JCB Journal of Cell Biology

SPOTLIGHT

Killing cells using light (activated) sabers

Stephen W.G. Tait[1,2]

Many types of regulated cell death exist, however the non-cell autonomous effects of specific forms of cell death remain poorly understood. Addressing this, Shkarina et al. (2022. *J. Cell Biol.* https://doi.org/10.1083/jcb.202109038) describe an optogenetic method to activate distinct modes of cell death in select cells.

In broad terms, cells die in our bodies through passive (necrotic) or regulated means. Varied forms of regulated cell death (RCD) exist including apoptosis, necroptosis, and pyroptosis. Why are there so many ways for a cell to die? One reason is that different types of cell death dramatically impact the immune response. For instance, apoptosis is typically considered immunosilent—given the billions of cells that die through apoptosis daily, this makes intuitive sense. In contrast, pyroptosis is an inflammatory type of cell death that can be triggered by pathogen infection, thus alerting the host to the presence of danger. Nonetheless, how different forms of cell death impact the microenvironment is largely unknown. Tackling this, Shkarina et al. (1) develop a new approach to induce different types of RCD in specific cells following exposure to light. This optogenetic cell death toolbox opens many research avenues, not least in understanding and contrasting how different forms of cell death exert non-autonomous effects.

A unifying mechanism theme in RCD is the requirement for dimerization or oligomerisation of proteins to initiate and execute cell death. For instance, dimerization of caspase-8, -9, or inflammatory caspases (-1, -4, and -5 in humans) is sufficient to drive extrinsic, intrinsic apoptosis, and pyroptosis respectively. In necroptosis, dimerization of RIPK3 initiates cell death whereas oligomerisation of the effector protein MLKL executes necroptosis. Shkarina and colleagues (1) exploited this knowledge to develop an optogenetic method to trigger distinct forms of RCD. Specifically, they fused RCD effector and initiator proteins to Cry2olig, a photosensitive protein modified from the plant *Arabidopsis thaliana* (2). Crucially, Cry2olig undergoes oligomerization upon exposure to blue light. Therefore, cells expressing what the authors collectively term optogenetically controlled cell death effectors (optoCDEs) undergo specific forms of cell death in response to light. They validate this method extensively in human and mouse cells in vitro as well as zebrafish in vivo.

A major benefit of the optoCDE method is its reversibility; in the absence of light, Cry2olig reverts to its monomeric state. This permits sub-lethal activation of opto-caspases RIPK3 and MLKL by transiently pulsing light, providing new opportunities to understand how cells tolerate sub-lethal stress. Sub-lethal caspase activity has numerous purported cellular roles; therefore, opto-caspases may also be exploited to investigate these non-lethal functions. Interestingly, the authors find extensive variation in apoptotic sensitivity between cell lines following activation of optogenetic caspase-8 and caspase-9. Understanding why cells live or die in response to a similar apoptotic cue is of extensive interest, not least because it may provide new ways to revert cancer cell evasion of apoptosis inducing therapy.

Importantly, optoCDEs allow investigation of how dying cells interact with neighboring viable cells through specific induction of cell death in select cells. For instance, the authors use optoCDEs to investigate the fate of dying cells within a confluent epithelial monolayer by engaging cell death within defined cells and following their fate. Interestingly, dependent on cell type, apoptotic cells can either be extruded from the monolayer or phagocytosed by their neighbors (a process called efferocytosis). Exactly what underlies these cell type–specific responses requires further investigation. Optogenetic apoptosis induction in the context of an intact epithelial layer, but not in isolated cells, led to apoptotic cell breakdown generating cell fragments called apoptotic bodies—this suggests that neighboring viable cells may actively participate in promoting the apoptotic program of dying cells. Intriguingly, this finding shares analogy to previous observations in the nematode *Caenorhabditis elegans*, where phagocytic uptake of apoptotic cells promotes their demise (3). Investigating signaling mechanisms required for efferocytosis or extrusion of apoptotic cells, the authors identified a key role for sphingosine-1-phosphate, a sphingolipid, that is released by apoptotic cells. Extending these analyses, optoCDEs will greatly facilitate our understanding of how different types of RCD impact neighboring cells, perhaps most importantly permitting analysis in vivo. For instance, apoptotic stress in vitro has recently been shown to cause release of pro-survival FGF2 and EGF, facilitating survival

[1]Cancer Research UK Beatson Institute, Glasgow, UK; [2]Institute of Cancer Science, University of Glasgow, Glasgow, UK.

Correspondence to Stephen W.G. Tait: stephen.tait@glasgow.ac.uk.

of surrounding cells—whether such processes occur in vivo remains an open question, but something optoCDEs are well positioned to address (4, 5).

What are the potential advantages of optoCDEs compared to other methods of cell death induction? Firstly, optoCDEs offer a way to "cleanly" induce (and therefore better investigate) select types of RCD, by avoiding ancillary (off-target) effects often seen with cell death–inducing treatments such as staurosporine. Various methods to optogenetically induce RCD have previously been reported, e.g., light induced re-localization of pro-apoptotic BAX to the mitochondrial outer membrane (6). One major advantage of optoCDEs relative to these approaches, is that different cell death modalities can be directly compared under similar experimental conditions. A potential limitation of optoCDEs may relate to their direct induction of cell death effector activity in the absence of the biologically relevant initiating event. For instance, caspase-9 is normally activated by mitochondrial outer membrane permeabilization, a process that has additional biological effects alongside caspase-9 activation—therefore direct activation of caspase-9 fails to fully capture its endogenous activation processes. Nonetheless, these are aspects that can be controlled for and, as the authors highlighted, light-induced oligomerization may be applicable to other components of cell death pathways. As with most things in life, there is usually a Star Wars analogy; referring to lightsabers, Obi-Wan Kenobi once said, "This is the weapon of a Jedi knight. Not as clumsy or random as a blaster; an elegant weapon for a more civilized age." If cell death researchers can be considered Jedi knights (debatable), optoCDEs represent our light (activated) sabers, whilst staurosporine and its ilk are blasters. As Shkarina and colleagues (1) demonstrate, optoCDEs serve as a powerful new method, not least to understand sub-lethal signaling but also to investigate non-cell autonomous effects of different types of cell death.

Acknowledgments

The author declares no competing financial interests.

References

1. Shkarina, K., et al. 2022. *J. Cell Biol.* https://doi.org/10.1083/jcb.202109038
2. Taslimi, A., et al. 2014. *Nat. Commun.* https://doi.org/10.1038/ncomms5925
3. Reddien, P.W., et al. 2001. *Nature.* https://doi.org/10.1038/35084096
4. Bock, F.J., et al. 2021. *Nat. Commun.* https://doi.org/10.1038/s41467-021-26613-0
5. Gagliardi, P.A., et al. 2021. *Dev. Cell.* https://doi.org/10.1016/j.devcel.2021.05.007
6. Hughes, R.M., et al. 2015. *Angew. Chem. Int. Ed. Engl.* https://doi.org/10.1002/anie.201506346