Developmental Cell

CellPress

Voices

The story behind the emergence of different forms of cell death

Various types of cell death program are needed for cells to respond to changes in physiological conditions. In this collection of Voices, we asked scientists to tell the story behind their contributions to the identification and mechanistic dissection of cell death pathways and to discuss future directions for such research.

Barbara Conradt
Faculty of Life Sciences, University College London

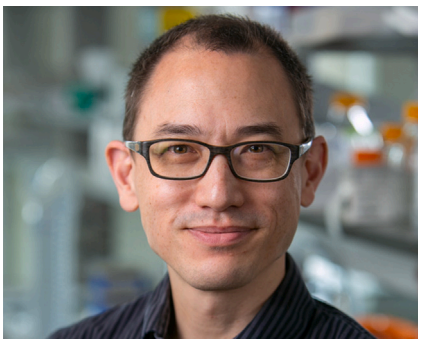
Different levels of engagement

In the nematode *Caenorhabditis elegans*, progenitors of "unwanted" cells engage the apoptosis pathway to ensure that "perfect" unwanted cells are generated.

During *C. elegans* development, 131 cells reproducibly commit suicide, primarily via apoptosis. Most of these unwanted cells are the smaller of two daughters generated through asymmetric division of a progenitor cell. Unexpectedly, we discovered that the apoptosis pathway (comprising *egl-1* BH3-only, *ced-9* Bcl-2, *ced-4* Apaf-1, and *ced-3* caspase) is already engaged by the progenitor cells. However, the degree of pathway engagement within these cells is insufficient to trigger apoptosis.

Why then, do the progenitors already engage the apoptosis pathway?

We found that this low level of engagement is necessary for the ability of the progenitor cells to divide asymmetrically and to ensure that the unwanted cells generated have a certain small size. Small cell size promotes apoptosis and an increase in size can delay—or even block—the death of unwanted cells. A low level of engagement of the apoptosis pathway in progenitor cells therefore ensures that "perfect" unwanted cells are reproducibly generated. These can then be efficiently killed and eliminated once the apoptosis pathway has been fully engaged.



Edward A. Miao
Duke University School of Medicine

Pyroptosis: Burning down the house

Pathogenic bacteria invade host cells and turn them into cozy habitats for replication. Host cells that detect this nefarious scheme engage in altruistic suicide, lest they spew forth more invaders. Pyroptosis is a form of regulated cell death that is an especially rapid, inflammatory, and decisive defense against intracellular infection. The discovery that inflammatory caspases activate a gasdermin D pore to execute pyroptosis was an inflection point, revealing that multiple gasdermins can execute pyroptosis.

Pyroptosis efficiently halts intracellular replication, but only if a cell actually detects the invaders. Under such intense evolutionary pressure, host-adapted pathogens evolved elegant virulence mechanisms to evade the sensors that trigger pyroptosis. Bona fide pathogens are thus one step ahead of innate pyroptotic defenses. In contrast, environmental pathogens have the ability to replicate intracellularly but fail to evade detection and are efficiently eliminated by pyroptosis.

Our understanding of pyroptosis has often focused on macrophages—one current challenge is to understand why other cell types use different gasdermins to accomplish pyroptosis. Understanding how pyroptosis fits into a complex multicellular immune response is another challenge. In this regard, we recently showed that pyroptosis is a prerequisite to the formation of a fully functional granuloma, in which macrophages and other cell types organize to surround infected tissues. We seek to understand why pyroptosis is an essential step in forming a granuloma that sterilizes infection.

Junying Yuan
IRCBC, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences

Discovery of necroptosis

In the mid-1990's, apoptosis was a red-hot topic after we revealed the critical role of caspases in mammalian apoptosis. We soon realized that inhibiting caspases could block apoptosis, but was not enough to inhibit cell death, as mammalian cells instead could die with necrotic morphology. The textbooks described necrosis as passive unregulated cell death, but we wondered if mammalian cells could have a regulated necrotic cell death mechanism. Fortunately, Marc Kirschner and Stuart Schreiber's chemical biology idea provided an opportunity to investigate cell-based mechanisms using small molecules. By screening for inhibitors of caspase-independent necrotic cell death, we discovered Necrostatin-1 (Nec-1), which could effectively inhibit caspase-independent necrosis. Based on these results, we proposed necroptosis as a regulated necrotic cell death mechanism in mammalian cells. Three years later, we showed that the RIPK1 kinase was the target of Nec-1.

