Science Education Collection

An Introduction to Cell Death

URL: https://www.jove.com/science-education/5649

Abstract

Necrosis, apoptosis, and autophagic cell death are all manners in which cells can die, and these mechanisms can be induced by different stimuli, such as cell injury, low nutrient levels, or signaling proteins. Whereas necrosis is considered to be an “accidental” or unexpected form of cell death, evidence exists that apoptosis and autophagy are both programmed and “planned” by cells.

In this introductory video, JoVE highlights key discoveries pertaining to cell death, including recent work done in worms that helped identify genes involved in apoptosis. We then explore questions asked by scientists studying cell death, some of which look at different death pathways and their interactions. Finally, several methods to assess cell death are discussed, and we note how researchers are applying these techniques in their experiments today.

Transcript

Paradoxically, cell death helps shape an organism’s life. Just like any whole organism, cells can die as a result of aging, due to accidental injury, or following a pathogen infiltration a cell can sacrifice itself to prevent the spread of infection. Under these circumstances, cells can follow different death pathways like apoptosis, autophagy, or necrosis. All these types display specific morphological characteristics. Apoptosis or programmed cell death leads to membrane “blebbing” and nuclear fragmentation. Autophagy, which is also regulated, leads to formation of large vacuoles enclosing cellular components. Lastly, necrosis, which is “unplanned” or accidental, ends in cell lysis.

This video will discuss important discoveries that led to the identification of these pathways, explore questions that researchers are still asking about cell death, discuss tools they use to answer them, and finally review a few example experiments.

First, let’s review some key researchers who helped to decipher different cell death pathways.

Modern terms used to describe these paths can be traced back to Hippocrates, a physician in ancient Greece. He used the term apoptosis, meaning “falling off,” to describe bone “shredding” observed following a fracture. Coming to the modern era, the first noticeable mention of “necrosis” occurred in 1859, when Rudolf Virchow—in his compilation called *Cell Pathology*—used this term to describe “advanced tissue breakdown.”

With advances in microscopy and histology over the next decade, in 1877 Carl Weigert and Julius Cohnheim were able to study necrosis at the cellular level. They provided insight into the morphological features associated with this type of death, like the loss of nuclei.

Almost 70 years later, Christian de Duve discovered “autophagy,” a process in which cellular components are engulfed and broken down by membrane-bound organelles called autophagosomes, which fuse with another type of organelle—lysosomes—to further destroy their contents. We now know that autophagy actually plays a dual role in the cell, either facilitating survival or inducing death.

In 1972, John Kerr, A. R. Currie, and Andrew Wyllie observed another type of cell death with peculiar morphology. Since this process involved pieces “falling off” of dead cells, they gave it the ancient Greek name apoptosis. Later, apoptosis was recognized as a form of “programmed cell death” in 1977, when H. R. Horvitz and John Sulston were studying *C. elegans* development. They noticed that specific cells would undergo apoptosis at the same time in different worms.

Since this was happening early on during development, it hinted that genes may guide apoptosis. This hypothesis was confirmed by Horvitz’s group in the 1980’s, when they observed that cells with mutations in certain *ced* or “*C. elegans* death” genes didn’t die during the development of these worms. Later, Horvitz showed that the *ced-3* gene encodes a protein-degrading enzyme called a caspase. Now, we know that there are several caspases, and they play major roles in cell death.

These advances in the cell death field opened new roads for researchers to explore. Let’s look at some of them.

There has always been interest in finding out what factors trigger cell death. To identify them, researchers are currently exposing cells to radiation, chemicals, and signaling molecules, and then searching for changes in the degree or type of death.

Other scientists are interested in elucidating the biochemical pathways involved in each cell death mechanism. Currently, we know that apoptosis follows a pathway where caspases are the key enzymes, whereas autophagy involves proteins that are necessary for autophagosome formation. However, there are components in these pathways that are unknown, and researchers are trying to figure out ways to explain them. In addition, researchers are also studying whether any “crosstalk” occurs between cell death pathways. If crosstalk is present, then the same signal can factor in apoptosis, as well as autophagy.

Lastly, a popular area of research deals with understanding why certain cells—like cancer cells—become immortal. Scientists are constantly looking for mutations in cancer cells, and assessing whether any of them affect genes encoding factors involved in death pathways.

These are all complicated questions, but luckily researchers have a variety of tools at their disposal to answer them.

The trypan blue assay is a commonly used screening tool to assess the effect of a compound on cell death. The assay relies on a stain that cannot enter live cells, as they posses “selective membranes,” but can easily enter dead cells as their membranes are “ruptured.” This assay identifies cell death, but fails to pinpoint the specific cell death pathway.

Therefore, scientists have designed techniques like caspase activity assays. Since caspases are activated during apoptosis, scientists can add substrates for these enzymes that fluoresce when they’re activated by caspases. This helps in the identification of apoptotic cells.

Similarly, DNA fragmentation that happens during apoptosis can be easily identified using the TUNEL assay, which relies on reagents that tag the “nicked” ends of damaged DNA. As this method is relatively easy to perform, it is a commonly employed assay in the field.

When scientists want to determine the cell death mechanism occurring in their population, they can pair annexin V and propidium iodide (PI) stains with flow cytometry analysis. Annexin V binds to phosphatidylserine residues in the membrane, whereas PI enters through the damaged membranes to associate with DNA. By studying the resulting data, scientists can separate cells undergoing different death pathways.

Lastly, scientists can use live cell imaging to view the cell death process in real time. This is an all-encompassing technique that can be used to identify autophagic, necrotic, or apoptotic cells based on unique morphological features.

As you’ve seen, there are several methods to detect cell death, some of which are not specific, others that can help identify apoptotic cells, and some that distinguish between different pathways.

Now, let’s see how scientists are using these techniques to study more about cell death.

Diet plays an important role in health, and may affect cell death in different tissues. In this *in vitro* assay, researchers exposed mouse neurons to palmitic acid, a saturated fatty acid present in both dairy products and meat, and then used a caspase assay to evaluate apoptosis. They discovered that palmitic acid-treated cells demonstrated increased caspase activity and cell death.

Other researchers are using these assays to determine how drugs induce different death mechanisms. Here, transgenic mice with fluorescently labeled cancer cells were injected with doxorubicin, an anti-cancer drug. Scientists then imaged cells in live animals, and by looking for changes in cancer cell morphology, determined that drug treatment triggered both apoptosis and necrosis.

Finally, some scientists are investigating whether cell death can be reversed. In this experiment, researchers exposed human cancer cells to ethanol, and confirmed through a variety of assays that this treatment caused them to embark on the apoptosis pathway. Upon washing off the ethanol, affected cells were able to recover from apoptosis through a process called “anastasis.” This provided insight into how cancers can return following drug treatment.

You’ve just watched JoVE’s introduction to cell death pathways. This video reviewed the rich history of cell death research—from ancient times to the 20th century—and then discussed a few current questions. We also explained popular methods to assay cell death, and demonstrated how these techniques are being used to better understand the connection between environment, disease, and cell death. As always, thanks for watching!