Tools for Studying Cell Death

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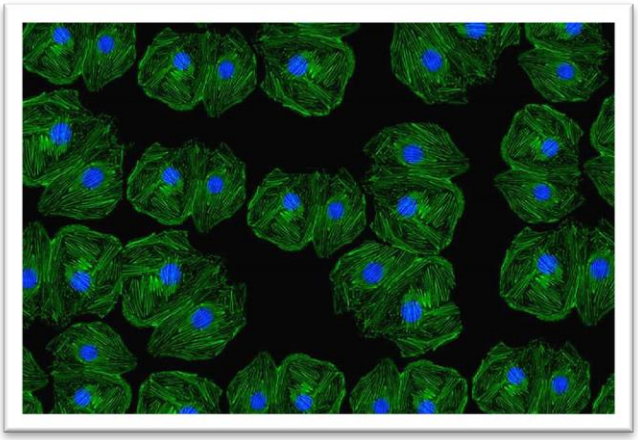
Overview of Cell Death

In adult tissues, cell death balances cell division. The number of cells in multicellular organisms is tightly regulated. Cellular homeostasis is achieved by controlling the rate of cell division and the rate of cell death. Cells die as a result of chemical and physical events, or "insults," such as radiation, heat, toxic substances, bodily trauma, or injury and lack of oxygen. Injured cells swell up, burst, and spill their contents, undergoing unplanned cell death. For many years, biologists have known that cells die at predictable points during development and this represents programmed cell death (PCD).



Cell death can be classified into a number of types including apoptosis, autophagy, necrosis, and others. Of the three major pathways, Apoptosis (Programmed Cell Death Type 1) is an ordered and orchestrated cellular process that occurs in physiological and pathological conditions. It is also one of the most studied topics among cell biologists and recognized as a critical regulator of development, differentiation, regulation and function of the immune system, as well as organ and tissue proliferation/homeostasis. Dysregulation of apoptosis can play a primary or secondary role in causing diseases such as cancer. Excessive apoptosis can contribute to neurodegeneration, autoimmunity, etc. Various means of detecting apoptotic cells have been explored and made available over recent years. Autophagy, in essence, is a biological recycling mechanism where misfolded proteins are ubiquitinated and targeted for degradation by the lysosomal pathway. Autophagy (Programmed Cell Death Type 2) is selective degradation of intracellular targets, such as misfolded proteins and damaged organelles, thereby serving as an important homeostatic function. Necrosis (Programmed Cell Death Type 3) is characterized by cell swelling and destruction of the plasma membrane and subcellular organelles. Necrotic cell death is considered a heterogeneous phenomenon including both programmed and accidental cell death.

Overview of Apoptosis

Apoptotic cell death is known to involve a cascade of proteolytic events accomplished mainly by a family of cysteine proteases called caspases. These proteases are synthesized as inactive proenzymes that are activated after cleavage at specific aspartate residues. Because caspases exist in healthy cells as inactive precursors (procaspases), events that regulate caspase activation and downstream activity constitute critical control points in apoptosis. Caspases are organized in hierarchical activation networks, with some caspases playing a role as upstream initiators of caspase activation and others playing a role as downstream effectors of substrate proteolysis. There are three pathways by which caspases can be activated. The two commonly described initiation pathways are the intrinsic (or mitochondrial) and extrinsic (or death receptor) pathways of apoptosis. Both pathways eventually lead to a common pathway or the execution phase of apoptosis. A third lesser-known initiation pathway is the intrinsic endoplasmic reticulum pathway.

Methods of Detecting Apoptosis/Necrosis

The display of phosphatidylserine (PS) on the extracellular face of the plasma membrane is the hallmark of early apoptosis. Phospholipid binding proteins such as Annexin V possess a high affinity for phosphatidylserine and readily bind in the presence of Ca2+. Given that Annexin V is not cell permeable, the binding of externalized PS is selective for early apoptotic cells. As a cell trends towards necrosis, membranes continue to degrade, allowing non-permeable detection reagents, such as Necrosis Detection Reagent, to intercalate into guanine rich regions of the DNA and provide additional information. Cells that are in late apoptosis or early necrosis demonstrate signal for both Annexin V and Necrosis stain. Determining the stage of apoptosis or necrosis is of particular interest to several research areas ranging from target identification and validation to small molecule efficacy and toxicity. The binding of fluorochrome-conjugated Annexin V to exposed PS can be detected by flow cytometry or fluorescence microscopy. While fluorescence microscopy allows the visualization of the event, flow cytometry is the most useful method as it allows for a quick and accurate quantification of cells with exposed PS. Our GFP-CERTIFIED® Apoptosis/Necrosis detection kit distinguishes between healthy, early apoptotic, late apoptotic and necrotic cells and is compatible with GFP and other green fluorescent probes.

Enzo provides over 40 years of experience in the manufacturing and supply of research kits, biochemicals, and biologicals. Our growing list of over 1000 IHC validated antibodies includes those for the detection of key signaling proteins, cell surface markers, mediators of cell death, oxidative stress, heat shock proteins, proteasomes, and more.