Cell death

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The many paths ascending to ferroptosis

Marcus Conrad & Adam Wahida

In 2012, a study revealed a unique form of cell death, termed ferroptosis, that is dependent on iron and unregulated lipid peroxidation. We revisit how this paper changed the trajectory of ferroptosis research from its origins to its current state.

For many years, cell death was defined as apoptosis mediated by caspase activation. However, additional forms of cell death with cellular characteristics divergent from those of apoptosis were later identified and termed non-apoptotic cell death. The existence of these initial modes, such as necroptosis and pyroptosis, raised the possibility of other modes of non-apoptotic cell death that had yet to be characterized. In 2012, a decisive shift occurred when Brent Stockwell's group introduced the term 'ferroptosis' in a seminal study linking iron-catalyzed lipid peroxidation to cell death[1] (Fig. 1).

Hints of ferroptosis had been present even before then. Cysteine is the essential building block for the biosynthesis of glutathione (GSH), the primary antioxidant in mammalian cells. Studies in the 1970s found that cysteine deprivation causes cell death in cultured cells through GSH depletion and increased reactive oxygen species (ROS) generation and can be mitigated with lipophilic antioxidants such as vitamin E.

Around the turn of the millennium, two studies — one in hematology and the other in oncology — linked cystine addiction (cystine dependence) in cell lines derived from Burkitt lymphoma to the discovery of glutathione peroxidase 4 (GPX4, formerly called PHGPx) and a 'putative peroxiredoxin-type NADH oxidase', now known as ferroptosis suppressor protein-1 (FSP1)[2] (Fig. 1). Parallel efforts to identify targets in *KRAS*-mutant cell lines from solid tumors uncovered compounds capable of triggering what would later be coined ferroptosis. Notably, molecule names such as erastin or RSL3 are portmanteaus or abbreviations for 'eradicator of RAS and ST-expressing cells' and 'RAS-selective lethal-3', respectively. Yet the precise mechanisms behind their actions remained unknown[3–5] (Fig. 1).

Upon determining that erastin acts by targeting the system x_c^- antiporter and triggers an iron-dependent form of cell death, Stockwell's team introduced the term 'ferroptosis' as a distinct addition to the family of cell death paradigms, drawing from the Latin ferrum (iron)[1]. The team successfully integrated diverse research strands into a cohesive tapestry of findings encompassing biochemistry, developmental genetics, cancer biology, medicinal chemistry and, particularly, redox biology. Additionally, they introduced a novel clade of radical-trapping antioxidants (RTAs) called ferrostatins, which mitigate peroxidized lipid species, thus solidifying the link between lipid peroxidation and iron as central factors in ferroptosis. Stockwell's expertise in modulating cell fate decisions is now seen as providing a unique approach to uncovering fundamental biological mechanisms while providing a

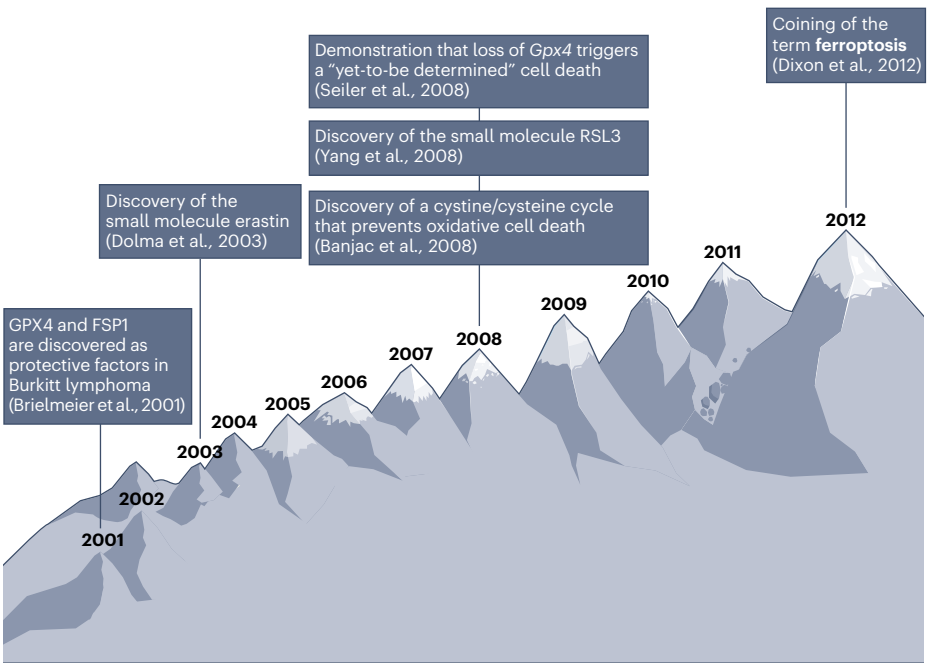

Fig. 1 | The many paths ascending to ferroptosis. Multiple significant milestones[2,4,5,7,14] led Stockwell and colleagues[1] to conceptualize 'ferroptosis' as a unique form of cell death in 2012.

