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Review article

Apoptosis : Searching for the detection techniques

Sharma M, Kumar R, Dhingra R

**Department of Anatomy, All India Institute of Medical Sciences, New Delhi - 110029.*

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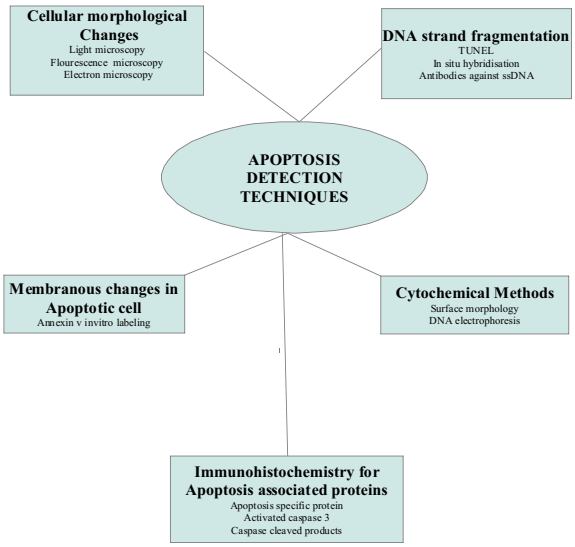
ABSTRACT

Major tool of apoptosis detection, even today is the routine morphology based on already established histological techniques. Therefore, validation of new techniques becomes critical. Various aspects of apoptotic events have been extensively researched to elucidate the common biochemical pathways leading to this critical and unique phenomenon. Till now, apart from prototypical apoptotic morphology , techniques like DNA fragmentation estimation, specific sera against apoptotic components have been established. With each of these techniques, the essential requirements for apoptotic detection have been tried to establish like differentiating the apoptotic cells from non apoptotic cells, stages of apoptotic events and sensitivity of these techniques for apoptotic cells. In this review, we have not only tried to encompass various recent technique advancements in apoptosis detection, but also elaborate the pitfalls of all the techniques that shake the interpretation of results starting from the routine to the latest ones.

1.Introduction:

Various fields of modern biology including cancer development and degenerative diseases have been working in exploring the knowledge regarding the pathways and detection methods of apoptosis (programmed cell death). It has been already confirmed that apoptosis is a highly organized phenomenon and this event is responsible for various pathological conditions. Therefore, the area of cell death research is highly upcoming and exploring field and require a constant insight into the cell biology that lead to the expansion of this field. Detection of apoptotic cells which was previously based on the cell morphological changes and DNA fragmentation, has now advanced to the more specific methods depending on various events occuring during apoptotic pathway. Proteins expressed or fragmented during apoptosis process are recognised by the specific antibodies directed against them. Moreover, antisera against various death receptors, ligands, pro and antiapoptotic proteins are being used to identify the apoptotic cells. Also, in this review, we will focus on the recent cytochemical methods extensively used in the tissues.



Schematic flow chart showing various techniques of detection of apoptosis in tissues

* Corresponding Author : Sharma M
Department of Anatomy,
All India Institute of Medical Sciences,
New Delhi-110029.
Cell: 9968147821
Email: dr.mona18sharma@gmail.com

Table describing various techniques of apoptosis detection.

Techniques	Principles	Positive Findings	Negative Aspects	References
1.Morphological staining- By using H andEstain;Nisslstain;Methyl green under lightmicroscope;electronmicroscope;and confocallaser scanning (Fig:I)	Apoptotic morphology is specific for the dying cells. Nucleus and cytoplasm shows distinct features of apoptotic process	Cytoplasm is condensed and shrunken;nuclear chromatin gets condensed along the nuclear membrane making a crescent; plasma membrane forms blebs and dissolves;breaking of condensed nuclei into apoptotic bodies	Lymphocytes and oligodendrocytes with smaller condensed nuclei may mimic apoptotic bodies, results may vary among the observers	[1]
2.Identification of DNA Fragmentation (a)TUNEL(terminal deoxynucleotidyl transferase mediated dUTP biotin nick end labeling) (Fig:II)	Identification of DNA fragmentation by addition of labeled nucleotide to the sites of single or double stranded DNAbreaks with the help of terminal deoxynucleotidyl transferase.	DNAbreaks in nucleus is characteristic of cell death and give a color stained nucleus depending on the chromogen used	Chances of false positivity is more as necrotic cells also undergo DNA fragmentation. As endonuclease activation occurs very late, so can be bypassed during apoptotic assesment assays.	[2,3]
(b)Antibodies against ssDNA	The antibodies detect early stages of apoptosis	Identifies apoptotic cells even after secondary necrosis	Non specific background staining has been reported	[4,5]
(c)Insitu DNA hybridisation	There is use of labeled polyA probes which bind to sequences which are prone for denaturation	Identifies early stages of double stranded DNA fragmentation.	Reported use in formalin fixed and paraffin embedded tissues but use in cell culture is not being reported	[6]
3.Membranous changes Annexin v labeling	The process of apoptosis brings about transport of phosphotidylserine to the outer membrane;annexin v binds to it and detected by flow cytometry	Detects apoptotic process early as externalization occurs early in the cascade	Its use has been reported in flow cytometric detection but not in tissue sections	[7]
4.Detection of apoptosis associated proteins (a)Specific proteins of apoptosis process	Antibody acts against Nterminal aminoacids of transcription factor c-jun		This technique is not specific for apoptotic cells as the antibody cross react with various other cytoplasmic contents	[8]
b)Caspase antibody	CM-1 antibody recognises specifically subunit of caspase 3 which is an effector caspase during apoptotic cascade	Sensitive and specific marker. Does not stain necrotic cells		[9]
(c)Caspase cleaved fragments (Fig. III)	Cytokeratin 18 fragments formed during apoptosis cascade identifies specifically in apoptotic epithelial cells by M30 antibody. Antisera against caspase cleaved actin and amyloid precursor protein has also been identified	M30 antibody is more sensitive for apoptotic cell as cytokeratin cleavage occurs early during apoptosis cascade and remains positive for a longer time M30 antibody does not stain the necrotic cells and so specific for apoptotic cells		[10,11]

