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APOPTOSIS (PROGRAMMED CELL DEATH): A REVIEW

Tajamul Islam Shah*[1] and Zahoor Ahmad Lone[2]

[1*]Department of Zoology, Government Boys Degree College Baramulla, Jammu and Kashmir, India.
[2]Department of Botany, Government Boys Degree College Sopore, Jammu and Kashmir, India.

ABSTRACT

The process of programmed cell death or apoptosis is an integral part of various processes. It plays a pivotal role in numerous cellular activities which includes, proper embryonic development, normal cell turnover, normal functioning of bodies defense mechanism, chemical induced cell death and also in the removal of cancer cells. Inappropriate apoptosis may lead to genesis of many life threatening human conditions including neurodegenerative diseases, ischemic damage, autoimmune disorders and many types of cancers. The regulation of the cell death and cell survival is important for normal functioning of the body and is controlled by many apoptotic key factors. Any abnormality in the factors may have grave consequences and make apoptosis an unusual process. Researchers across the globe continue to focus on cell cycle machinery and signaling pathways that control cell cycle arrest and apoptosis. To that end, the field of apoptosis research is increasing at an alarming rate and heads a way in the elucidation of many diseases their treatment and eradication including dreaded cancer. In this review an approach has been made to provide an overall view of apoptosis and its pivotal role in the formulation of new drugs in cancer therapy.

KEYWORDS

Apoptosis, Programmed cell death, Intrinsic/extrinsic pathway and Cancer.

Author for Correspondence:

Tajamul Islam Shah,
Department of Zoology,
Government Boys Degree College Baramulla,
Jammu and Kashmir, India.

Email: taju.zoology@gmail.com

INTRODUCTION

The term apoptosis (a-po-toe-sis) was first used in a now-classic paper by Kerr, Wyllie, and Currie in 1972 to describe a morphologically distinct form of cell death, although certain components of the apoptosis concept had been explicitly described many years previously[1-3]. Our understanding of the mechanisms involved in the process of apoptosis in mammalian cells transpired from the investigation of programmed cell death that occurs during the

development of the nematode *Caenorhabditis elegans*[4-7]. In this organism 1090 somatic cells are generated in the formation of the adult worm, of which 131 of these cells undergo apoptosis or "programmed cell death." These 131 cells undergo apoptosis at appropriate time in a controlled manner with sharp accuracy. However, it is important to note that other forms of programmed cell death have been described and other forms of programmed cell death may yet be discovered[8-10]. Apoptosis is also important in maintaining homeostatic mechanism of ageing and development and ensure normal cell populations in tissues. Apoptosis also occurs as a defense mechanism such as in immune reactions or when cells are damaged by disease or noxious agents[11-13]. The cells respond to variety of stimuli to maintain balance between cell death and cell survival and also not all cells will necessarily die in response to the same stimulus. The conventional methods used to treat cancer cells do so by mutating the genetic makeup of same cells which ultimately result into apoptotic death through a *p53*-dependent pathway. Some hormones, such as corticosteroids, may lead to apoptotic death in some cells (e.g., thymocytes) although other cells are unaffected or even stimulated. Some cells express Fas or TNF receptors which are actually called as death receptors that can lead to apoptosis via ligand binding and protein cross-linking. Other cells use default death pathway and must be inhibited by action of survival factors such as a hormone or growth factor. There is also the issue of distinguishing apoptosis from necrosis, two processes that can occur independently, sequentially, as well as simultaneously[14-17]. In some cases it's the type of stimuli and/or the degree of stimuli that determines if cells die by apoptosis or necrosis. At low doses, a variety of injurious stimuli such as heat, radiation, hypoxia and cytotoxic anticancer drugs can induce apoptosis but these same stimuli can result in necrosis at higher doses. Finally, apoptosis is a coordinated and often energy-dependent process that involves the activation of a group of cysteine proteases called "caspases"[17-20] and a complex cascade of events that link the initiating stimuli to the final demise of the cell.

Apoptosis vs. necrosis

Broadly speaking, there are two ways that cells die in a multi-cellular organism such as you:

They die when attacked by harmful things (such as toxic chemicals or physical injury), a process called necrosis.

They are triggered to undergo programmed cell death and the best-understood form of programmed cell death is called apoptosis.

Necrosis and apoptosis occur under different circumstances and involve different steps. Simply put, necrosis is messy and causes an immune response of inflammation, while apoptosis is tidy and splits the cell into little parcels that can be taken up and recycled by other cells.

