

Back to the roots of regulated necrosis

Vladimir Todorov and Andreas Linkermann

Division of Nephrology, Department of Internal Medicine III, University Hospital Carl Gustav Carus at the Technische Universität Dresden, 01307 Dresden, Germany

In recent years, our knowledge of how cells die by regulated pathways of necrosis has increased tremendously. In this issue, Distéfano et al. (2017. *J. Cell Biol.* <https://doi.org/10.1083/jcb.201605110>) provide yet another milestone in our understanding of regulated necrosis as they identify a ferroptosis-like cell death in *Arabidopsis thaliana*.

A decade ago, apoptosis was assumed to be the exclusive mode of cellular demise that followed genetically determined signaling pathways. Today, however, we understand that in most pathophysiological circumstances, necrosis also represents a regulated process of cell death that can follow distinct signaling pathways that define necroptosis, pyroptosis, or ferroptosis (Vanden Berghe et al., 2014). In contrast to apoptosis, in which the contents of dying cells remain sequestered within apoptotic bodies, necrosis is characterized by the rupture of the plasma membrane and the release of damage-associated molecular patterns that may elicit an immune response. Different effector molecules execute the endpoint of these pathways of regulated necrosis; for example, membrane rupture during necroptosis and pyroptosis may be mediated by pore formation driven by the mixed lineage kinase domain-like protein (Sun et al., 2012) and gasdermin D (Ding et al., 2016), respectively. In contrast, ferroptosis results from the iron-dependent generation of toxic lipid reactive oxygen species (ROS) in the plasma membrane by lipoxygenases when glutathione peroxidase activity is diminished (Dixon et al., 2012; Yang et al., 2014). Whether or not ferroptotic cell death involves formation of a specific protein membrane pore is unknown. Therefore, these three pathways of regulated necrosis are clearly distinct but how their upstream signaling pathways may be intercalated and how they differ in their effects on the immune system are currently matters of debate (Vanden Berghe et al., 2014).

The reason for the conservation of several regulated pathways of necrosis is unclear and, potentially, other unknown pathways may exist. As a means to protect the organism in general, necrosis may appear counterintuitive at first glance given that the dying cells violently burst. However, as with many cellular processes that arise during evolution, host microbe interactions may be key to our understanding. Indeed, the most likely reason for the evolutionary conservation of pathways of regulated necrosis is for defense against microbes as it is very clear that necroptosis functions to fight viruses (Kaiser et al., 2013) and pyroptosis defends against bacteria (Lamkanfi and Dixit,

2010). However, why and how we have conserved the ferroptosis pathway currently remains elusive as a function to defend against microbes has not yet been convincingly demonstrated. Instead, ferroptosis can be induced artificially as a potent driver of tumor cell death (Dixon et al., 2012) and by ischemic injury during kidney damage or transplantation (Linkermann et al., 2014). But why would eukaryotes preserve such a dangerous program for cell death in their genome? In this issue, Distéfano et al. add important insights to our understanding of this question by identifying ferroptosis-like death by plant cells in response to a more physiological stimulus: heat stress.

Distéfano et al. (2017) used models of plant stress and cell death in *Arabidopsis thaliana* to ask whether a process similar to ferroptosis might occur because plant cell death processes can be necrotic and driven by ROS (Huysmans et al., 2017). Whereas plant cell death during root development or reproduction was independent of any obvious ferroptosis-like features, heat shock-induced regulated cell death (HS-RCD) exhibited striking similarities to ferroptosis. When 6-d-old seedlings were pretreated with ferrostatins (Fer-1), small molecules that inhibit ferroptosis by blocking lipid ROS (Dixon et al., 2012), the plant cell death in root hairs that normally occurs when they are subjected to a temperature of 55°C was completely prevented. In contrast, ferrostatins had no effect on the induction of cell death at 77°C, or by H₂O₂ or high salt treatment, which are thought to initiate nonregulated forms of oxidative cellular necrosis. HS-RCD at 55°C could also be inhibited by treatment of plant cells with the iron chelator ciclopiroxolamine or polyunsaturated fatty acids resistant to oxidation, just like ferroptosis in mammalian cells. Closer examination of the morphology of root cortical cells by transmission electron microscopy revealed that the cytoplasm of plant cells dying by HS-RCD at 55°C had retracted from the cell wall and accumulated lytic vacuoles, and the mitochondria displayed a more condensed matrix, which resembled changes in the mitochondria of mammalian tumor cells undergoing ferroptosis (Dixon et al., 2012). Distéfano et al. (2017) observed that HS-RCD occurred in the parts of the root that are known to contain more iron and correlated with the disappearance of the antioxidant reduced glutathione (GSH) and the appearance of lipid and cytoplasmic ROS. In line with a critical role for GSH in preventing ferroptosis, inhibition of GSH biosynthesis accelerated HS-RCD, whereas the exogenous application of GSH to plants blocked HS-RCD as effectively as the ferrostatins. Given the striking overlap in these requirements for iron and ROS to trigger HS-RCD in plant cells with mammalian ferroptosis, the authors termed this form of

