XV. APOPTOSIS, CELL DEATH, CANCER

XV.1. APOPTOSIS

Apoptosis is considered a vital component of various processes including development and functioning of the immune system, normal cell turnover, hormone-dependent atrophy, embryonic development and chemical-induced cell death.

Apoptosis was first discovered in embryos where it plays an important part in shaping various developing organs or body regions (the free spaces between embryonic fingers and toes).

Definition. (Gr. apo=off+ptosis=afalling) is the process of cell suicide or the process of programmed cell death. Apoptosis is a rapid process with a highly regulated cellular activity that shrinks and eliminates defective and unneeded cells. The results are smalls membrane inclosed bodies called ***apoptopic body*** which undergo phagocytosis. It is generally characterized by distinct morphological features and energy dependent biochemical mechanisms.

Cells that died as a result of acute injury typically swell and burst and spill their contents all over the neighbours (a process called cell necrosis), causing a potentially damaging inflammatory response.

By contrast a cell that undergo apoptosis dies neatly, without damaging its neighbours. The cell shrinks and condenses and eliminates defective and unneeded cells.

Apoptosis occurs normally during development and aging and as a homeostatic mechanism to maintain cell population in tissues

Some characteristic of apoptosis:

- the cytoskeleton collapses
- the nuclear anvelope disassembles
- the nuclear DNA breaks up in two fragments
- the cell surface is altered

Those are properties that cause the dying of cell. This dying cell will be phagocyted immediately, both by its neighbours or by macrophages. Apoptotic cells do not rupture and release none of their contents, unlike damaged cells that undergo necrosis as a result of injury.

The machinery that is responsible for programmed cell death it is similar in all animal cells. It involves a family of protease which are themselves activated by proteolytic cleavage in response to signals that induce apoptosis. The activated proteases cleave, and their by activate other members of the family, resulting an amplifying proteolytic cascade. The activated proteases then cleave other key proteins in the cell, killing it quickly and neatly (ex. protease cleaves the nuclear lamina causing the irreversible breakdown of the nuclear lamina). The suicide machinery is regulated by signals from other cells (some are killer signals like increased thyroid hormones which acts in this way in the tadpole tail at metamorphosis of the tail of the frog; others are survival signals, suppressing the suicide machinery so as to keep the cell alive.

Most animal cells require continous signaling from other cells to avoid apoptosis; this may be a mechanism to ensure the cells survive only when and where they are needed.

The control system

Apoptosis is controlled by cytoplasmic proteins in the Bcl-2 family, which regulate the release of death- promoting factors from mitochondria.

Specific Bcl-2 proteins induce a process with the following characteristics:

- **Loss of mitochondrial function and caspase activation:** Bcl-2 proteins associated with the outer mitochondria membrane compromise membrane integrity and realizing cytochrome c into the cytoplasm where it activate proteolytic enzymes called caspases. Caspases are enzymes which act by the cysteine mechanism. They are some caspases which are activated by some mechanisms with start events from interior or exterior of the cell. The initial caspases activate a cascade of other caspases resulting the protein degradation.
- **Fragmentation of DNA**. Endonucleases are activated, which cleave DNA between nucleosomes into small fragments. The new ends produced in the fragmented DNA allows specific histochemical staining of apoptotic cells.
- **Shrinkage of nuclear and cell volumes:** Destruction of cytoskeleton and chromatin causes the cell to shrink quickly producing small structures with dense, darkly stained pyknotic nuclei.
- **Cell membrane changes:** The plasmamebrane of the shrinking cell undergoes dramatic shape changes because the membrane proteins are degraded and lipid mobility increases.
- **Formation and phagocytic removal:** Membrane-bound remnants of cytoplasm and nucleus separate as very small apoptotic bodies. Newly exposed phospholipids on these bodies induce their phagocytosis by neighboring cells or white blood cells.

Physiologic Apoptosis

The role of apoptosis in normal physiology is as significant as that of its counterpart, mitosis. It demonstrates a complementary but opposite role to mitosis and cell proliferation in the regulation of various cell population. It is estimated that to maintain homeostasis in the adult human body, around 10 billion cells are made each day just to balance those dying by apoptosis. And that number can increase significantly when there is increased apoptosis during normal development and aging or during disease.

Apoptosis is important during various developmental processes. As examples, both the nervous system and the immune system arise through overproduction of cells. This initial overproduction is then followed by death of those cells that fail to establish functional synapses or productive antigen specificities, respectively.

Apoptosis is a vital component of wound healing in that it is involved in the removal of inflammatory cells and evolution of granulation tissue into scar tissue. Dysregulation of apoptosis during wound healing can lead to pathologic forms of healing such as excessive scarring and fibrosis. Apoptosis is also needed to eliminate activated or auto-aggressive immune cells during maturation in central lymphoid organs or in peripheral tissues.