Necrosis (the messy way)

When cells are damaged by harmful factors (such as injury or toxic chemicals), they usually "spill their guts" as they die. Because the damaged cell's plasma membrane can no longer control the passage of ions and water, the cell swells up, and its contents leak out through holes in the plasma membrane. This often causes inflammation in the tissue surrounding the dead cell.

Apoptosis (the tidy way)

Cells that choose the path of apoptosis have to go through a different and much more orderly manner. They shrink and develop bubble-like protrusions called as "blebs" on their surface. The DNA in the nucleus gets chopped up into small pieces, and some organelles of the cell, such as the endoplasmic reticulum, break down into fragments. In the end, the entire cell breaks up into small fragment and chunks, each neatly enclosed in a package of membrane.

What happens to the chunks? They release signals that attract debris-eating (phagocytic) immune cells, such as macrophages. Also, the fragments of the dying cell display a phospholipid molecule called phosphatidylserine on their outer surface which is usually present on the inside of the membrane, and when it is on the outside, it acts as eat me signal and

lets the phagocytes bind and "eat" the cell fragments[20-23].

Apoptosis pathways

Apoptosis can be initiated by one of two separate pathways; the intrinsic or extrinsic pathway. Both of these pathways end with a final common effecter pathway, known as the execution phase.

Intrinsic pathway

The intrinsic pathway mainly triggers apoptosis in response to internal stimuli which may include:

- Biochemical stress
- DNA damage (this activates the p53 gene which halts the cell cycle and initiates DNA repair. If this repair attempt is unsuccessful, apoptosis can be induced)
- Lack of growth factors

The intrinsic pathway is modulated by two groups of molecules, Bcl-2 and Bax. Activation of Bax leads to the formation of Bax-Bax dimers, which in turn enhances the action of a variety of apoptotic stimuli - increasing a cell's susceptibility to apoptosis. The Bcl-2 family consists of both pro- and anti-apoptotic members, and it is the balance between these that determines how susceptible a cell may be to apoptosis.

To maintain the balance between these groups of pro-apoptotic and anti-apoptotic factors is essential to determines whether a cell will survive or undergo apoptosis in response to internal stimuli,

Extrinsic Pathway

The extrinsic pathway triggers apoptosis in response to external stimuli, usually by ligand binding at 'death' receptors on the cell surface. These receptors are typically members of the Tumour Necrosis Factor Receptor (TNFR) gene family, such as TNFR1 or FAS. Binding at these receptors leads to receptor polymerisation to initiate downstream caspase activation.

Execution Phase

The initiation of apoptosis by either pathway results in a cascade activation of caspases. These are specialized proteases which normally reside as inactive precursors within the cell.

The apoptosis triggers by first activating other initiator caspases, which includes caspase 8 which in turn activates other pro-caspases by cleaving them into active "executioner" caspases. These executioner caspases then lead to degradation of a variety of internal cellular structures, such as the cytoskeleton and nucleus. For example, caspase 3 activates DNAse leading to fragmentation of DNA. These entire processes have effect on morphology of a particular cell which includes DNA fragmentation and nuclear shrinkage. Despite, the cell itself shrinks but retains an intact plasma membrane. The dead cells are either immediately phagocytosed by adjacent neighboring cells or break down into smaller, membrane bound vesicles, known as apoptotic bodies which are eventually phagocytosed.

Regulation of apoptosis

There are a variety of factors responsible for regulating apoptosis, both intracellular and extracellular. External signals can include growth factors or specific signals from other cells, whereas internal factors can include DNA damage or failure of cell division.

Why do cells undergo apoptosis?

Most of the cells in our body have inherited ability to undergo apoptosis like their ability to replicate their genetic material. Moreover, apoptosis is a convenient way to get rid of unnecessary cells that are no longer functional.

- Some cells need to be "deleted" during embryonic development e.g., to carve out cells from a larger block of tissue between digits of hand.
- Some cells are abnormal and need to be wiped out to prevent serious effects on adjacent cells, such as cells with viral infections or DNA damage.
- Cells in an adult organism may be eliminated to maintain balance - to make way for new cells or remove cells needed only for temporary tasks.

Microscope images from a scientific paper, showing a developing mouse paw. The cells between the developing digits are stained by a marker that indicates apoptotic cells.

Image modified "Duplication of digit 4 is preceded by reduced apoptosis and expanded chondrogeneis in the posterior limb mesenchyme." By Farin *et al.* CC BY 4.0.

