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Review Article

Annexin V and apoptosis – A review

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ABSTRACT: Apoptosis is an important mechanism happening in our body that basically is a form of programmed cell death developed to remove unwanted, damaged or senescent cells from tissues. Analysing the number of cells undergoing cell death helps us understand the underlying mechanisms of pathobiology. Annexin V has an affinity to apoptotic cells by binding to cell membrane proteins. Annexin V assays have been developed to further analyse the degree of apoptosis occurring during health and disease.

Keywords: Annexin V, apoptosis, plasma membrane, programmed cell death, DNA damage

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INTRODUCTION

To maintain tissue homeostasis, a balance between cell proliferation and cell death must be preserved. Abnormal cell accumulations, like in cancer, frequently result from a breakdown of this equilibrium [1]. Cell death and growth should be given equal weight. Necrosis and apoptosis are the two types of cell death. Different types of cell death are referred to by the word apoptosis and necrosis. The term "apoptosis," was first used to describe a particular kind of physical cell death by Kerr, Wyllie, and Currie in 1972 [2]. There are two potential apoptosis mechanisms. There are both internal and exterior pathways at play.

Cytotoxic stress causes the activation of BH3-only and multidomain pro-apoptotic Bcl-2 family members, which in turn activates the intrinsic pathway (mitochondrial) [3]. In particular, the multidomain Bcl-2 proteins Bax and Bak are directly activated by certain BH3-only proteins, such as tBid [4]. Anti-apoptotic Bcl-2 proteins like Bcl-2, which

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can be inhibited by a different group of BH3-only proteins known as indirect activators, are adversely regulated by this component. Cytochrome c and other pro-apoptotic intermembrane proteins are released when activated Bax/Bak oligomerise and cause mitochondrial outer membrane permeabilization (MOMP). Once in the cytosol, cytochrome c binds and converts pro-caspase 9 into an active initiator caspase 9 by causing Apaf-1 (apoptotic protease activating factor 1) to oligomerize into a huge protein structure known as the apoptosome. The executioner caspases, including caspase 3, are ultimately activated by the initiator caspase 9, which causes the cell to undergo apoptosis [3].

Specific receptors that are found on the cell surface activate the extrinsic route (death receptor). These receptors come from the family of tumour necrosis factors, which has sections known as death domain. Type I TNF receptor and Fas are examples of death receptors (CD95). Membrane-expressed on activated T cells, this Fas ligand is a protein. By binding adaptor proteins through the death domain, Fas molecules crosslink with each other and activate caspase-8. These caspases could join the intrinsic route by activating the pro-apoptotic Bcl-2 family member Bid [5].

In order to remove cells during development, tissue homeostasis, infection, or in reaction to injury, apoptosis, a type of programmed cell death, is frequently used. Apoptotic cells' altered plasma membranes cause phagocytes to recognise and engulf these cells. An effective method for identifying apoptotic cells is to measure the externalisation of plasma membrane phosphatidylserine using fluorescently tagged annexin V.

The plasma membrane of the apoptotic cell is occasionally exposed at their outer surface along the pathway. However, the mechanisms underlying it are not well explained [1]. Annexin V is a protein of significance that plays a key role in apoptotic mechanism. It detects and binds to phosphatidylserine, located on the apoptotic cell and serves as a marker for the identification of apoptotic cells. The plasma membrane of an apoptotic cell changes, exposing the phosphatidylserine on the surface membrane and preserving the cell's integrity [1].

APOPTOSIS – PHYSIOLOGIC CAUSES:

- The controlled cell death that occurs during embryogenesis.
- Upon hormone deprivation, hormone-dependent tissues evolve.
- Increase in the cell populations to keep the number of cells that have accomplished their activity constantly
- Self-reactive lymphocytes that may be harmful
- Cytotoxic T cells cause death

APOPTOSIS - PATHOLOGIC CAUSES:

- DNA damage
- a buildup of improperly folded proteins
- Infections

- Parenchymal organ atrophy caused by pathology.

This article's main goal is to review the fundamental relationship between apoptosis and annexin V.

CHANGES IN PLASMA MEMBRANE OF APOPTOTIC CELLS: Plasma membranes are composed of lipid bilayers. Examples of lipids include cholesterol, sphingolipids, and glycerophospholipids. When lipids are distributed unevenly between two leaflets, glycerophospholipids and shingolipids generate lipid assymetry, and when lipids are distributed unevenly within a single leaflet, cholesterol and shingolipids cause lipid microdomain. It is important to maintain the plasma membrane's adherence to mechanical stability.

Changes in the assembly are brought on by platelet coagulation, cell death, and cell cycle advancement [6]. Proteins with C2 or gamma-carboxyglutamic domains can attach to phosphatidylserine, one of the negatively charged lipids in the inner leaflet of the plasma membrane, which also makes it easier for polycationic ligands to interact electrostatically with cellular membranes [7]. It serves as a marker for apoptosis. Phosphatidylserine asymmetry is an early indicator of annexin V for apoptosis. It is believed that the so-called flippases, membrane proteins that make it easier for lipid molecules to move from one leaflet to the other, are responsible for maintaining the plasma membrane's lipid asymmetry in healthy cells [8].

