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A Review on Apoptosis: When Death Precedes Life

Dr. Pragya Panda[1], Dr. Subhashree Ray[2], Dr. Sudeshna Behera[3], Dr. Subhrat kumar Tripathy[4], Dr. Sangeeta S Bhanja[5], Dr. Viyatprajna Acharya[6]

[1]Final year post-graduate student, [2]Professor & HOD, [3]Associate Professor, [4]Associate Professor, [5]Assistant Professor, [6]Professor- Dept. Of Biochemistry, IMS and SUM Hospital, S'O'A University,Bhubaneswar]

pandapragya123@gmail.com

Abstract: Apoptosis process occurs when a cell decides to die by itself. This often happens for the better development and morphological changes of the whole organism, mostly to prevent cell damage and various cancers. Morphological and biochemical changes such as energy dependent mechanism plays a pivotal role in apoptosis or PCD(programmed cell death). It has a significant role in development of embryos, normal and proper cell turnover, homeostasis and its functioning. By this process atrophy of a cell occurs which may be hormone dependent or by chemicals. Due to disturbance in apoptosis or failure of regulation of apoptotic signals may lead to condition like damage of neurons, heart, blood regulation, autoimmune disorders or cancers. Study of apoptosis might play a vast development and acknowledgment of the huge therapeutic and palliative aspects of this. Many researches are going on for to understand thoroughly the mechanism of cell cycle and its pathways that is the key regulator of the apoptosis. Apart from cell cycle, the key proteins and enzymes and their mechanism remained to be explained. This review article mainly aims for better overview of current knowledge on the topic, mechanism of programmed cell death with health problems and detection methodologies, which is vast topic to explain but this article aims to concise and simplify it for better understanding.

Keywords: Intrinsic pathway, extrinsic pathway, Caspases, Apoptosome

1. INTRODUCTION:

Programmed cell death is very much crucial or important for normal growth and aging process of human beings as well as acts as A homeostatic tissue cell population maintenance mechanism. [1, 2] DNA of the cell receives various biochemical signals or instructions to complete the process of apoptosis. It is strictly controlled procedure that permits a cell to self-destruction for eliminating the dysfunctional cells. The process of apoptosis is somewhat similar to necrosis with few changes in it. Necrosis is where cell dies not by itself but due to outside trauma or other factors which deprive the cell morphology.

The period "Apoptosis" creates from Greek language "απόπτωσις" which earnings "dropping off" or "falling off" of leaves or petals from trees or flowers respectively.[3] so basically this language imaginarily explains the cell demise triggered by various physiological and pathological signals.

Starting from 19th century, number of theories have depicted that death of a cell possesses A central physiological and pathological process function in eukaryotic organisms, mainly in development of embryo and metamorphosis. In sixties the term 'programmed cell death' was introduced by Lockshin & Williams in the context of insect development pointing the fact that cell demise in the process of development is not by chance, but it follows a series of regulated steps resulting in locally and temporally self-degradation.[4,5]

John Kerr introduced the term 'apoptosis' in 1972, when by ultrastructural analysis he noticed a peculiar form of cell death with markedly different morphology to necrosis. In necrosis there occurs cell swelling, ruptured cell membrane and release of cellular components; whereas in apoptosis, the cell shrinkage occurs with integral but irregular cell membrane and nuclei are condensed and fractionated.

The biochemical events resulting in cell changes (morphology) and death include: cell shrinkage, membrane blebbing, nuclearfragmentation,chromatin condensation,chromosomal DNAsegregation.