critical vantage point for translational drug discovery. For instance, Stockwell's work using brain tissue slices confirmed the high susceptibility of neurons to ferroptosis. This observation has increased interest in targeting ferroptosis in neurodegenerative diseases as a means to protect neurons from degeneration. The paper, however, left an open question: what is the target of RSL3? Unlike erastin, RSL3 does not inhibit cystine uptake, yet it still triggers an iron- and ROS-dependent ferroptotic death pathway. The authors notably flagged this uncertainty with a question mark in the paper's final panel, resolving it two years later in a significant follow-up study that identified GPX4 as the target of RSL3 – and thus as the guardian of ferroptosis[6].

Of note, earlier studies had identified a novel form of non-apoptotic cell death in mice linked to the genetic deletion of *Gpx4*. At the time, this was merely described as a "yet-unrecognized cell-death pathway"[7] (Fig. 1). Subsequent work established that inducible global *Gpx4* deletion is also a primary driver of renal ischemia–reperfusion injury in adult mice, mimicking acute kidney injury in transplant patients. This work also led to the discovery of the first in vivo active ferroptosis inhibitor, liproxstatin-1[8].

The following decade yielded an avalanche of insights into the mechanistic regulation of ferroptosis and its implication in disease. Unlike other forms of cell death, ferroptosis lacks a specific activating trigger. Instead, several cellular surveillance systems continuously work to restrain it. Genetic screens identified FSP1 as a second central regulator of ferroptosis[9,10], alongside activators such as acyl-CoA synthetase long-chain family member 4 (ACSL4)[11,12]. These discoveries were complemented by increasingly granular insights into the biology of ferroptosis, such as the role of selenium in the form of selenocysteine in the active site of GPX4, the involvement of various organelles in initiating ferroptosis, the propagation of ferroptotic signals across cells and the significance of specific lipid components in the plasma membrane for a cell's susceptibility to ferroptotic death. Ferroptosis has been repeatedly implicated in human diseases, including cancer, particularly in relation to epithelial–mesenchymal transition (EMT) and drug resistance, underscoring its potential as a pharmacological target and inspiring novel therapeutic strategies actively being explored.

Nonetheless, several crucial questions remain unanswered, motivating the next generation of ferroptosis researchers to strive for significant advancements. Fundamentally, we still do not fully understand the role of lipid peroxidation in normal physiology, if such a role exists. If ferroptosis is a form of uncontrolled lipid peroxidation, what physiological functions might lipid peroxides serve in an organism? Moreover, the physiological context of ferroptosis is still unclear, underscoring the urgent need for models to explore these cues – especially as efforts intensify to leverage ferroptotic cell death as a cancer treatment strategy. A notable practical challenge in ferroptosis research is the persistent difficulty of reliably detecting ferroptosis through a robust biomarker. Identifying a definitive 'proof of death' biomarker would enable more clinically relevant studies, allowing researchers to link ferroptosis to human disease. Such a breakthrough would also expedite clinical trials for drugs targeting the ferroptosis pathway, whether to inhibit ferroptosis in degenerative diseases or trigger it in cancer. Additionally, it would provide a more precise way to evaluate therapeutic success in clinical trials.

Finally, we still have few effective therapeutic strategies for modulating ferroptosis. Beyond establishing suitable therapeutic windows and clinical outcomes, there is an urgent need to identify specific targets that can modulate ferroptosis without putting patients at undue risk, as could be expected with the indiscriminate targeting of GPX4, which may pose grave side effects. Today, ferroptosis is increasingly being explored from a translational perspective, with growing insight into its implications for human disease. This shift underscores the potential of ferroptosis research to deepen our understanding of cell death mechanisms and advance the diagnosis and treatment of various conditions[13].

As with any scientific breakthrough, the initial discovery may not immediately change practice but expands our understanding of the world. Stockwell and colleagues' pivotal paper[1] galvanized numerous research avenues under a unifying theme: the reconciliation, synchronization and development of new scientific and clinical concepts centered on ferroptosis.

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Competing interests

M.C. is a co-founder and shareholder of ROSCUE Therapeutics GmbH and holds patents for some of the compounds described herein.