Additionally, apoptosis is central to remodeling in the adult, such as the follicular atresia of the postovulatory follicle and post-weaning mammary gland involution. Furthermore, as organisms grow older, some cells begin to deteriorate at a faster rate and are eliminated via apoptosis. One theory is that oxidative stress plays a primary role in the pathophysiology of age-induced apoptosis via accumulated free-radical damage to mitochondrial DNA. It is clear that apoptosis has to be tightly regulated since too little or too much cell death may lead to pathology, including developmental defects, autoimmune diseases, neurodegeneration, or cancer.

Pathologic Apoptosis

Abnormalities in cell death regulation can be an important component of diseases such as cancer, autoimmune lymphoproliferative syndrome, AIDS, ischemia and neurodegenerative diseases such as Parkinson´s disease, Alzheimer ´s disease, Huntington´s disease, and

Amyotrophic Lateral Sclerosis. Some conditions feature insufficient apoptosis whereas others feature excessive apoptosis.

XV.2. CELL DEATH

In humans, as in all other multicellular organisms the rates of cell proliferation and cell death determine the net cell production. An abnormality in any of these rates can cause **disorders of cell accumulation** , which represents when the rate of cell division is higher than the rate of cell death, determining to a variety of disorders of cell accumulation (hyperplasia, cancer, autoimmune diseases), or **disorders of cell loss** (atrophy, degenerative diseases, AIDS, ischemic injury). If the rate of cell death is higher than that of cell division, will occur a loss of cell number. This is why the homeostasis between cell production and cell death must be carefully maintained.

Cell death can be a result of acute cell injury (necrosis) or an internally incoded suicide program (apoptosis).

Necrosis or accidental cell death, is a pathologic process, It occurs when cells are exposed to an unfavorable physical or chemical environment (hypothermia, hypoxia, radiation, low ph, cell trauma) that causes acute cellular injury and damage to the plasma membrane. These damages of the plasma membrane may be initiated by viruses or proteins called perforins. Some characteristics of necrosis are cell swelling and lysis. As a result of cell injury, damage to the cell membrane leads to an influx of water and extracellular ions. Mitochondria, RER and nucleus undergo irreversible changes that are caused by cell swelling and cell lysis. After this breakdown of the plasma membrane the lysosomal enzymes and cytoplasmic contents are released into the extracellular space. This is why necrotic cell death is associated with surrounding tissue damage which determine an intense inflammatory response.

Other Forms of Programmed Cell Death

- **Autophagy** is a regulated cellular process that enables cells to turnover their contents by lysosomal degradation of their own components. An intracellular membrane (part of SER) wraps around an organelle, forms a closed double membrane bound vacuole (autophagosome), fuses with lysosomes and initiates digestion.
- **Mitotic catastrophe** it is a kind of cell death during mitosis. It results from a malfunction of several cell cycle checkpoints (G1, S, G2, damage checkpoints). Failure to arrest the cell cycle before mitosis occurs causes problems with chromosome separations which triggers the apoptotic pathway and cell death.
- **Paraptosis** is an alternative, nonapoptotic cell death that may be induced by growth factors receptors (insulin growth factors receptors). Paraptosis is characterized by deformation of multiple large vacuoles within the cell cytoplasm along with mitochondrial swellings.
- **Piroptosis** is a form of cell death induced by infections with some microorganisms that generate inflammatory reactions. This process is dependent by caspase 1(which is not involved in apoptosis) which activates the inflammatory cytokines that mediate intense inflammatory reactions in the neighbouring tissue.
- **Necroptosis** is a regulated caspase independent cell death mechanism induced in different cell types. Necrostatin 1 is a specific inhibitor of necroptosis that significantly reduced ischemic damage in affected tissue.

Microscopic studies of dying cells in the tissue reveals that different forms of cell death can occur simultaneously and that dying cells can share features of different types of cell death.

XV.3. CANCER

Cancer malady (neoplasm or malignant tumor) is a genetic or epigenetic disease of somatic cells that is manifested by a cell accumulation as a result of clonal expansion. It is also a disease of cell differentiation, because these cells, which continue to proliferate, remain relatively immature in function, coming out of control of mechanisms of regulation of function and cellular structure.

In view of the many biomedical implications, the study of the cancer cell is of the paramount importance in cell and molecular biology. This subject could have been treated in a holistic manner; we analyze the special characteristics of the cancer cell because they differ from the normal cell in their various cellular components.