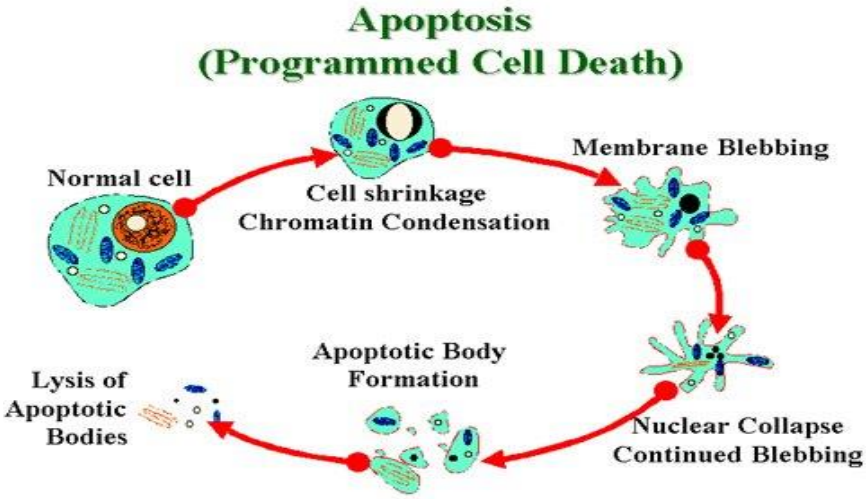

Figure 1 - different stages of apoptotic cell

In an average adults around billions of cell die per day by virtue of apoptosis to secure the functionality of the entire organism.[6] In order to maintain cell homeostasis, a stability among the increase (either due to differentiation from precursors or due non-proliferation) and decrease (may be due to differentiation) in the population of cell must be perfectly maintained.

Necrosis is a toxic process where the cell follows an energy independent mode of death. Different factors play a key role to differentiate between apoptosis and necrosis in which type of tissue, nature, developmental processes of the cell is included ,whereas physiological environment determines whether the cell dies by necrosis or apoptosis. Necrosis may be uncontrolled or controlled, passive or energy dependent, they may affect individual cell or cluster of cell or may affect large field of cells. The two issues that can change an on-going apoptotic procedure into a necrotic course are reduced obtainability of caspases and intracellular ATP. [7]

Cell swelling is a variety of morphological changes in necrosis;

Cytoplasmic vacuol formation; endoplasmic distended reticulum; creation of

Cytoplasmic blebs; ruptured mitochondria or swollen; Dismissal and

Ribosome objectivity; organelle membranes disrupted; lysosome swollen and ruptured; and cell membrane disturbance at the eventual time. Cell membrane withdrawal leads to the removal in the surrounding tissue of cytoplasmic contents which cause chemical signals. This lead to recruitment of inflammatory cells**.**

Macrophages eat the cellular content which released by apoptotic cells, so inflammatory reaction cannot be seen in contrast to apoptotic cells. [8]

2. NEED OF APOPTOSIS:

Apoptosis is a kind of regulated programmed cell death occurring by a series or chain of signal cascades. It involves in animal development. Its function or mechanism is reverse of a mitosis or cell proliferation but they are complementary to them. To maintain homeostasis in adult

Human body, it has estimated that each day billions of cell are formed to maintain crucial balance between the dying apoptotic cell and again regenerating new one. During normal development as age increases the rate of apoptosis also increases so ultimately leading to increase in apoptosis.

Programmed cell death plays an essential role in many physiological and developmental processes. For example, both the central nervous system and immunological system that produce due to overproduction of cells gradually followed by demise of those cells which cannot establish any functional synaptic connection or produce any antigen specificity respectively. [9]

During wound healing, apoptosis act as a vital component due to its active role in removing cells which causes inflammation and progressive granular tissue into scarred one. [10]

Elimination of activated or auto-aggressive immune cells , follicular atresia of post ovulatory follicles, involution of mammary gland are also mediated by apoptosis.[11,12]

Withdrawals of positive or negative signals are one of the reasons of apoptosis

Examples of Inter-leukin-2 (IL-2), positive signals are neuron growth factors.

Example of Negative signals include elevated cellular oxidants rates, oxidant damage to the DNA, death triggers: Tumour necrosis influence alpha (TNF-a), Lymph toxin (TNF-β), Fas ligand (FasL), Inducers of Apoptosis, TNF family, Growth factor withdrawal, Calcium, Oncogenes, Nutrient deprivation, Toxins, UV radiation, Gamma radiation.