Apoptosis is part of development

In many organisms, programmed cell death is a normal part of development. In some cases, apoptosis during development occurs in a very predictable way: As already explained in the introductory part that in the worm *C. elegans*, 131 cells will die by apoptosis as the worm develops from a single cell to an adult (and we know exactly which ones they are). Apoptosis also plays a key role during embryonic of human. For instance, as we saw in the introduction, our hand started growing out as webbed and paddle-like block of tissue in an embryo, later on the block was "carved" in between the digits of fingers by apoptosis of the cells in between the developing fingers.

This process is usual in all vertebrate species that have finger- or toe-like digits, and less occurrence of apoptosis end up in to more webbing between the digits or fingers. Sometimes, a subtle hindrance in the process of apoptosis during finger or toe development, results in to fused toes. Other examples we can cite is the loss of tail in tadpole larvae into tailless adult frog.

Apoptosis can eliminate infected or cancerous cells

In certain circumstances, an infected cell can pose a threat to the rest of the body if it survives. For instance, this may be the case for cells with DNA damage, pre-cancerous cells, and cells infected by viruses. If these cells become the victim of apoptosis, the threat to the rest of the organism (such as cancer or spread of a viral infection) is removed. When a cell's DNA is damaged by somehow, it will call upon DNA repair enzymes and will try to fix it. If the damage is beyond repair, the cell will normally call upon into activation of pro-apoptotic factors and inhibition of anti-apoptotic factors and finally undergo apoptosis, ensuring that it will not pass on its damaged DNA to daughter cells. If cells with damaged DNA escape the process of apoptosis, they may produce new cells with all damaged section of DNA and may form cancer. Sometimes, pre-cancerous cells that have avoided internal apoptosis cues are detected by immune cells, which try to trigger apoptosis through an external signaling pathway. Cancer cells, however, manage to escape both internal and external cues that would normally trigger apoptosis. This adaptation allows successful cancer cells to lose control over cell cycle and proliferate in uncontrolled fashion and accumulate mutations in genetic material.

Apoptosis is key to immune function

Apoptosis also plays an important role in maintaining the healthy body of an organism in accordance with its healthy immune system. When B and T cells (immune cells that bind specific molecules) are first produced, they're tested to see if they react against any of the body's own "self" components. Cells that have high affinity or react with body's own cells or components are eliminated right away by apoptosis. If this process fails, self-reactive cells may be released into the body, where they can attack tissues and cause autoimmune conditions[24-27]. Apoptosis also has a role in switching off the immune system with no response to a pathogen. When a pathogen is detected, the immune cells that recognize the pathogen divide extensively, undergoing a huge increase in numbers with the purpose of destroying the pathogen. Once the pathogen is cleared from the body, the large numbers of pathogen-specific immune cells are no longer needed and must be removed by apoptosis to maintain homeostasis (balance) in the immune system.

Apoptosis and cancer therapy

A number of anticancer agents currently in vogue are designed to identify agents that selectively kill tumor cells. Until recently, most research into drug action focused on their intracellular targets, the nature of the cellular damage produced by the drug-target interaction, or resistance mechanisms that prevent the drug target interaction. However, in the 1970s pathologists noticed that radiation and chemotherapy can induce cell death with

