Apoptosis And Necrosis Through Differential Staining With Hoechst 33342 And Propidium Iodide

Apoptosis and necrosis

Cellular death is a naturally occurring phenomenon. Cells often die due to a harmful environment or through a regulated process of death, with the former termed necrosis and the latter termed apoptosis. While apoptosis is regarded as cell death resulting from normal healthy processes, necrosis results from external factors or disease. (Fink and Cookson, 2005)

When cells are exposed to toxins or extreme conditions, damage of the internal cellular environment occurs. Environments such as increased temperatures or decreased oxygen levels may lead to a loss of cell membrane integrity. This may cause the cell to swell, and eventually lyse in the ATP-independent process known as necrosis. As these conditions often affect several cells, necrosis leads to the death of groups of cells as opposed to individual cells. (Elmore, 2007)

Apoptosis is triggered by physiological changes such as a higher amount of free radicals or the depletion of growth factors. On the external surface of the plasma membrane, phosphatidylserine is exposed as a signal for apoptosis. Cytochrome c, a necessary component of the mitochondria, is extruded to the cytosol, and cellular components are degraded by caspases. (Jiang and Wang, 2004) The cell shrinks, and a loss of cell-cell contact occurs before the cell is phagocytosed, allowing for an easy clearance. Apoptosis, often termed as programmed cell death, is an ATP-dependent process vital to the cell cycle as it allows for normal cell turnover. Cells die naturally to maintain a balance of the cellular multiplication. This is also necessary for the growth and development of an organism. As observed in human fetuses, over time, the webbing formed around phalanges is degraded to make way for separate digits. (Elmore, 2007)

Autophagy is a less common form of programmed cell death observed during nutrient stress. Autolysosomes are formed from the fusion of lysosomes and autophagosomes to promote cell survival. Starved cells are provided nutrients through the digestion of some cellular components. (Gustafsson and Mentzer, 2013)

Methods used to characterize cell death

Hydrogen peroxide can be a source of reactive oxygen species which damages cell components. Lower concentrations of hydrogen peroxide predominantly induce apoptosis while higher concentrations induce necrosis. Exposure of a cell to hydrogen peroxide at lower doses causes the loss of mitochondrial membrane potential, cytochrome c release, and caspase activation, thus inducing apoptosis. At higher doses, hydrogen peroxide is known to cause damage to all macromolecules, thus inducing necrosis. (Uhl et al., 2015)

Differential staining is a versatile assay to characterize cell death. For this experiment, the mechanism for which cell death ensues can be elucidated by the use of two different stains:

Hoechst 33342 and propidium iodide. In the case of apoptotic cells with intact membranes, Hoechst 33342 is able to permeate through the membrane to stain the DNA of cells. Propidium iodide, on the other hand, can only stain the DNA of cells with cell membranes that are not intact, as in the case of necrotic cells. Live cells are normally not stained as Hoechst 33342 is actively transported out of the cell while propidium iodide is not able to permeate through the membrane. Upon viewing cells, however, live cells may still be observed to be stained by Hoechst 33342 as it can readily cross cell membranes. For this reason, apoptotic and live cells may not be highly differentiated from the said procedure alone. (Lema et al., 2011)

Electron microscopy can be utilized in order to observe the morphological changes that come with apoptosis. Cell shrinkage, loss of cell-cell contacts, and condensation of chromatin can be seen through the microscope. This technique is suitable for uniform and enriched cell populations. In intact tissues, however, apoptosis may occur to individual cells distant to each other, and for this reason, cell death may be difficult to observe given the small density of cells undergoing the process. Quantitation will also not be accurate as the number of cells observed per section is relatively few. (Mesner and Kaufmann, 1997)

Apoptotic cells are known to decrease in size as water is lost. Larger cells scatter light more effectively as compared to smaller cells. This can be monitored by using flow cytometry. Upon exposure to an apoptosis-inducing stimulus, cells are fixed at various times then measured and compared with the control. For the reason that in the cell cycle, a wide variation of cell size can also be observed, this parameter may not be sufficient enough to distinguish apoptotic and live cells. (Mesner and Kaufmann, 1997)

Apoptotic cells can be quantified by hematoxylin and eosin staining. As the chromatin condenses in apoptotic cells, hematoxylin is able to densely stain the area. This technique allows for a large number of cells to be examined in a tissue. Nuclei of cells such as small lymphocytes may be mistaken for dense chromatin. (Mesner and Kaufmann, 1997)

Acridine orange combined with Hoechst 33342 (AO/HO Staining) is used to provide better contrast as well as a more distinctive identification of apoptotic and live nuclei. This technique is able to differentiate between the early and late apoptosis stage based on the color emitted, with yellow indicative of an earlier stage and orange indicative of a later stage. However, this procedure is not capable of differentially staining necrotic nuclei. (Mpoke and Wolfe, 1997)

References

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