Because necroptosis was identified in cells, it was another challenge to elucidate its *in vivo* relevance. We conducted a genome-wide small interfering RNA (siRNA) screen for 16,873 genes that regulate necroptosis and found that knockdown of multiple human disease-associated genes showed sensitization with negative *Z* scores in this screen, including OPTN (ALS, amyotrophic lateral sclerosis), ABIN1(psoriasis) and NAT1/2 (diabetes). These clues led us to demonstrate the role of RIPK1 and necroptosis in ALS, psoriasis, multiple sclerosis, and cerebromicrovascular damage. These studies directly promoted human clinical trials for developing RIPK1 inhibitors for the treatment of relevant diseases.

Thirumala-Devi Kanneganti
St. Jude Children's Research Hospital

Defining PANoptosis: Innate immune cell death

PANoptosis is a lytic, innate immune cell death pathway initiated by innate immune sensors and driven by caspases and RIPKs through PANoptosomes. PANoptosome complexes assemble in response to innate immune sensors detecting pathogens, pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns (DAMPs), and cytokines released during infections, inflammatory conditions, and cancer, making PANoptosis critical in multiple diseases. The mechanistic delineation of PANoptosis built on decades of genetic, molecular, and biochemical studies from multiple groups aimed at understanding NLRs and caspase-1 inflammasome activation. These studies identified extensive crosstalk among molecular components of pyroptosis (inflammasome-dependent cell death), apoptosis, and necroptosis. Discovery of ZBP1 as an innate immune sensor that activates the NLRP3 inflammasome and caspase-8/RIPK3-mediated cell death that is not prevented by inhibition of pyroptotic or necroptotic executioners was the catalyst to define PANoptosis as a distinct cell death pathway activated by innate immune sensor(s). Subsequent genetic and biochemical studies identified additional innate immune sensors that form PANoptosomes to drive PANoptosis (e.g., AIM2, RIPK1, NLRC5, NLRP12) and physiological conditions where PANoptosis is critical, such as in response to diverse pathogens and homeostatic disruptions (e.g., cytokine storms, hemolytic conditions). Understanding how the innate immune system drives PANoptosis and how genetic mutations in this pathway affect development of infectious, inflammatory, and autoimmune diseases and cancers is critical for developing therapeutics.

Brent R. Stockwell
Columbia University

Ferroptosis: Death by iron-driven lipid damage

In 2003, my lab described and named a small molecule, erastin, that caused non-apoptotic cell death. In 2007–2008, we found related compounds, and that this mechanism was driven by iron-dependent oxidation of lipids. In 2012, we showed this was a new form of cell death distinct from apoptosis, necroptosis, and necrosis.

To name this cell death, we considered that "-osis" was a suffix for a biological process (e.g., mitosis) and "-ptosis" was a specific suffix for a form of cell death (apoptosis, necroptosis, pyroptosis). Using the Latin for iron (*ferrum*), we coined the term *ferroptosis* for this iron-dependent cell death.

Earlier and parallel studies by the groups of Eagle, Mitchell, Ursini, Bannai, and Conrad converged on similar observations of cysteine deprivation causing cell death that depended on glutathione, vitamin E, and GPX4, supporting a framework linking iron oxidation of lipids to cell death.

There were three waves of ferroptosis discoveries. First, key regulators were found, including GPX4, SLC7A11, FSP1, ACSL4, and LPCAT3, revealing genetic control of this form of cell death and an intimate link with metabolism. Second, ferroptosis was implicated in many diseases. Third, natural roles for ferroptosis, such as intrinsic tumor suppression by p53 and $CD8^+$ T cells and control of plant immunity were uncovered. To enable these breakthroughs, technical advances included genetic screening, lipidomics, and ferroptosis marker discovery.

The future of ferroptosis likely involves deeper understanding of its biological functions, as well as discovery of ferroptosis-driven therapeutics.