In the cancer cell they were identified **morphological alterations, genetic alterations and cell proliferation, differentiation, growth and proliferation anomalies.**

1. Morphological alterations

a. Membrane abnormalities

- disappearance of GAP junctions;
- loss of coupling;
- changes in glycolipids, glycoproteins, reduction in gangliozides, abundant amount of GAG which increase the negative charge of the cell coat;
- higher mobility of surface receptors, increased transport of sugars, growth of new antigens;
- fibronectine, a large glycoprotein found in normal cell, is reduced in cancer cells, playing an important role in metasthasis.

b. Cytoplasmic abnormalities:

- less cytoplasm than in normal cells;
- cytoplasm basophilia by a higher content in nucleic acids and proteins;
- the cytoskeleton, which is composed of a network of microtubules, actin microfilaments and intermediate filaments is reduced or disorganized.

c. Nucleus abnormalities:

- the size of the nucleus is increased and the nucleo-cytoplasmic ratio is also increased;
- anisocariosis: nuclei of varying sizes from one cell to another for the same amount of cytoplasm;
- hyperbasophilic nuclei with high concentration of heterochromatin distributed all over the
- thick and irregular nuclear membrane;
- multiple, bulky, irregular nucleoli;
- high rate of mitosis, with atypical mitosis;
- the kariotype is frequently abnormal with changes in chromosome number or with chromosomal alteration.

2. Genetic abnormalities

The mutations that make cancer cells defective in this respect affect two grow category of genes:

- proliferation genes – encode proteins that normally help to promote cell division
- antiproliferation genes - encode proteins that normally help to apply the brakes that halt the cell cycle at the checkpoints.

A mutation in a proliferation gene that causes the protein produced by the gene to be overexpressed or hyperactive results in excessive cell multiplication.

The mutant gene is then classified as an **oncogene** (cancer promoting gene) while the normal gene is known as a **protooncogene.**

Oncogenes are genes that encode proteins to stimulate cellular proliferation.

Oncogenes encode proteins called onco-proteins that do not have regulatory systems, and their production is independent of external factors (growth or other signals).

The mechanisms of transformation of proto-oncogenes into oncogenes are represented by:

- modifying the gene structure with the synthesis of an abnormal protein that has an aberrant function,

- altering the regulation of gene expression without alteration of function, but with increased or inappropriate synthesis of a normal protein that exaggerates cell growth.

Mutations occurring in genes encoding growth factors can confer oncogenic characters - proto-oncogenes c-sis (coding for B-chain of PDGF) or viral oncogenes v-sis. Altered PDGF synthesis has been identified in numerous human tumors (osteosarcoma, astrocytoma, bronchial carcinoma). In carcinomas was identified the expression of EGF and TGFα growth factors. Excessive cellular proliferation is one that determine an increased risk of spontaneous mutations and, therefore, the risk of cancer.

On the inner membrane of the cytoplasmic membrane have been identified several oncoproteins that reproduce the function of the cytoplasmic proteins that ensure the transmission of the signal (the RAS family). Approximately 20% of human neoplasms show mutations of RAS proteins.

A large number of oncogenes encode nuclear transcription factors that have the ability to activate or inhibit transcription of the corresponding genes: myc, myb, jun and fos oncogenes.

For other genes, the danger lyse in mutations that destroy gene function. These mutations are generally recessive: both gene copies must be lost or inactivated before an effect is seen; the affected gene is called a **tumor suppressor gene. Tumor suppressor genes** were first identified by studies of human genetics. Occasionally individuals are encountered who have inherited a mutation in a tumor suppressor gene; although one gene copy is enough for normal cell behavior.

Proto-oncogenes and tumor suppressor genes are of many sorts, corresponding to the many different kinds of misbehavior that cancer cell display. Some of these genes code for growth factors, for receptors or- like RAS-for components of the intracellular signaling pathways that growth factor activate. Other code for DNA repair proteins, for mediators of the DNA damage response, such as p53, or for regulators of the cell cycle or of the apoptosis.

An example of a tumor suppressor gene is the retinoblastoma gene (Rb) that plays a role in the cell cycle. Inactivation of both copies of this gene causes cell cycle braking, drastically increasing the risk of cancer, especially of the retina.

Another tumor suppressor gene is the p53 gene that has the role of preventing the propagation of altered genetic cells. Mutations of this gene have been identified in breast cancer, leukemias, cerebral malignancies, adrenal cortical carcinomas.

