The Cell Cycle and Apoptosis

The cell cycle is the series of events that take place in a cell leading to its division and duplication of its DNA to produce two daughter cells. Transmission of genetic information from one cell generation to the next requires genome replication during the S-phase, and its segregation to the two new daughter cells during mitosis or M-phase. The cell cycle has four distinct phases: Mitosis, and three Interphase periods termed G_1 (the time gap between mitosis and DNA replication), S (the period of DNA synthesis), and G_2 (the gap between DNA duplication and the next mitosis).

1- During the G_1 phase: there is active synthesis of RNA and proteins, including proteins that control the cell cycle, and the cell volume, that was reduced to 1/2 by mitosis, grows to its previous size.

2- The S phase: is characterized by the synthesis of DNA and histones and by the beginning of centrosome duplication.

3- G_2 phase: is relatively short, proteins required for mitosis accumulate.

G_0 phase: cell cycle activities may be temporarily or permanently suspended and the new cells begin to specialize and differentiate, some differentiated cells, such as those of the liver, renew cycling under certain conditions; others, including most muscle and nerve cells are *terminally differentiated*.

In a normal cell cycle, S-phase is always preceded by M-phase and M-phase does not occur until S-phase is complete. Between the S- and M-phases, there are two preparatory gaps. G1 separates M from S, and G2 is between S and M. When the cell undergoes differentiation, it exits from the G1 phase of the cell cycle to enter into a quiescent state referred to as G0.

Cell cycle checkpoints

The checkpoints are a series of control systems enabling proliferation only in the presence of stimulatory signals such as growth factors. The timing and order of cell cycle events are monitored during cell cycle checkpoints that occur at:

1. G1/S boundary, also knowing as restriction point, As the cell progresses through G1, depending on internal and external conditions, it can either delay G1, enter a quiescent state known as G0, or cross the restriction point.

2. S-phase, also known as the DNA damage checkpoint, ensures that the cell underwent all of the necessary changes during the S and G2 phases and is ready to divide.
3. G2/M-phases : ensures that DNA replication is complete.
4. M phase checkpoint: (The mitotic spindle checkpoint). Check that all the chromosomes should be aligned at the mitotic plate and be under bipolar tension.

   In this case, the growth arrest caused by checkpoints allows the cell to repair the damage. After damage repair, progression through the cell cycle resumes. If the damage cannot be repaired, the cell is eliminated through apoptosis.

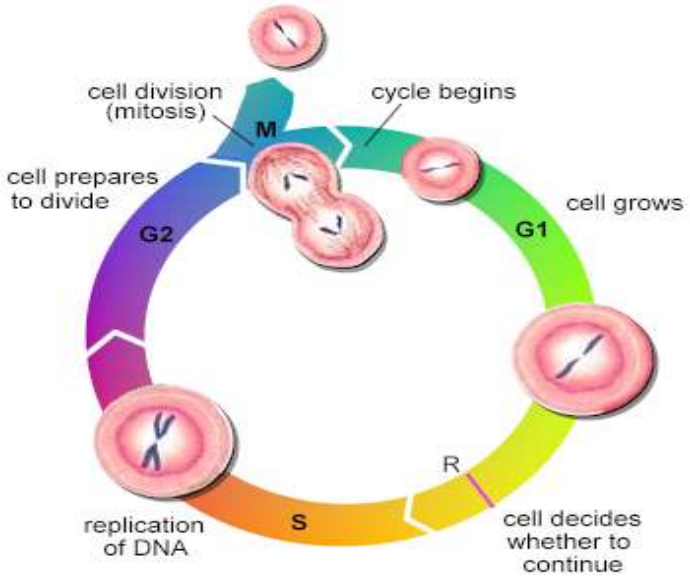

Figure 1: The cell cycle

Apoptosis

Apoptosis is the Programmed cell death, serves as a major mechanism for the precise regulation of cell numbers and as a defense mechanism to remove unwanted and potentially dangerous cells. Despite the striking heterogeneity of cell death induction pathways, the execution of the death program is often associated with characteristic morphological and biochemical changes, and this form of programmed cell death has been termed apoptosis. Apoptosis is an important means of eliminating cells whose survival is blocked by lack of nutrients, by damage caused by free radicals or radiation, or by the action of tumor suppressor proteins. In all examples studied apoptosis occurs very rapidly, in less time than required for mitosis, and the affected cells are removed without a trace. The main important features of apoptosis are summarized as:

1- Loss of mitochondrial function: Mirochondrial membrane integrity is not maintained, causing the end of normal activity and release of cytochrome c into the cytoplasm where it activates proteolytic enzymes called caspases. The initial caspases activate a cascade of other caspases, resulting in protein degradation throughout the cell.
2- Fragmentation of DNA: Endonucleases are activated which cleave DNA between nucleosomes into small fragments.(The new ends produced in the fragmented DNA allow specific histochemical staining of apoptotic cells using an appropriate enzyme that adds labeled nucleotides at these sites.)
3- Shrinkage of nuclear and cell volumes: Small dark-stained (pyknotic) nuclei can sometimes be identified with the light microscope.
4- Cell membrane changes: The integrity of the plasma membrane is maintained, but the cell undergoes dramatic shape changes, such as "blebbing", as membrane proteins and cytoskeleton are degraded. Phospholipids normally found only in the inner layer move to the outer layer, serving as signals to induce phagocytosis.
5- Formation and phagocytic removal of these apoptotic bodies.

