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SPOTLIGHT

Cell death leaves a new TRAIL

Michael Overholtzer

Cell death involves numerous mechanisms that can be cross-regulated through a complex signaling network. In this issue, Bozkurt et al. (2021. *J. Cell Biol.* https://doi.org/10.1083/jcb.202010030) identify a new connection in the network: signaling from TRAIL, a canonical inducer of apoptosis, can also induce a form of cell death called entosis, which has implications for cancer progression.

"I will not follow where the path may lead, but I will go where there is no path, and I will leave a trail" (1).

Cell death, it turns out, is complicated. While it was once thought there was a single mechanism, one path that could lead to the death of metazoan cells, now up to 12 or more different paths are known. Regulated forms of necrosis (e.g., necroptosis, pyroptosis, and ferroptosis), a type of cell ingestion called entosis, and at least seven other mechanisms can, like the classical apoptosis, eliminate damaged or infected cells or remove supernumerary cells during development (2).

Although some forms of cell death have unique properties that make them well suited for specific contexts, many forms appear to occur more broadly. Cell death responses to stress or infection, for example, can involve the parallel induction of numerus forms of cell death—apoptosis, forms of necrosis, and entosis—each occurring at different frequencies within a cell population (3, 4, 5). While crosstalk between some mechanisms (e.g., apoptosis, necroptosis, and pyroptosis) has been extensively studied (2), little is known about how other forms of cell death, such as entosis, are regulated within mixtures. How mixed death profiles might impact physiology is also not well understood. In this issue, Bozkurt et al. uncover an unexpected path that may reveal some important new clues: signaling from the TNF-related apoptosis-inducing ligand (TRAIL), a well-known inducer of apoptosis, can initiate entosis as well (Fig. 1; 6).

To examine the spatiotemporal dynamics of cell death induced by TRAIL, the authors used colon cancer cells expressing a fluorescence resonance energy transfer–based reporter of caspase activity, a hallmark of apoptosis, as well as tetramethylrhodamine methyl ester (TMRM) staining to indicate mitochondrial membrane potential, and examined cells by time-lapse microscopy. Some cells underwent apoptosis, as indicated by the induction of caspase activity and loss of mitochondrial membrane potential, but others showed different patterns, with slower kinetics of caspase activation or in some cases increased TMRM staining, an unusual observation that prompted further examination. By carefully inspecting cell morphology, the authors observed engulfment events involving whole cells within the TRAIL-treated cultures that were reminiscent of entosis, a death mechanism that results from the ingestion of live cells by their neighbors. Indeed, through further examination of the localization of cell adhesion proteins and functional requirement for the cytoskeletal regulator Rho-kinase, which mediates entotic cell ingestion, the authors showed that entosis is induced by TRAIL treatment.

The induction of entosis by a canonical apoptosis-inducing ligand was surprising and presented an opportunity for the authors to identify new regulators of this unusual mechanism. By using knockout and inhibitor-based strategies, they showed that the death receptors to which TRAIL binds, called death domain–containing receptors 4 and 5 (DR4 and DR5), were required for entosis induction, and that, intriguingly, the presence of caspase-8, but not its activity, was required for entosis as well. They further demonstrated that while ingested cells underwent what is called entotic cell death, characterized by the recruitment of lysosomes and acidification of the large endocytic compartment (an activity that also accounted for the increased TMRM staining they initially observed), apoptotic factors were involved in the execution of cell death in this context. The knockout of *BAX* and *BAK*, whose protein products function at mitochondria to control apoptosis, as well as the inhibition of caspase activity, reduced the percentage of entotic cells that died and increased the percentage that escaped from their hosts and were rescued.

To begin to investigate how the regulation of entosis by TRAIL might relate to pathophysiology, the authors examined colorectal cancer specimens, where entotic cell structures could be identified by histology and the expression levels of components of the TRAIL signaling pathway could also be quantified (6). Notably, correlations were identified between entotic structures

Cell Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY.

Correspondence to Michael Overholtzer: overhom1@mskcc.org.