Correspondence to Andreas Linkermann: andreas.linkermann@uniklinikum-dresden.de

© 2017 Todorov and Linkermann This article is distributed under the terms of an Attribution-Noncommercial-Share Alike-No Mirror Sites license for the first six months after the publication date (see <http://www.rupress.org/terms/>). After six months it is available under a Creative Commons License (Attribution-Noncommercial-Share Alike 4.0 International license, as described at <https://creativecommons.org/licenses/by-nc-sa/4.0/>).



cell death “ferroptosis-like,” thereby describing a novel entity of regulated necrosis in plants.

Our knowledge of the signaling pathways and effector molecules that control regulated cell death in plants is quite limited but Distéfano et al. (2017) provide some preliminary insights into what might regulate plant ferroptosis. Calcium is an important regulator of plant cell death in many circumstances (Huysmans et al., 2017) and the authors observed much lower sensitivity to HS-RCD in root hairs treated with the calcium chelator EGTA. This finding is of particular interest because the ability to maintain calcium homeostasis has been reported to affect heat-induced necrotic cell death in *Caenorhabditis elegans* and other types of necrosis in diverse species (Kourtis et al., 2012). The mechanisms by which calcium controls this ferroptosis-like HS-RCD are elusive but interesting to investigate, particularly because ferroptosis induced in mammalian cells by the pharmacological agent erastin cannot be blocked by calcium chelators (Dixon et al., 2012). Of interest, heat shock proteins have been reported to regulate ferroptosis in mammalian cells (Sun et al., 2015). There are likely other key differences in the cellular machinery that mediates ferroptosis-like cell death in plants versus mammalian cells. Unlike ferroptosis in mammals, plant enzymes that resemble apoptotic caspases appear to be required for HS-RCD, as Distéfano et al. (2017) observe that HS-RCD is blocked by a caspase inhibitor. It will be interesting to find out what substrates these enzymes might be targeting, if they affect iron-dependent lipid ROS generation, and how they lead to cellular demise. Specific transcriptional programs can control plant cell death, particularly during development and reproduction (Huysmans et al., 2017). Distéfano et al. (2017) examined whether higher temperatures triggered changes in the expression of *Arabidopsis* homologues of the genes proposed to be markers of mammalian ferroptosis such as the asparagine synthetases, mitochondrial voltage-dependent channels, and regulators of cation transport. The authors found that up-regulation of the kiss of death (*KOD*) gene, which was already known to be induced by heat stress in plants, was abrogated by treatment with Fer-1, which suggests that this may be a mediator of ferroptosis-like cell death. Future work will be required to identify the other key components of the plant cellular machinery that controls ferroptosis and how they function together with KOD. Intriguingly, although the majority of the experiments were done in nonphotosynthetic cells lacking chloroplasts, Distéfano et al. (2017) found that exposure to light and chloroplasts may be important in triggering HS-RCD in the parts of the plant exposed above the soil. Therefore, many more details of the underlying cell biological processes that control ferroptosis in plants are waiting to be dug up.

In conclusion, the identification of a ferroptosis-like cell death process in plants suggests the possibility that this is a form of regulated necrosis with ancient evolutionary origins. Unlike necroptosis and pyroptosis, a protective role for ferroptosis has yet to be identified; therefore, the evolutionary pressure that has led to the conservation of this pathway is unclear. Ferroptosis has been demonstrated to be of paramount importance when directly compared with necroptosis and pyroptosis as a major

driver of organ damage in response to ischemia/reperfusion during stroke or kidney transplantation in mammals (Linkermann et al., 2014). Similarly, ferroptosis-like cell death seems to be occurring as a regulated form of iron-dependent necrosis in response to stress that can result in plant pathology and damages plant viability. In their last set of experiments, Distéfano et al. (2017) demonstrate that blocking ferroptosis in plants increases their thermo-resistance and they can survive being exposed to higher temperatures. Although a beneficial function to host cells of maintaining the pathway for ferroptotic cell death during evolution has yet to be identified, it appears obvious that we may use the ability to block ferroptosis in plant and mammalian cells to our advantage.