The study of apoptosis under fluorescence microscopy is classified into five different categories, which are; viable non-apoptotic (viable), viable apoptotic (early apoptotic), non-viable apoptotic (late apoptotic), necrotic and chromatin free (ghost) cells as shown in (Fig. 2 in the power point). Acridine orange (AO) and propidium iodide (PI) are intercalating nucleic acid specific fluorochromes which emit green and orange fluorescences, respectively, when they are bound to DNA. Only AO can cross the plasma membrane of viable and early apoptotic cells. Late apoptotic cells and necrotic cells will stain with both AO and PI.

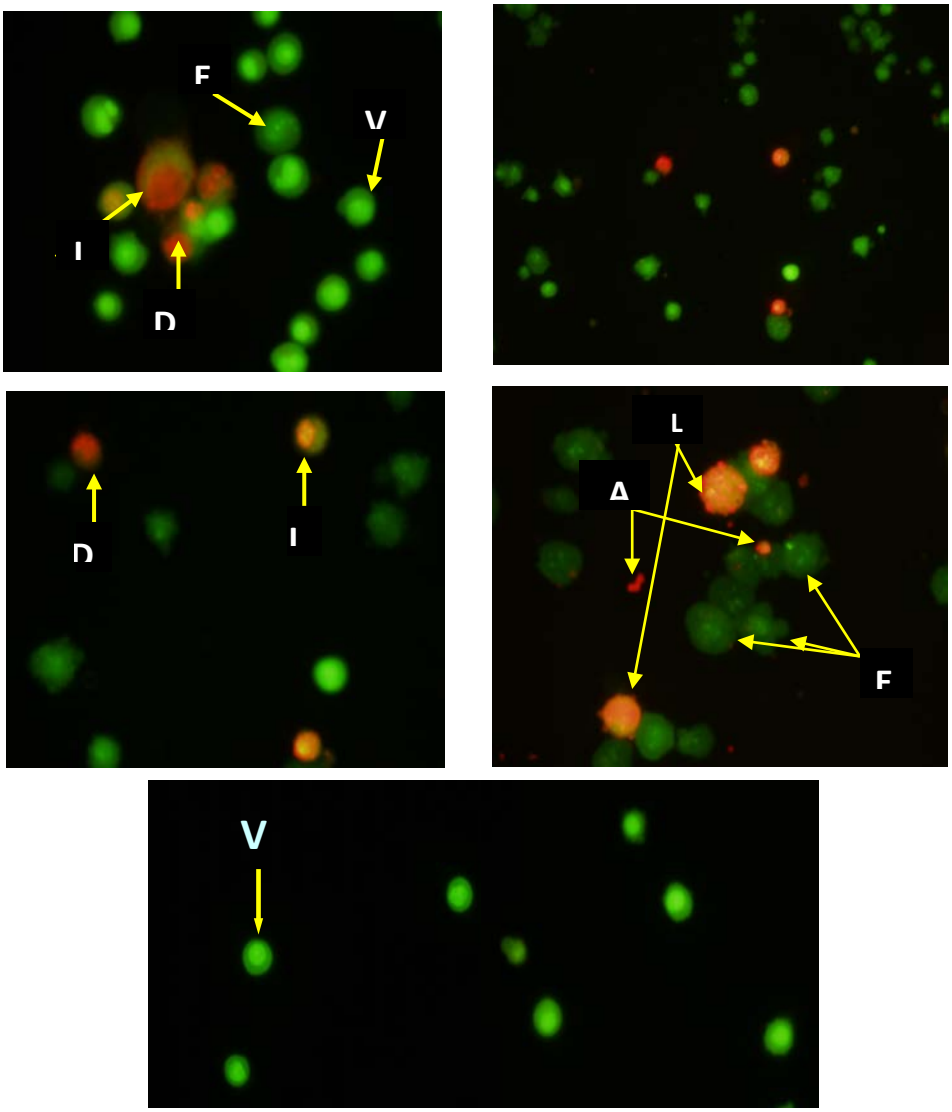

Figure 2: fluorescence microscopy images of cells. Where V: viable cells; E: early apoptotic cells; L: late apoptotic cells; AB: apoptotic bodies; D: dead cells