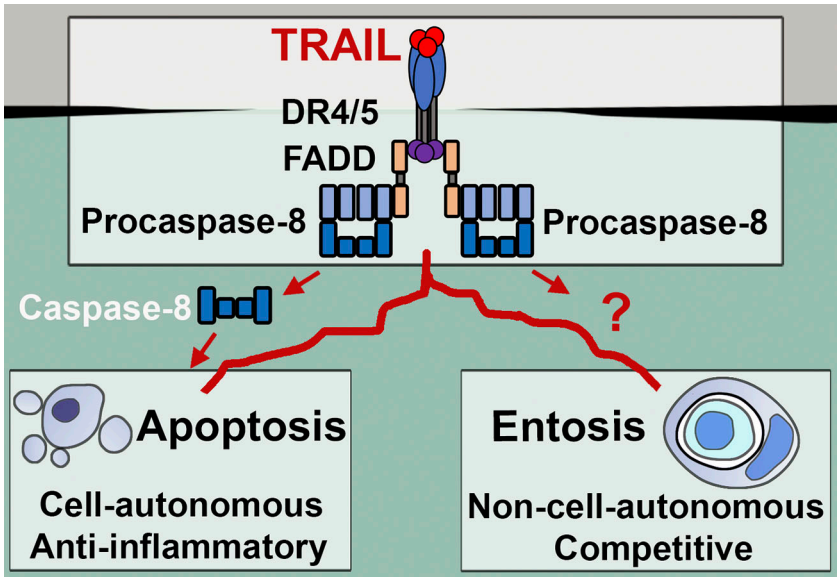

Figure 1. **A new TRAIL of cell death.** Signaling from TRAIL through its receptors (DR4/5) is well known to induce apoptosis (left path) by a mechanism involving formation of a death-induced signaling complex involving fas-associated death domain protein (FADD) and caspase-8, and subsequent activation of caspase-8 activity that leads to cell death. Bozkurt et al. now discover that TRAIL can also induce entosis (right path) through a mechanism requiring DR4/5 and a noncatalytic function of caspase-8 (6). Whereas apoptosis is executed in a cell-autonomous manner and is largely anti-inflammatory, entosis is executed in a non-cell-autonomous manner and is competitive between cells.

and the expression of TRAIL and cellular FLICE-like inhibitory protein, a factor that binds to a TRAIL-induced signaling complex, with a trend toward caspase-8 as well, consistent with TRAIL-mediated regulation of entosis in colorectal cancer. The authors further identified a correlation between the presence of entotic structures at the invasive front of stage III colon cancer specimens and poor patient outcomes, suggesting that the induction of entosis in response to TRAIL signaling could promote the development of more aggressive disease.

These findings by Bozkurt et al. uncover an important new path that leads to entosis, underscoring newfound complexity and the parallel nature of death signaling (Fig. 1). Different death mechanisms have unique properties and physiological effects (2). Apoptosis, for example, occurs mostly silently, or undetected by the immune system, a feature that is particularly well suited for death in normal tissues. Forms of regulated necrosis, on the other hand, spew intracellular contents and can alert immune responses to infection (2). Entosis may be the most unusual because it involves the ingestion and killing of individual cells by their neighbors, a form of death that is non-cell-autonomous in nature and intrinsically competitive between individual cells within a population. The findings in this study identify an important signaling node involving caspase-8 that now connects each of these multiple forms of cell death. While caspase-8 activity is required for apoptosis, here entosis is shown to be unaffected by caspase activity but curiously requires the presence of the caspase-8 protein. Noncatalytic functions for caspase-8 have been reported, including, intriguingly, regulation of the activity of the Rac-GTPase, a known regulator of entosis (7), through interaction with the PI-3-kinase scaffolding subunit p85 (8), as well as control over inflammatory signaling through formation of a complex called the "FADDosome" (9). Whether these or other reported noncatalytic functions of caspase-8 might contribute to entosis regulation will be important to uncover in future studies.

The parallel induction of different death mechanisms suggests that the physiological effects of cell death, for example during cancer therapy, relate not only to the extent of death that occurs but also to the types of death and their relative proportions within a population. Treatments inducing more necrosis than apoptosis, for example, could generate more pronounced immune responses. A more predominant induction of entosis is predicted to select for cells that can ingest their neighbors, called "winners," which have been shown to have competitive advantages and could promote the development of more aggressive disease (10). While the authors show that TRAIL receptors are required within the cells that are internalized when *DR4/5* knockout cells are mixed with control cells, they also find that the winner cells exhibit lower levels of caspase activation than others in response to TRAIL, suggesting, overall, that entosis may select for cells with resistance to TRAIL-induced cell death. TRAIL treatment is known to result in the fractional killing of tumor cells (11), which may limit the efficacy of TRAIL in cancer therapy. Its newfound control over entosis raises the possibility that the fraction that survives might also be selected through this competitive mechanism.

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