Numerous other genes in this category have been individualized for example brca genes are tumor suppressor genes associated with breast and ovarian cancer,

Genes that control programmed cell death, blocking it or inducing it, play a role in controlling tumor cell mass. Several gene families contribute to the control of apoptosis: some such as bcl-2, bcl-xl inhibit apoptosis, others such as bax, bad and bclxS favor programmed cell death. Apoptosis is the endpoint of a cascade of molecular events, induced by various stimuli that lead to the activation of proteolytic enzymes (caspases) that are responsible for cell death. The ratio of forces between the agonist and antagonist genes of cell death determines the cell's response to apoptotic stimuli.

3. Growth and proliferation anomalies.

Cancer Cells Disobey the Socials Controls on Cell Proliferation and Survival

Cancers are the product of mutations that set cells free from the usual controls on cell proliferation and survival. A cell in the body mutates through a series of chance accidents and acquires the ability to proliferate without the normal restraints. Its progeny inherit the mutations and give rise to a tumor that can grow without limit.

Cellular homeostasis is determined by the balance between proliferation (cells in the division) and physiological cell death (apoptosis). The characteristic of neoplastic cells is the disruption of mechanisms to regulate these processes, resulting in increased cellular proliferation.

a) Although there are abnormalities of differentiation, the cancer cells exhibit (in the case of primary tumors) the phenotypic characters of the origin cells. The differentiation of a tumor is defined by the presence of phenotypic characters that allow the origin of the tumor to be determined. From this point of view, cancers can be differentiated (cells close to their home tissue), slightly differentiated (cells expressing few particular phenotypic characters but sufficient to indicate origin) and undifferentiated (origin can not be specified). The assessment of the degree of differentiation of a tumor is important because it can establish the prognosis of the disease (the more the tumor is differentiated, the better the prognosis) and also allows the treatment to be adapted to the type of tumor.and essential feature.

b) Cancer cell adhesion is diminished due to qualitative and quantitative abnormalities of adhesion molecules, which promotes the mobility of tumor cells, causing invasion of neighboring tissues and distant dissemination.

c) Neoplastic cells lose contact inhibition.

d) Immortality. Normal somatic cells are divided into cultures for a limited number of times, depending on the cell type and donor age (e.g., fibroblasts are divided 50 times), after which they die by apoptosis. Malignant cells, once stabilized in crops, will live for an indefinite number of generations if they are supplied with growth factors and nutrients.

e) Cancer cells are characterized by uncontrolled cell growth, invasion of other tissues and dissemination to other sites of the organism producing secondary tumors.

All these characteristics suggest that cancer cell have escape from the controls that regulate normal growth. Cancerous tumors are monoclonal, derived from the division of the single cell which has been transformed into a cancer cell.

The role of telomerases

After a defined number of cell divisions, somatic cells get into senescence. This number of divisions, constant for the same cell type, is controlled by specialized structures located at the ends of the chromosomes called telomere. These structures are shortened at every cell cycle scroll, shortening that causes the accumulation of genetic abnormalities. As a rule, shortening of telomere is one of the determinants of leaving the cell cycle and activating apoptosis.

In germ cells, shortening of chromosomes is avoided by an enzyme called telomerase; this compensates for the reduction of the telomere's waist. The introduction of telomerases into somatic cells significantly increases their life span. The telomerase activity was observed in the vast majority of cancers. It can be considered that the reduction of the telomere size has a tumor suppressing effect, and telomerase activity is a molecular marker of cancer.

Some different features between normal cells and cancer cells:

1. Cancer cells have a reduced dependence on signals for growth, survival and division. This is because they contain mutations in components of the cell signaling pathways through which cell respond to different signal.

2. Cancer cells are less susceptible than normal cells to kill themselves by apoptosis. About 50% of all human cancers have lost or suffered a mutation in the p53 gene (the protein p53 normally acts as part of a checkpoint mechanism that causes cells either to cease dividing or to die by apoptosis when their DNA is damaged. Chromosome breakage if not repaired, will cause a cell to commit suicide, but if the cell is defective in p53, it may survive and divide , producing abnormal daughter cells that can became malignant

3. Cancer cells can proliferate indefinitely. Most normal somatic human cells will divide a limited number of times in culture, after that the division process ceased. Is happening because of the absence of synthesis of telomerase enzyme, so the telomeres on the ends of their chromosomes become too short. Cancer cells reactivate the production of the telomerase enzyme that maintains telomere length.

Telomere – structure at the ends of linear chromosomes, associated with a characteristic DNA sequence that is replicated in a special way, and it is shorten with each round of replication

Telomerase – enzyme that elongates telomeres.

4. Cancer cells are genetically unstable with a greatly increased mutation rate.

5. Cancer cells are abnormally invasive, because they lack cadherins (cell adhesion molecule) that hold normal cells in their proper place.

6. Cancer cells survive and proliferate in foreign tissues to form secondary tumors (metastases), whereas most normal cells die when misplaced (an ability that is not understand).