5.Cytochemical changes in cell cultures (a)Surface morphology (Fig:IV)	The cellular morphological changes are analysed using video time lapse microscopy defining the kinetics of apoptosis in cell cultures.	There is loss of adhesion and cell rounding, followed by surface blebbing,leading to shrinkage of cell. There is spike formation from cell surface and blistering of membrane and lysis of cell	Some substrates used in cultures like staurosporine have effects on cell motility and other surface changes making the interpretation difficult.	[12]
(b)DNA gel electrophoresis (Fig.V)	The nuclear DNA breakdown into multiples of 200 bp oligonucleosomal size fragments is the hallmark of apoptosis. The detection of apoptosis in cultured cells relies heavily on techniques involving the extraction of nuclear DNA and characterization of such oligonucleosomal ladders by gel electrophoresis	The characteristic DNA ladder are seen	This procedure requires extraction of DNAfrom large no. of cells. DNA breaks can occur during or after the extraction procedure. Therefore, false positive results may occur. Signals are generated very late in apoptotic	[12]

Fig I: Hematoxylin and Eosin staining- Arrows represent apoptotic trophoblastic nuclei in placental tissue

Scale bar 100µm

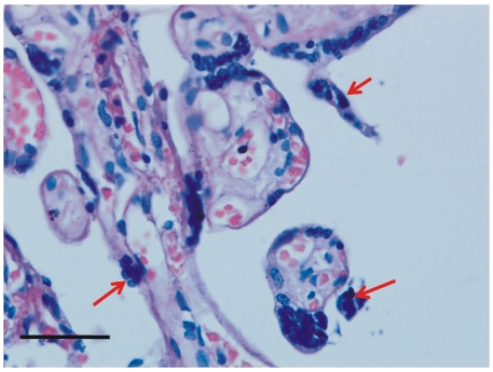


Fig II: TUNEL Assay- Arrows represent apoptotic trophoblastic nuclei in the placental tissue

Scale bar 100µm

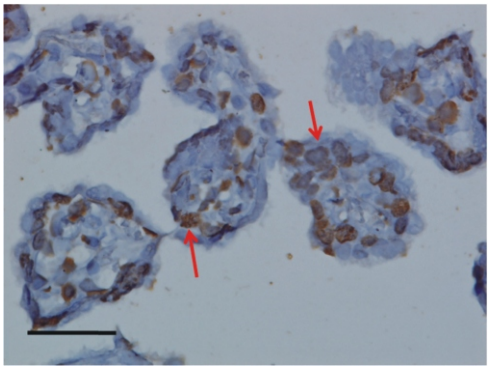


Fig III: M30 Antibody staining- Arrows represent stained cytoplasm of apoptotic cells in placental tissue

Scale bar 100µm

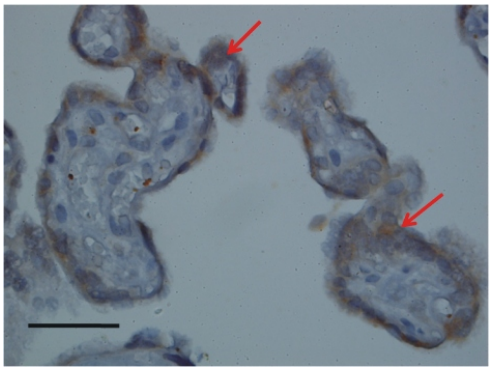


Fig IV: Morphological changes of apoptotic cells in cell culture [Collins JA et al. 1997]

