{2+} uptake experiments demonstrated that the kinetic profile of erastin followed a biphasic behavior, while RSL3 elicited a monophasic kinetic similar to the second wave induced by erastin.

In ferroptosis, membrane pore formation leads to cell collapse due to water influx by pore formation. This event could be counteracted by osmoprotectants, such as polyethylenglycols (PEGs) of the appropriate size. Authors demonstrated that addition of such PEGs prevented the increases of cytosolic Ca^{2+} and cell rounding, but it did not prevent lipid peroxidation. Moreover, removal of PEGs resulted in cell death, indicating that this osmotic protection was only transient. Osmoprotectants prevent the entry of water molecules during the final steps of ferroptosis [4]. PI incorporation and Draq7 experiments are somewhat discrepant as both readouts (DNA stain) for the effect of PEG are different at 7 h. Although authors mention that cell death cannot be averted at 24 h following Erastin treatment, a 24 h PI uptake kinetic measurement with PEG800 would further elucidate PEGs role in ferroptotic cell death.

The fact that the cell death secondary to membrane permeabilization (namely, pore number and size) is only delayed by PEGs, makes us think that there could be another cellular mechanism that drives this membrane damage, not only pore formation. As PEGs were not able to decrease lipid peroxidation, perhaps the inhibition of lipid peroxidation could further enhance the protective capacity of osmoprotectants by limiting membrane damage. In a previous study [5], we demonstrated

* Corresponding author at: Faculty of Science and Engineering, Groningen Research Institute of Pharmacy, Department of Molecular Pharmacology, University of Groningen, Antonius Deusinglaan 1, Groningen, The Netherlands.
E-mail address: a.m.dolga@rug.nl (A.M. Dolga).

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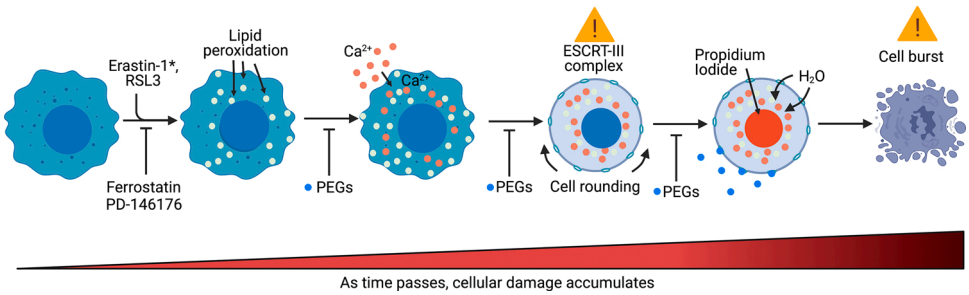

Fig. 1. Ferroptotic cell death follows a timeline of hallmarks. First, lipid peroxidation, then calcium increases, ferroptotic pore formation, cell reounding and cell death. Polyethinelglycols (PEGs) of the adequate size are able to prevent cell death and calcium increases but fail to prevent lipid peroxidation.

that erastin-induced ferroptosis in neuronal cells was accompanied by BID transactivation to mitochondria, loss of mitochondrial membrane potential, mitochondrial fragmentation and reduced ATP levels. All these paramenters were prevented by ferrostatin-1 and indicate the involvement of mitochondrial functions to ferroptotic cell death. It would be of interest to investigate the timeline of mitochondrial dysfunction, lipid peroxidation, and pore formation in ferroptosis.

Finally, Pedrera and colleagues demonstrated that the endosomal sorting complexes required for transport (ESCRT-III) machinery is protective against ferroptosis and may affect inflammation. Previous research reported that ESCRT-III is required to limit inflammation related to NF-kB signaling [6], suggesting that ESCRT-III machinery contributes to inflammatory processes in ferroptosis.

What is the point of no return in the ferroptotic cascade? A new piece of evidence is added to the ferroptotic death pathway: ESCRT-III complex formation is shown to be implicated in ferroptosis (Fig. 1). Previous scientific discussions on ESCRT-III implications in ferroptosis [7,8] can find a follow-up with this research. Further experiments that evaluate the assembly of the ESCRT-III complex in ferroptosis will be valuable to delineate key players in this process. Whether the secretion of cytokines in ferroptosis occurs during early or late stages of ferroptosis is not clear yet. Stimulation of RAW 264.7 macrohpages with supernatant of RSL3-treated NIH-3T3 cells with and without downregulation of CHMP4B suggests that there is a pro-inflammatory phenotype dependent on membrane damage (late stages).

In the field of ferroptosis, the use of prototypic synthetic drugs such as erastin or RLS3 is a potential limitation of the ferroptotic model. Two parallel approaches can be considered to strengthen the findings by Pedrera et al.: 1) the use of a physiological compound such as glutamate, to inhibit the system Xc-, inducing ferroptosis in an adequate cell model, such as cell types lacking NMDA/AMPA receptors [9,10], 2) characterizing ferroptosis *in vivo* demonstrating that the pro-inflammatory and propagatory phenotype in tissue parenchyma can be prevented by activating the ESCRT-III complex in models of chronic disease.

Overall, the study of ferroptotic pores and ESCRT-III complex brings more knowledge to the field and will certainly stimulate further research!

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