Caspases : Caspases are aspartate-directed cysteine proteases (where "C" stands cysteine protease mechanism, and "aspase" for its ability to cleave after aspartic acid) involves as a significant part in the process of initiation and execution pathways of programmed cell death, inflammation and necrosis etc. It is therefore believed that decreased level or impairment of caspase function may cause tumour development and several autoimmune diseases. [13, 14]

Caspases are endopeptidases that have high specificity for substrates containing Aspartate, and they use catalytic Cysteine residues present in their active sites to catalyze the peptide bond cleavage. They are synthesized in the cell in their precursor form called procaspase which are only activated following appropriate stimulus.

There are 3 types of caspases such as: [15]

Inflammatory Caspases: 1, 4, 5 and 12 (mainly involved in the process of inflammation),Initiator Caspases: 8, 9 and 10 and Effector Caspases: 3, 6, 7 and 14 (both initiator and effector caspases are involved in apoptosis).These caspases initiate cell death, play an important part in apoptosis by cutting off contact with adjacent or surrounding cells, reorganizing the cell skeleton, it hampers the DNA's replication, repair, and interrupt splicing and disrupt the DNA and nuclear structure and make it suitable for phagocytosis.

Caspases in Apoptosis:

Apoptosis is controlled or up regulated by deconstructing the intracellular components to avoid inflammation and damaging of surrounding cells. As inactive procaspase monomeric forms, the initiator caspases-8 and -9 usually exist. The working principle of initiator caspases is activated by the phenomenon of dimerization which ultimately increases autocatalytic breakdown or cleavage of this caspase monomer. This cleavage gives stability to dimer by breking into one large and one small subunits. [16, 17]

Execution caspases are present in their inactive dimeric forms that need to be cleaved for activation. Due to the cleavage a conformational changes takes place which permits the two active site that is large and small subunits of the dimer, that comes together and form a very

much mature and functional protease which further cleaves and activates other execution caspases. [18]

A series of caspases activation occurs which include activation of executioner caspases by initiator caspases which ultimately coordinated for destruction of proteins and enzymes for eg- DNA fragmentation and membrane blebbing.

3. PATHWAYS

Apoptosis has 2 pathways namely : INTRINSIC or EXTRINSIC, either one of the two can initiate apoptosis whereas both of this pathway leads to 'execution phase' which is the final stage of apoptosis that occurs through activation and function of various caspases

Intrinsic pathway:

- Intrinsic pathway gets initiated from within the cell in the mitochondria. It triggers apoptosis in response to various internal stimuli like [19,20]
- Biochemical stress
- DNA damage (p53 gene is activated in response to DNA damage that halts cell cycle and initiates repair of DNA. If repair attempt is ineffective, the cell enters into apoptosis)
- Deficiency of growth factors

The two groups of molecules that modulate the intrinsic pathway are Bcl-2 and Bax .When Bax is activated, it will lead to formation of Bax-Bax dimer which ultimately enhances the action of varieties of apoptotic stimuli. This increases the susceptibility of a cell to apoptosis.

The BCL-2 protein family

Some 30 years ago various Studies have shown that a fusion of the strong immunoglobulin chain with the BCL2 loci in acute B-cell leucemia and follicular lymphoma cells contributes to the overexpression of BCL-2 occurs between chromosome 14 and 18 t (14; 18). [21-25] .Later it was found that BCL2 has some suppressing action on apoptosis for which the survival period of these cells improves.[26-29] Some other genes that code for both pro- and anti-apoptotic have since been identified having varying degree of homology with BCL2. [30] The anti-apoptotic BCL2 proteins are Bcl-2, Bcl-x, Bcl-x_L, Bcl-X_S, Bcl-w, and BAG which share BCL2 homology (BH) 1 , 2, 3 and 4 areas of structural homology; The Bax, Bak, Bid, Bad, Bim, Bik, Hrk, and Blk pro-apoptotical proteins share homogeneity only in the BH3 domain.

