Review Article

Overview of makers for apoptosis

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ABSTRACT

The various markers for the apoptosis pathway reviewed in this article are DNA fragmentation, caspase activation, bid cleavage, and cytochrome c release. Apoptosis is a pathway of programmed cell death that may occur in multicellular organisms. Biochemical events leads to characteristics cell changes and death. The apoptosis assays, based on methodology, are of six groups cytomorphological alterations, DNA fragmentation, detection of caspases, cleaved substrate, regulators and inhibitors, membrane alterations, detection of apoptosis in whole mounts, mitochondrial assays.

KEY WORDS: Apoptosispathway, Caspases, DNA fragmentation, Markers

INTRODUCTION

In multicellular organisms, cells that are no longer needed or are a threat to the organism are destroyed by a tightly regulated cell suicide process known as programmed cell death, or apoptosis.[1] Defective apoptosis is implied in a number of diseases, hence an insight of the various markers for apoptosis is important, for us to understand the pathogenesis of diseases. The various markers for the apoptosis pathway will be overviewed in this article.

APOPTOSIS

Apoptosis is characterized by nuclear chromatin condensation,cytoplasmicshrinking,dilatedendoplasmic reticulum, and membrane blebbing and mitochondria remains unchanged morphologically.[2] Increased apoptosis is characteristic of AIDS; neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis; ischaemic injury after myocardinal infarction, stroke and reperfusion; and in autoimmune diseases such as hepatitis and graft versus host disease. Decreased or inhibited apoptosis is a feature of many malignancies, autoimmune disorders such as systemic lupus erythematous and some viral infections.[3]

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MARKERS OF APOPTOSIS PATHWAY

Apoptosis is the process of programmed cell death that may occur in multicellular organism. Biochemical events lead to characteristics cell changes and death. These changes are mentioned above. Caspases are the initiators of apoptosis which are protein that are highly conserved, cysteine dependent aspartate-specfic proteases. There are two types of caspases; initiator caspase, caspases 2, 8, 9, 9, 10, 11, 12 and effector caspases, caspases 3, 6, 7.[1] There are various biochemical techniques for analysis of cell surface markers, cellular markers such as DNA fragmentation, caspases activation, bid cleavage, and cytochrome C release. Acridine orange (AO)/ethilidium bromide staining, cleaved caspase 3 measured by western blot or by cytometry, annexin V assay are the few well known markers for apoptosis.[4]

Apoptosis assays, based on methodology, can be classified into six major groups:

1. Cytomorphological alterations
2. DNA fragmentation
3. Detection of caspases, cleaved substrates, regulators and inhibitors

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4. Membrane alterations
5. Detection of apoptosis
6. Mitochondrial assays.[5]

CYTOMORPHOLOGICAL CHANGES

Cells undergoing apoptotic cell death reveals a characteristic sequence of cytological alterations including membrane blebbing and a nuclear and cytoplasmic condensation. Transmission electron microscope is considered gold standard to confirm apoptosis. This is because categorization of an apoptotic cell is irrefutable if the cell contains certain ultrastructural morphological characteristic.[6] These characteristics are (1) electron-dense nucleus (marginalization in the early phase). (2) Nuclear fragmentation; (3) intact cell membrane even late in the cell disintegration phase; (4) disorganized cytoplasmic organelles; (5) larger clear vacuoles; and (6) blebs at the cell surface.

DNA FRAGMENTATION

One of the major characteristics of apoptosis is the degrading of DNA after the activation Ca dependent endonuleases. This DNA cleavages leads to strand breaks within the DNA. However necrosis can also result in similar DNA cleavage. Therefore an additional methods should be used to confirmapoptosis.[7] Since DNA fragmentation occurs in the later phase of apoptosis, in the absence of a DNA ladder does not elimate the potential that cells are undergoing early apoptosis.[8]

DETENTION OF CASPASES, CLEAVED SUBSTRATES, REGULATORS AND INHIBITORS

There are more than 13 known caspases (procaspases or active cysteine caspases) that can be detected using various types of caspases activity assays. The cleaved substrate such as PARP and known cell modifications such as phosphorylated histones are markers that are detected by immunohistochemistry assays.[9] Caspasesactiation can be detected in a variety of ways including western blot, immunoprecipitation and immunohistochemistry.[10]

MEMBRANE ALTERATIONS

Externalization of phosphatidylserineresidues on the outer plasma membrane of apoptotic cells can be detected via Annexin V in tissues, embryos or cultured cells.[11] The disadvantage is that the membranes of necrotic cells are labelled as well. A critical control is there to demonstrate the membrane integrity of the phosphatidylserine-positive cells. The loss of membrane integrity is a pathognomonic feature of necrotic cell death, necrotic cells will stain with specific membrane impermeant nucleic acid dyes such as propidium iodide and trypan blue. And the membrane integrity of the apoptotic cells can be demonstrated by the exclusion of these dyes. The transfer of phosphatidylserine to the outside of the cell membrane will also permit the transport of certain dyes into the cells in a unidirectional manner. As the cell accumulates dye and shrinks in volume, the cell dye content becomes more concentrated and can be visualised with light microscopy. This dye uptake bioassay works on cell cultures, does not label necrotic cells and has a high level of sensitivity.[5]

DETECTION OF APOPTOSIS IN WHOLE MOUNTS

Apoptosis can be detected in whole mounts also, in embryos or tissues using dyes such as AO, Nile blue sulphate (NBS), and neutral red (NR).[12] These dyes are acidophilic, and are concentrated in areas of high lysosomal and phagocytotic activity. But these dyes cannot distinguish between lysosomes degrading apoptotic debris from degradation of other debris such as microorganisms.[13] Hence, needs other assays to confirm the results. Although all of these dyes are fast and inexpensive, they have certain disadvantages. AO is toxic and mutagenic and quenches rapidly under standard conditions whereas NBS and NR do not penetrate thick tissues and can be lost during preparation for sectioning.[9]

MITOCHONDRIAL ASSAYS

Mitochondrial assays and cytochrome c release allow the detection of changes in the early phase of the intrinsic pathway.[14] Mitochondrial permeability transition, depolarization of the inner mitochondrial membrane, Ca2+ fluxes, mitochondrial redox status, and reactive oxygen species can all be monitored with this laser scanning confocal microscopy.[9,15-19] Cytochrome c release from the mitochondria can also be assayed using fluorescence and electron microscopy in living or fixed cells. However cytochrome c becomes unstable once it is released into the cytoplasm. Therefore a nonapoptotic control should be used to ensure that the staining conditions used are able to detect any available cytochrome c.[19-24] Apoptotic or anti-apoptotic regulator proteins such as Bax, Bid and Bcl-2 can also be detected using fluorescence and confocal microscopy. Since, the fluorescent protein tag may alter the interaction of the native protein with other proteins, other apoptosis assays should be used to confirm the results.

CONCLUSION

The importance of apoptosis was underestimated for many years. Today, apoptosis is implicated in

biological process ranging from embryogenesis to ageing, from normal tissue homeostasis to many human diseases, and it has become one of the hottest fields of biomedical research.[25] Understanding the mechanisms involved in physiological as weel as in disturbed or dysregulated apoptosis may lead to the development of new methods of preventive treatment of various developmental abnormalities.[26] Hence, it is also important to study the various markers of apoptosis pathway and the assays used in these cases, which has been overviewed in this article.

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