morphological features of apoptosis, although the significance of these observations was not widely appreciated. In particular, the premise that anticancer agents induce apoptotic cell death implies that cellular responses occurring after the drug-target interaction can have impact on drug-induced cell death. It is now well-established that anticancer agents induce apoptosis, and that disruption of apoptotic programs can reduce treatment sensitivity. Since agents with distinct primary targets can induce apoptosis through similar mechanisms, mutations in apoptotic programs produce multi-drug resistance. For example, many agents activate p53, and that p53 loss can attenuate drug-induced cell death. Moreover, *p53* mutations reduce therapy-induced apoptosis and tumor regression in experimentally generated and spontaneous murine tumors[27-30], whereas re-introduction of normal p53 to *p53* mutant tumor lines and xenographs cooperates with chemotherapy to induce apoptosis and tumor regression. p53 is not strictly required for drug-induced cell death; indeed, at sufficient doses virtually all anticancer agents induce apoptosis (and other types of death) independently of p53. In fact, the contribution of p53 to drug-induced apoptosis is determined by a variety of factors, including agent, dose, tissue and mutational background of the tumor. In short-term assays, Bcl-2 can promote resistance to a wide range of anticancer and can even prevent p53-independent deaths. Because Bcl-2 is considered as a general apoptosis inhibitor, these results argue for the broad importance of apoptosis in treatment sensitivity. Additionally, death receptor pathways may also contribute to therapy-induced apoptosis, although the relative contribution of these effects is controversial. In human cancer, the most compelling links between apoptosis and treatment sensitivity occur in patients with leukemia or lymphoma. In these malignancies, *p53* mutations correlate with short remissions and drug resistance following therapy. Also, *INK4a*/*ARF* mutations, which reduce cyclophosphamide-induced death in murine lymphomas, are associated with poor treatment outcome in acute lymphoblastic leukemia. The extent to which apoptosis contributes to treatment sensitivity in carcinomas is less clear: whereas some studies identify striking correlations between *p53*mutations and poor treatment response, others see no effect. Few studies have associated Bcl-2 with drug resistance in patients and, in fact, high Bcl-2 levels may be a good prognostic indicator for breast cancer. Finally, in some settings loss of Bcl-2 and p53 delays therapy-induced apoptosis but does not enhance long-term survival in clonogenic assays. What could explain these discrepancies? Though it is possible that apoptosis does not contribute to treatment sensitivity in solid tumors, some caveats are worth mentioning. First, clinical studies typically examine single alterations (e.g. *p53* mutation) relying on detection methods that are not perfect and cannot exclude extragenic mutations in the same pathway. This makes it virtually impossible to determine negative results. As an example, murine lymphomas harboring *INK4a*/*ARF* mutations are chemo resistant, display defective p53 function, but retain wild-type *p53* genes. These tumors would be mistakenly classified as `p53 normal' by current technologies. Secondly, while clonogenic survival is often considered the `gold-standard' of cytotoxicity assays, this readout does not always reflect the *in vivo* response. It is possible that extracellular survival factors influenced by cell density or microenvironment can affect drug-induced death. Anticancer agents induce apoptosis in normal tissues as well as in tumors. In fact, many of the pathologists who identified apoptosis in tumors realized that apoptotic cell death was induced in a subset of normal tissues (e.g. bone marrow and intestine), and it was suggested that the process might contribute to the `toxicity' associated with chemotherapy. Studies using mouse models provide strong support for this idea. For example, moderate doses of radiation and chemotherapy induce apoptosis in the murine thymus, spleen, bone marrow and intestine, the same tissues that account for the deleterious side-effects of chemotherapy. However, these tissues in p53

`knockout' mice display much reduced apoptosis and cell loss following radiation or chemotherapy, and these animals are resistant to otherwise lethal doses of ionizing radiation. Similarly, ectopic expression of Bcl-2 in bone marrow cells achieves a similar effect. Together, these studies strongly suggest that drug-induced apoptosis causes loss of normal cells and contributes to the side effects of cancer therapy.

Table No.1: Comparison of morphological features of apoptosis and necrosis

S.No	Apoptosis	Necrosis
1	Single cells or small clusters of cells	Often contiguous cells
2	Cell shrinkage and convolution	Cell swelling
3	Pyknosis and karyorrhexis	Karyolysis, pyknosis, and karyorrhexis
4	Intact cell membrane	Disrupted cell membrane
5	Cytoplasm retained in apoptotic bodies	Cytoplasm released
6	No inflammation	Inflammation usually present