The anti-apoptotic proteins directly interact with pro-apoptotic BH3-only proteins. Balance between the pro- and anti-apoptotic groups of proteins determines the susceptibility of a cell to apoptosis. In simple words the balance between these two groups establishes a 'molecular switch" that determines if a cell will survive or enter into apoptosis in response to internal stimuli. [31, 32]

Whenever there is any apoptotic stimulus, it up-regulates BH3-only proteins and/or down-regulates of anti-apoptotic BCL-2 family proteins.

Pro-apoptotic BH3-only proteins now activate BAX and BAK and induces conformational changes leading to assembly of BAX/BAK proteins forming multisubunit pores called permeability transition pore complex (PTPC) in The mitochondrial external membrane facilitates permeabilization of the mitochondrial outer membrane (MOMP). Several proteins stimulate or suppress apoptosis and also affect opening and closing of PTPC. MOMP is regarded as the "point of no return" for apoptosis. The steps leading up to MOMP can be blocked in their tracks by various inhibitor molecules, but once the stage of MOMP has been reached, the cell will definitely undergo its death process. Due to this outer membrane permeability, cytochrome C releases from intramembranous space to cytosol. [33-35]

Other pro-apoptotic proteins that are also escaped from room in the cytoplasm of the mitochondrial intermembranium AIF (apoptosis inducing factor),Smac (second mitochondria-derived activator of caspase), DIABLO (direct IAP Binding protein with Low pI) and HtrA2/Omi[36]

Cytochrome C now binds with ApaF1 and procaspase 9 forming apoptosome. Apoptosome comprises of seven units of cytochrome C and seven ApaF1. Apoptosome causes dimerization of procaspase 9 creating active caspase 9 dimer. Here cytochrome C shows 'MOONLIGHT EFFECT.'

The roles of HtrA2/Omi and Smac/DIABLO are to enhance apoptosis & promote caspase action by binding with IAP (Inhibitors of Apoptosis Proteins) which results in disruption of IAPs with caspase-3and 9. [37]

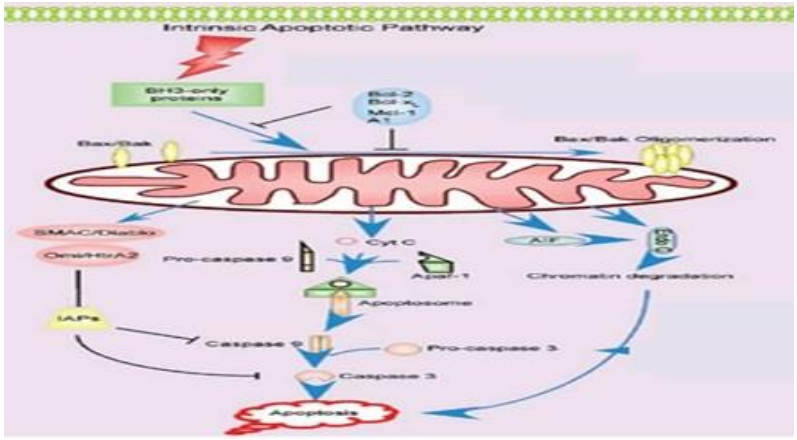

Figure 2 - the intrinsic pathway

Extrinsic pathway:

In Extrinsic pathway an external stimuli called ligand binds with the cell surface receptors and instructs cell to undergo apoptosis. It happens when a cell is either diseased or no longer required.The death factors or ligands that trigger extrinsic pathway to apoptosis belong to TNF personal (FasL,Fas Ligand;TNF-α,Tumor Necrosis Factor-α; TRAIL,) [38, 39]