Platelets and erythrocytes were the first cells to exhibit an irregular plasma membrane [9,10]. The erythrocyte membrane demonstrates actions that move aminophospholipids from one leaflet to the other, as was previously demonstrated serves as a sign of apoptosis [11]. In platelets, aminophospholipid translocation from one leaflet to another exhibits a comparable action, as shown by Comfurius et al [12]. In addition to having the capacity to translocate PS to the plasma membrane's outer leaflet with the aid of scrambalases, cells also have the capacity to maintain PS asymmetrically at the inner membrane leaflet via flippase and activity.

ANNEXIN V AND ITS ROLE IN DETECTING APOPTOTIC CELLS: Originally known as placental protein 4, annexin V was discovered by Bohn and colleagues in human placentas [13]. It also known as vascular-anticoagulant and have isolated it from umbilical cord [14]. Following the cloning and sequencing of the human annexin V cDNA, the protein was given its name because of its resemblance with the family of annexin proteins. Among annexin V's in vitro characteristics are its antiphospholipase, anticoagulant, antikinase, calcium channelling, and phospholipid binding capabilities [15,16,17,18,19]. Annexin V binds to PS when calcium is present. Furthermore, calcium is essential for Annexin V's ability to recognise apoptosis.

ANNEXIN V ASSAY: Koopman et al. developed the first technique for detecting apoptosis using an extrinsically applied hapten (such as FITC or biotin)-tagged annexin V. In the presence of calcium, hapten-labeled annexin V binds to PS on the outer leaflet of the plasma membrane of apoptotic cells. Important cells cannot be treated with annexin V due to the asymmetry of the lipids in the plasma membrane. However, because of the breakdown of the

plasma membrane in apoptotic cells, PS is shown moving from the inner to the outer leaflet, making it simple for annexin V to bind to PS in these cells [20]. The authors of this study used fluorescently-tagged annexin V, which binds to phosphatidylserine, to identify apoptotic cells using flow cytometry.

Due to the damage to the plasma membrane brought on by the attempt to separate cells into a single cell solution, this approach is not recommended for use with adherent or cells that have been isolated from solid tissue. Particularly adherent cells, which are typically separated from plastic dishes by enzymatic, chemical, or mechanical treatment, are susceptible to changes in Annexin V's adherence to PS. Any procedure that compromises the integrity of the plasma membrane will cause a cell to test positive for Annexin V. Plasma membrane integrity loss is demonstrated by PI staining. However, meticulous sample preparation can minimise the harm to the cell membrane.

They used Burkitt lymphoma cells and newly obtained germinal centre B cells. Normal, healthy cells did not take the dye, but chromatin condensation-affected cells did. This demonstrated that chromatin condensation happened concurrently with PS exposure [20].

Use the membrane-impermeable DNA dye propidium iodide to distinguish between apoptotic and dead cells. In light microscopy, necrotic cells exhibit both propidium iodide and annexin V positivity because the plasma membrane has deteriorated, but apoptotic cells preferentially stain with annexin V and do not show the propidium iodide stain [1]. Living cells are negative for PI and annexin V, but dead cells are positive for both.

Single cell suspension are the ideal technique to be employed with flow cytometry for the quantification of annexin V-positive apoptotic cells. Rat thymocytes treated with dxamethasone were used in this culture. After rats were exposed to dexamethasone for 4.5 hours, the number of apoptotic cells increased, whereas the quantity of dead cells remained constant. Van Engeland et al. demonstrated that the bivariate PI/annexin V analysis can be used to determine how well cells adhere in culture [21]. Using this method, membrane alterations are created that lead to PS exposure in the outer plasma membrane, most likely as a result of bleb formation during harvesting [22].

This technique for adhering cells uses adherent cell cultures that have been annexin V tagged before being harvested quantitatively. In order to detect and measure other intracellular antigens in the various cell populations, the annexin V-labeled cells can be preserved. Cells are labelled for this purpose using biotin-conjugated annexin V. With FITC-labeled streptavidin, annexin V binding is seen following fixation [21].

Using biotin-labeled annexin V, it is possible to perform in situ histochemical detection of apoptotic cells in mice. Following a 30-minute in-vivo biotinylated annexin V labelling procedure, the tissues were dissected, customarily fixed in formalin, and then embedded in paraffin. The tissue samples were dewaxed, rehydrated, and then given peroxidase-conjugated strepavidin. The outcomes were discovered using enzyme histochemistry. This is one way to use annexin V to recognise apoptotic cells [23].

The results of the Hammill et al investigation show that Annexin V staining and the ensuing loss of membrane asymmetry occur in this system prior to commitment to apoptotic death. They found that many of the cells that dye positively for Annexin V are still alive and can resume growth and phospholipid asymmetry after the signal is

removed [24]. Walton et al. conducted an animal study in rats to analyse the histochemistry of annexin V at various time points after ischemic insult. The CA1 pyramidal neurons that were affected by the hypoxic-ischemic injury that resulted in apoptosis showed a significant affinity for annexin V binding after the insult [25].

CONCLUSION

Regardless of the cell type, species, or reason, phosphotidylserine translocation from the inner to outer leaflet occurs frequently in apoptosis. One of the critical steps in studying medicine, making diagnoses, doing research, etc. is detecting apopotosis. It is among the easiest, fastest, and most productive methods for identifying apoptotic cells. The existence or absence of cellular features as well as other cellular characteristics like chromatic condensation can be ascertained using this technique.

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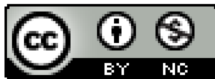

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