Acknowledgments

The authors declare no competing financial interests.

References

- Ding, J., K. Wang, W. Liu, Y. She, Q. Sun, J. Shi, H. Sun, D.C. Wang, and F. Shao. 2016. Pore-forming activity and structural autoinhibition of the gasdermin family. *Nature*. 535:111–116. <http://dx.doi.org/10.1038/nature18590>
- Distéfano, A.M., M.V. Martin, J.P. Córdoba, A.M. Bellido, S. D'Ippólito, S.L. Colman, D. Soto, J.A. Roldan, C.G. Bartoli, E.J. Zabaleta, et al. 2017. Heat stress induces ferroptosis-like cell death in plants. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201605110>
- Dixon, S.J., K.M. Lemberg, M.R. Lamprecht, R. Skouta, E.M. Zaitsev, C.E. Gleason, D.N. Patel, A.J. Bauer, A.M. Cantley, W.S. Yang, et al. 2012. Ferroptosis: An iron-dependent form of nonapoptotic cell death. *Cell*. 149:1060–1072. <http://dx.doi.org/10.1016/j.cell.2012.03.042>
- Huysmans, M., S. Lema A, N.S. Coll, and M.K. Nowack. 2017. Dying two deaths — programmed cell death regulation in development and disease. *Curr. Opin. Plant Biol.* 35:37–44. <http://dx.doi.org/10.1016/j.pbi.2016.11.005>
- Kaiser, W.J., J.W. Upton, and E.S. Mocarski. 2013. Viral modulation of programmed necrosis. *Curr. Opin. Virol.* 3:296–306. <http://dx.doi.org/10.1016/j.coviro.2013.05.019>
- Kourtis, N., V. Nikolettou, and N. Tavernarakis. 2012. Small heat-shock proteins protect from heat-stroke-associated neurodegeneration. *Nature*. 490:213–218. <http://dx.doi.org/10.1038/nature11417>
- Lamkanfi, M., and V.M. Dixit. 2010. Manipulation of host cell death pathways during microbial infections. *Cell Host Microbe*. 8:44–54. <http://dx.doi.org/10.1016/j.chom.2010.06.007>
- Linkermann, A., R. Skouta, N. Himmerkus, S.R. Mulay, C. Dewitz, F. De Zen, A. Prokai, G. Zuchtriegel, F. Krombach, P.S. Welz, et al. 2014. Synchronized renal tubular cell death involves ferroptosis. *Proc. Natl. Acad. Sci. USA*. 111:16836–16841. <http://dx.doi.org/10.1073/pnas.1415518111>
- Sun, L., H. Wang, Z. Wang, S. He, S. Chen, D. Liao, L. Wang, J. Yan, W. Liu, X. Lei, and X. Wang. 2012. Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. *Cell*. 148:213–227. <http://dx.doi.org/10.1016/j.cell.2011.11.031>
- Sun, X., Z. Ou, M. Xie, R. Kang, Y. Fan, X. Niu, H. Wang, L. Cao, and D. Tang. 2015. HSPB1 as a novel regulator of ferroptotic cancer cell death. *Oncogene*. 34:5617–5625. <http://dx.doi.org/10.1038/ncr.2015.32>
- Vanden Berghe, T., A. Linkermann, S. Jouan-Lanhouet, H. Walczak, and P. Vandenabeele. 2014. Regulated necrosis: The expanding network of non-apoptotic cell death pathways. *Nat. Rev. Mol. Cell Biol.* 15:135–147. <http://dx.doi.org/10.1038/nrm3737>
- Yang, W.S., R. SriRamaratnam, M.E. Welsch, K. Shimada, R. Skouta, V.S. Viswanathan, J.H. Cheah, P.A. Clemmons, A.F. Shamji, C.B. Clish, et al. 2014. Regulation of ferroptotic cancer cell death by GPX4. *Cell*. 156:317–331. <http://dx.doi.org/10.1016/j.cell.2013.12.010>