These ligands are type II membrane protein, having 3 identical unit of polypeptide (homotrimeric structure), which is soluble (when separating it from the membrane)

Death receptors binding on these ligands belong to the superfamily gene of the tumor receptors (TNF). [40] These Members have two domains; extracellular cyteine-rich domains and a cytoplasmic domain known as "death domain" consisting of about 80 amino acids. The later one plays an essential component to transmit death signals to the intracellular signal's pathway from cell surface. [41, 42]

The best characterised ligand-receptor combination that describes the sequence of events of extrinsic pathway is the FasL/FasR and TNF-*α*/TNFR1 models. When

Fas ligand binds to Fas receptor or TNF ligand binds to TNFreceptor, there occurs conformational change of intracellular death domain of the receptors .This leads to recruitment of the adapter proteins like FADD (Fas-associated death domain) in case of FasL/FasR model and TRADD (TNF receptor associated death domain) along with some downstream factors FADD and RIP in case of TNF-*α*/TNFR1 model respectively. [43]

Upon activation of FADD, two other proteins such as pro-caspase 8 and pro-caspase 10 are interacted. The inactive pro-caspases transform into active caspase-8 and caspase-10 which are romantically referred as "the beginning of the end" by the scientists as they play main role in starting cell death.

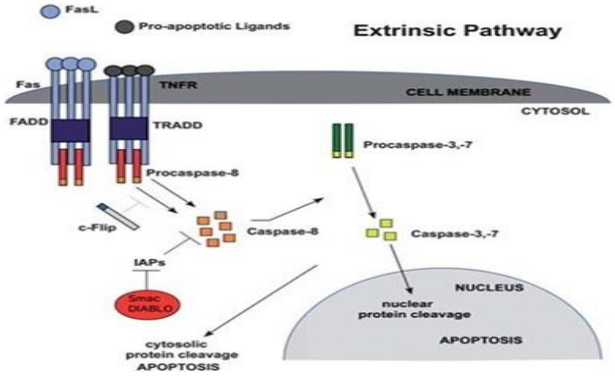

Figure 3 - the exrinsic pathway

These activated caspases scatter in the cytoplasm and transform another inactive molecule BID into active Tbid by removing the inactive part of BID. tBID now enters into mitichondia where it activates molecules BAX and BAK. After activation of BAX and BAK,the subsequent steps are the same between bothintrinsic and extrinsic pathways.

4. PERFORIN/GRAZYME PATHWAY:

Perforin/granzyme apoptosis path is the main gesturing way used by adaptive immune system that involves natural killer (NK) cells and CD8+ cytotoxic T lymphocytes (CTLs) to target and eliminate virus-infected and/or transformed cells.

Once the target cells are recognised, equally NK cells and CTLs perform comparable effector purpose to eliminate the board lockup. This is accomplished by releasing cytotoxic granules containing perforin and granzymes [44]. Perforin is a pore-forming protein whereas Granzyme is a family of structurally related serine proteases and both act cooperatively to induce target cell apoptosis. They attach to the surface of target cells along with serglycin as a single macromolecular complex which also prevents passive diffusion of granzymes.

Out of various granzymes like granzyme (A, B, H, K, and M) in human,) and granzyme B (GrB) remain greatest common as well as abundant ones and have been deeply studied. In cytoplasm Granzyme B mainly activates Bid(BH3 interacting domain death agonist)which is member of BCL-2 family of protein ,together with pro-apoptotic Bak and Bax protein result in leakage of cytochrome c into the cytosol. Cytochrome c the pro-formal caspase-3 turns into active caspase-3 that initiates Apoptosis Implementation Path through caspase-10 activation.

In contrast The Granzyme A path avoids the Implementation route, induces loss of mitochondrial inner membrane potential and liberates reactive oxygen species(ROS).It produces single stranded DNA nicks. In response to ROS, the ER-associated complex (SET, Ape 1, PP32, HMG2, TREX)trans locates to the nucleus, where granzyme A cleaves those members of the complex e.g. SET complex,that are involved in DNA repair resulting apoptosis inducing DNA degradation. [45, 46]

5. EXECUTION PATHWAY

The intangible, endogenous and perforated paths all end on the same terminal path known as the execution direction. This contributes to numerous changes in anatomy and biochemistry that illustrate apoptosis.