Peter Tsvetkov
Beth Israel Deaconess Medical Center

From cancer drug resistance to cuproptosis

Our journey to characterize cuproptosis was serendipitous. While studying the mechanisms of resistance of cancer cells to proteasome inhibitors, we found that drug-resistant cancer cells were sensitive to mitochondria-targeting copper ionophores. Copper overload was known for decades to be cytotoxic to bacteria, yeast, and human cells, although the mechanisms invoked to explain this cytotoxicity were diverse and contradictory. This sparked my curiosity about how an essential, yet overlooked, element such as copper kills cancer cells, but it was challenging to decide if dramatically pivoting my research to focus on this question was "wise". Ultimately, curiosity won.

Our research revealed that induced copper accumulation in the mitochondria targets both lipoylated and Fe-S cluster proteins, facilitating a distinct form of regulated cell death we termed cuproptosis. These findings naturally prompt many yet unresolved questions: What are the downstream effector mechanisms? Is cuproptosis naturally occurring? Can we leverage cuproptosis to develop therapeutics? While there is still much to discover, I have learned that the pioneering efforts of others—the fundamental work characterizing other regulated, non-apoptotic cell death pathways—laid both the experimental foundation and removed the psychological barriers for new discoveries that ultimately, enabled us to pursue cuproptosis. Whether cuproptosis will emerge as a critical regulated cell death pathway or a therapeutic target, only time and further research will tell. For now, I am excited to be part of this unfolding story.

Michael Overholtzer
Memorial Sloan Kettering Cancer Center

Entosis: A most unusual death

Entosis was discovered when different forms of death were just emerging. We were fortunate that mechanisms like necroptosis and pyroptosis were already published because entosis was so unusual. We could not deny what we were seeing, but why would cells do this?

Entosis felt simple. Cells were cultured in suspension and then they did this on their own. Its most basic mechanism involves cell adhesion and a tension differential, which allows entotic cells to enter their neighbors, stay for a while, and then get murdered. It feels primitive, even barbaric. We wrote a manuscript without naming it but ultimately found it too difficult to explain. Seeking a name that would feel as simple as the mechanism, Joan Brugge and I coined "entosis" after the Greek "entos", meaning inside or within.

In my lab we explored entosis from different angles. What causes this? How does it work? What does it mean for normal tissues or cancer? Over the years we and others have published a few answers. Entosis is observed often in aggressive carcinomas, consistent with its regulation by epithelial cadherins, and suggestive of a role in cancer progression. Entosis promotes competition between cells similar to "cell competition" in developing tissues, eliminating cells with high tension. Entosis also promotes aneuploidy and feeds cells when they are starved. Each of these properties can drive cancer progression, and together they may be particularly potent. We and others have also found entosis occurring in normal development where its adhesive properties and ability to eliminate cells may be important.

Boyi Gan
The University of Texas MD Anderson Cancer Center

Disulfidptosis: A new twist in cancer cell death

In 2016, when Pranavi Koppula, a junior student in the lab, showed me her finding that SLC7A11 overexpression drastically accelerated cancer cell death under glucose starvation, my first reaction was, "Did you accidentally switch the control and SLC7A11-overexpressing cell lines?" At the time, the prevailing belief was that SLC7A11-mediated cystine import promotes glutathione biosynthesis and generally plays a cytoprotective role under oxidative and other stress conditions. So, Pranavi's observation seemed completely counterintuitive.

We now have a better understanding of this phenomenon. Once cystine is imported into cells by SLC7A11, it is reduced to cysteine via an NADPH-dependent reaction, with cysteine then used to produce glutathione and other biomolecules. However, under glucose starvation, the NADPH pool becomes severely depleted. When combined with high SLC7A11 expression, this can cause a toxic buildup of cystine, triggering disulfide stress, actin cytoskeleton collapse, and rapid cell death. This form of cell death appears distinct from other known types of cell death, so we named it "disulfidptosis."

Moving forward, it is essential to deepen our understanding of the regulatory and execution mechanisms of disulfidptosis and its potential role in development. Since disulfidptosis occurs specifically in glucose-starved cells with high SLC7A11 expression, identifying other cellular conditions that can induce this form of cell death will be key. Future research should also explore the therapeutic potential of targeting disulfidptosis in cancer and other diseases.

DECLARATION OF INTERESTS

B.R.S. is an inventor on patents and patent applications involving ferroptosis; co-founded and serves as a consultant to ProJenX, Inc. and Exarta Therapeutics; holds equity in Sonata Therapeutics; serves as a consultant to Weatherwax Biotechnologies Corporation and Akin Gump Strauss Hauer & Feld LLP.