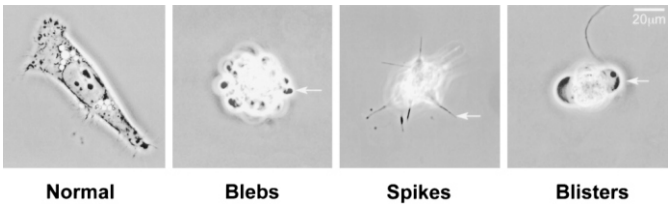
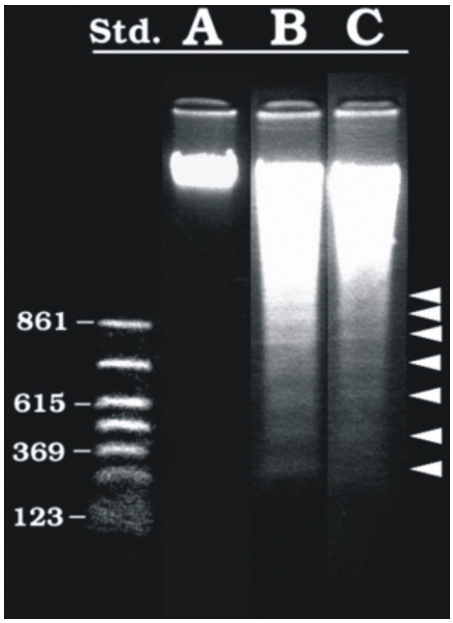


Fig V: Gel electrophoresis showing characteristic ladder pattern in apoptotic cells PanelA: no ladder pattern in non apoptotic DNA. Panel B and C: ladder pattern seen in apoptotic DNA [Collins JA et al. 1997]



2. Conclusion

The understanding of the molecular basis of the events taking place during apoptosis leads us to extensive research and analysis of apoptotic cells in tissues. The area of cell death research which started with just the morphological changes of apoptotic cells has now included studying various other parameters like DNA fragmentation, caspase cleaved products and even cytochemical tests. After considering the varying aspects of the previous and the developing new techniques , the multiparametric approach is considered to be the best as each test has got its limitations. Further understanding of the biochemical pathways of the apoptotic phenomenon(mitochondrial and death receptor pathways) may lead us to uncover various other techniques that would be more sensitive and specific in identifying various apoptotic cells in different phases of apoptotic pathway.

3. References

[1] Drachenberg CB, Ioffe OB, Papadimitriou JC. Progressive increase of apoptosis in prostatic intraepithelial neoplasia and carcinoma: comparison between in situ end-labeling of fragmented DNA and detection by routine hematoxylin-eosin staining. Arch Pathol Lab Med. 1997; 121: 54-58.

[2] Kerr JFR, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer. 1972; 26: 239-257.

[3] Ferrer I, Pozas E, Marti M, Blanco R, Planas AM. Methylazoxymethanol acetate-induced apoptosis in the external granule layer of the developing cerebellum of the rat is associated with strong c-Jun expression and formation of high molecular weight c-Jun complexes. J Neuropathol Exp Neurol. 1997; 56: 1-9.

[4] Frankfyrt OS 1998. Detection of apoptotic cells with monoclonal antibodies to single stranded DNA. Apoptosis Detect Assay Methods. ISS2:47-62

[5] Maeda M, Sugiyama T, Akai F, Hayashi Y,Takagi H. Single stranded DNA asan immunocytochemical marker for apoptotic change of ischemia in the gerbil hippocampus. Neurosci Lett. 1998; 240:69-72

[6]. Didenko VV, Tunstead JR, Hornsby PJ. Biotin-labeled hairpin oligonucleotides: probes to detect double-strand breaks in DNA in apoptotic cells. Am J Pathol. 1998; 152: 897-902.

[7] Van Engeland M, Nieland LJ, Ramaekers FC, Schutte B, Reutelingsperger CP. Annexin V-Affinity assay: A review on an apoptosis detection system based on phosphatidylserine exposure. Cytometry. 1998; 31: 0-9.

[8] Grand RJA, Milner AE, Mustoe T, Johnson GD, Owen D, Grant ML, Gregory CD. A novel protein expressed in mammalian cells undergoing apoptosis. Exp Cell Res. 1995; 218: 439-41.

[9] Srinivasan A, Roth KA,Sayers RO, Shindler KS, Wong AM, Tomaselli K. Insitu immunodetection of activated caspase 3 in apoptotic neurons in the developing nervous system. Cell Death Differ. 1998; 5:1004-1016.

[10] Leers MP, Kölgen W, Björklund V, Bergman T, Tribbick G, Persson B, Björklund P, Ramaekers FC, Björklund B, Nap M, Jörnvall H, Schutte B. Immunocytochemical detection and mapping of a cytokeratin 18 neo-epitope exposed during early apoptosis. J Pathol. 1999; 187:567-572.

[11] Yang F, Sun X, Beech W, Teter B ,Wu S, Sigel J,Vinters HV,Cole GM. Antibody to caspase cleaved actin detects apoptosis in differentiatedneuroblastoma and plaque asso9ciated neurons and microglia in alzeimers disease. Am J Pathol. 1998; 152:379-389.

[12] Mark CW. Cytochemical methods for the detection of apoptosis. J Histochem Cytochem. 1999;47:1101.

[13] Collins JA, Schandl CA, Young KK, Vesely J, Willingham MC. Major DNA event is a late event in apoptosis. J Histochem Cytochem. 1997;45:923-934.