The initiator caspases like caspase 8,10 of extrinsic pathway and caspase 9 of intrinsic pathway that get activated by specific triggering signals finally activate the executioner caspases of execution pathway.

Caspase-3, 6, 7 function as effector or "executioner" caspases, out of which the most important one is Caspase 3 and is activated by initiator caspases. Caspase-3 specifically activates the endonuclease CAD (caspase activated deoxynuclease) that is responsible for nuclear apoptosis. CAD translocates the nucleus to fragment DNA causes chromosomal condensation by degrading chromosomal DNA within the nuclei. Caspase-3 also persuades cleavage of protein kinases, Endonuclease family proteins and DNA reparation inhibitors. Along with it also brings about cytoskeletal reorganization and disintegration of the cell contributing to the characteristic morphological changes in apoptosis. [47]

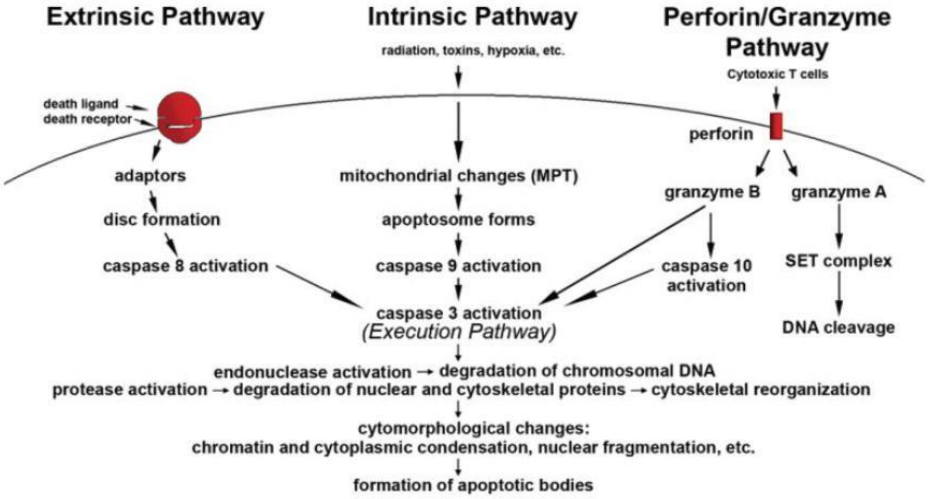

Figure 4 - the execution pathway

6. REGULATION OF APOPTOSIS

Apoptosis principals to a sequence of complex proceedings that includes activation of receptor complexes by external stimuli, expression of various genes in a well-controlled manner, activation of caspases and endonucleases to finally execute the procedure of involuntary cell demise. A huge numbers of genes and proteins play their role in different steps of apoptotic pathway and regulate apoptosis. [48]There exists a strict homeostatic regulation via number of regulators of modulators of the death pathway.

The irreversible checkpoint in apoptosis is achieved when caspases become enzymatically active and start cleaving the executioner proteins. Any inequity of pro-apoptotic and anti-apoptotic members of the Bcl-2 personal like Excessive anti-apoptotic protein expression or preoptic protein under expression can lead to dysregulated apoptosis in the cancer cell. [49, 50]