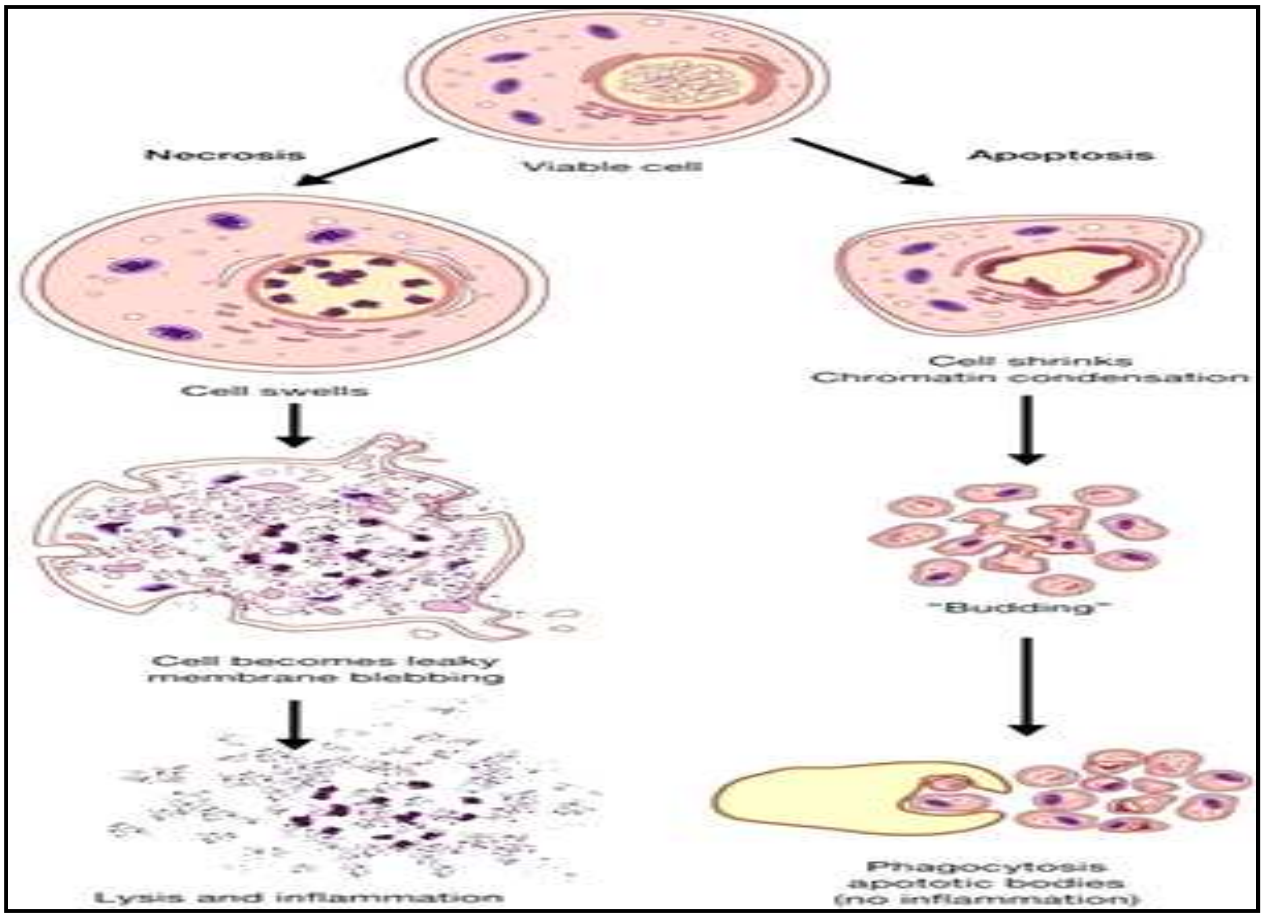


Figure No.1: Apoptosis VS Necrosis

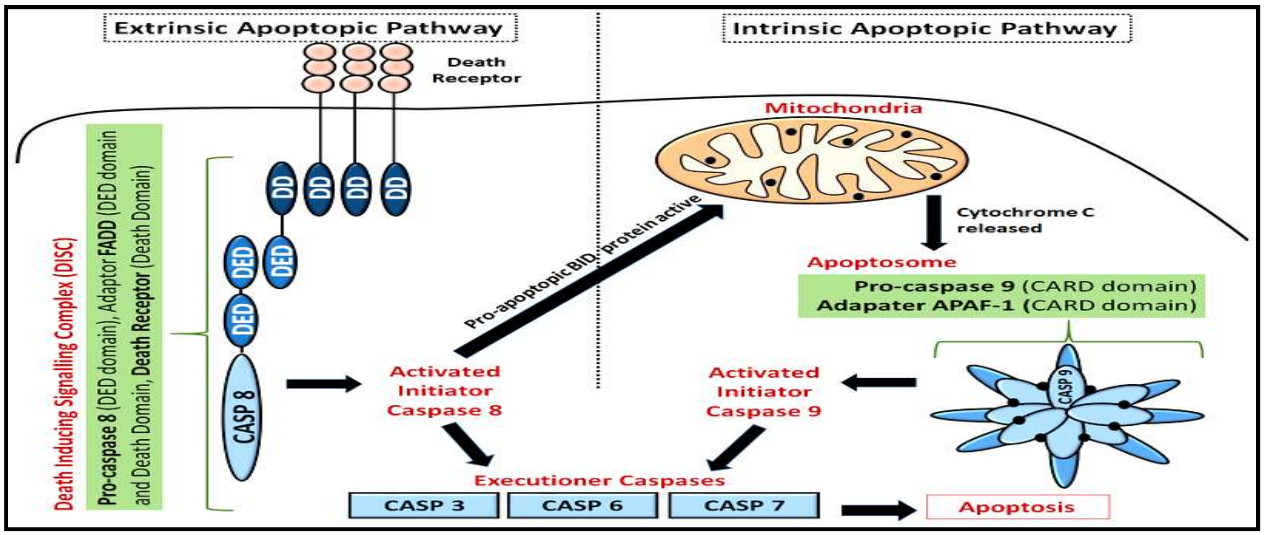
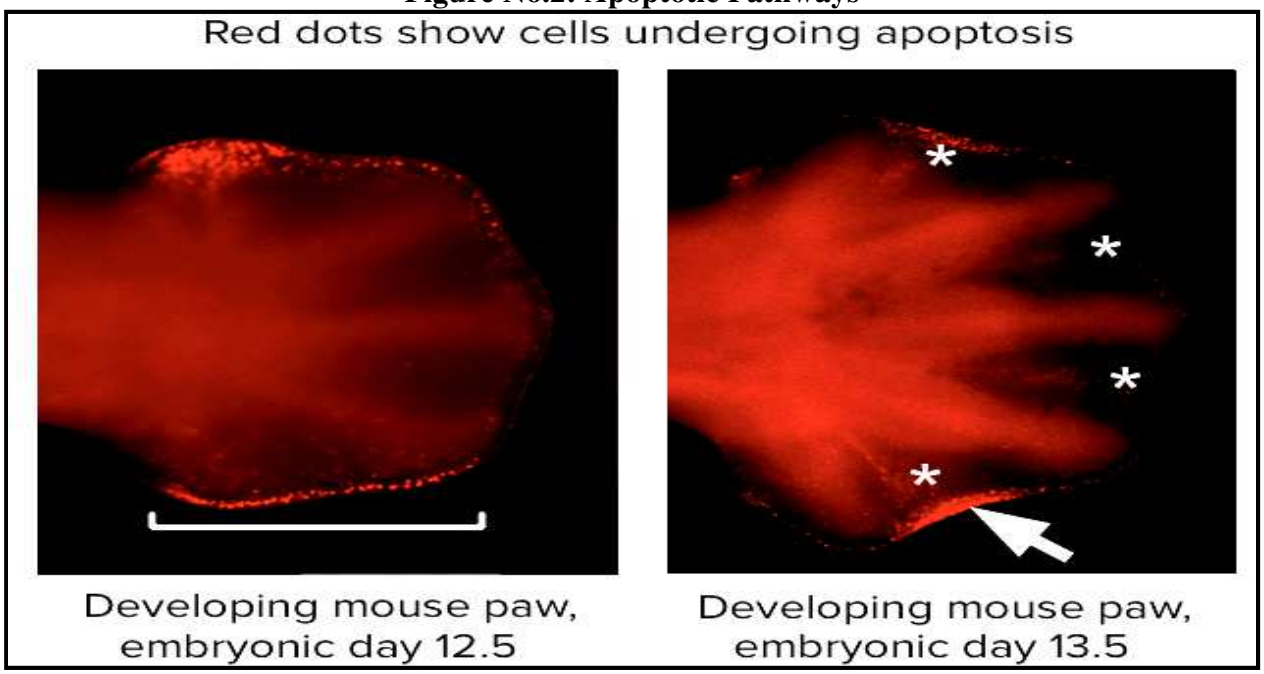


Figure No.2: Apoptotic Pathways



CONCLUSION

Apoptosis is a normal and important process of the body. It is controlled and regulated by a number of signals. The signals may comprise intrinsic as well as extrinsic depending upon cognate factors and lead to programmed cell death. Any avoidance to apoptosis due to certain biochemical insult which includes mutation may have grave consequences. To make the apoptosis normal process of the body, cells need to cross talk between themselves in order to screen the outside environment and function properly. However, due to certain mutations in the signaling as well as regulating factors, the proper communication between cells is lost which lead to changing response and production of altered molecules. The altered molecules in turn disturb the entire environment between the cells and loss of control over cell division which end up in the formation of abnormal mass of cells. It is now well-established that anticancer agents induce apoptosis in the cancer cells, and that disruption of apoptotic programs can reduce treatment sensitivity.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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