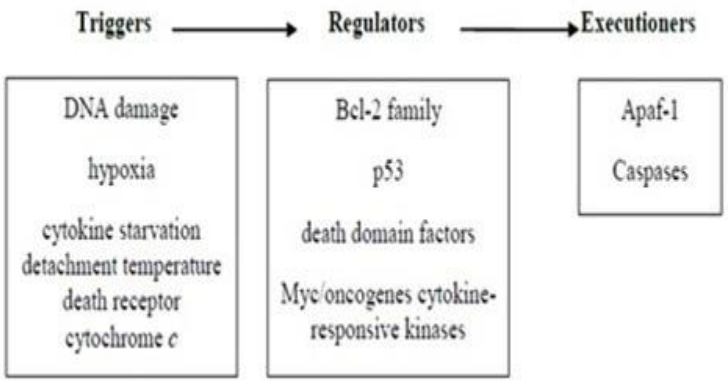

Figure 5 - the apoptotic cascade

Role of Bcl-2 family proteins: Any imbalance between the pro- and anti-apoptotic factors of Bcl-2 family may results in dysregulation of apoptosis in affected cells. As suggested by Raffo et al in his study, Protected prostate cancer cells from apoptosis overexpression of Bcl-2. Similarly Fulda et al found Bcl-2 resulted in inhibition of TRAIL-induced apoptosis in various cancerous conditions like neuroblastoma, glioblastoma, breast carcinoma cells.[51] In another study, researchers documented multidrug resistance phenotype in tumour cells that prevent them from undergoing apoptosis.[52] Similarly leukemogenesis in CLL (Chronic lymphocytic leukemia) is not due to increased proliferation of cells but due to High anti-apoptotic Bcl-2 and low pro-apoptotic proteins such as Bax leading to reduced autophagy. [53]

Role of p53: p53 also known as Tumour suppressor gene that encodes tumour suppressor protein which is otherwise known as p53 protein that is present in the petite arm of DNA 17. This protein is name is based afterward its molecular weight that is 53kDa. Since its discovery many studies have been done to find out significance and its functional role in cancer. More than 50% of cancerous conditions are associated with any impairment of p53 tumour suppressor gene. [54]

Melanoma cells can expresses some target gene of p53. p53involves in regulation of cell cycle and apoptosis. Both the mechanism may affect activity of p53 ultimately leading to proliferation of melanoma cells. [55]

Inhibitor of apoptosis proteins (IAPs)

Apoptosis is also regulated by IAPs, a group of related proteins in structure and functionality that illustrate a specific repeat domain called baculovirus IAP repeat (BIR) protein domain. These conserved BIR domains bind with the active sites of caspases and act as endogenous inhibitors of the caspases. They keep the caspases away from their substrates and promote their degradation resulting in impairement of the process of apoptosis. [56]

Lopes *et al.* reported development of resistance of pancreatic cancer cells towards chemotherapy is due to abnormal expression of the IAP family. [57]

Various other studies demonstrated the overexpression of living variety of IAPs in lymphoma and melanoma. [55, 58] Whereas Apollon in gliomas were responsible for cisplatin and camptothecin resistance.[59]

Detection of apoptic cells

Different Approaches of Apoptosis Finding Are: [60]

Electrophoresis (EA) of DNA agarose gel, Flow cytometry (FCM),Situ3-End labeling (ISEL) Light microscopy, Special stains / fluorescent stains, Immunohistochemistry

(IHC),electrone microscopies, Polymerase chain reaction (PCR), terminal deolynaic transferoscope-mediated dUTP-biotin nickel end labeling (TUNEL)

7. CONCLUSION:

Apoptosis is an extremely complicated as well as interesting phenomenon mainly to approach the innovation of the new anticancer therapies by the various mutations in the pathways i.e. extrinsic or intrinsic. Study of mechanism of apoptosis or programmed cell death is the crucial for cancer therapies. Apoptosis is a natural event for the development and homeostasis of the body. Apoptotic cells cause changes in the genes, its proteins and pattern of cell leading to its death. Detection and diagnostic approach of these cells and its regulation has openednew pathway for future in oncology and allied diseases. More in-depth study on tumour genesis, apoptosis and drug targets is required. In this article we have concluded in brief the development, significance, morphological features, molecular mechanism and diagnosis of apoptosis.

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