PROGRAMMED CELL DEATH (PCD) OR APOPTOSIS

Dr. M. Roy

MBOTCC-10
Unit-II

M.Sc. Sem-III
(2012-20)

Introduction:

Around 1950, it was established that cells die as a normal part of development and homoeostasis. This deliberate and orderly elimination of unwanted cells in a morphologically distinct manner is described as programmed cell death (PCD) or apoptosis, as against pathological cell death caused due to cell injury. Naturally occurring PCD is found in all animals, although its detailed analysis has been conducted only in some animal systems. Apoptosis has also been observed in microbial systems/cell cultures and therefore is ubiquitous.

Alterations in PCD have also been found to cause a number of human diseases. As a result, apoptosis has been an area of intensive research in recent decades.

Characteristic Changes during Apoptosis:

Execution of PCD or apoptosis is often associated with characteristic morphological and biochemical changes. They are as below:

(i) Shrinkage of cells.
(ii) Cell form tight sphere.
(iii) Cell membrane forms bubble-like blebs on the outer surface
(iv) Occurrence of nuclear membrane breakage
(v) Endonucleolytic clearance of DNA at internucleosomal sites leading to the degradation of chromatin
(vi) Breakdown of mitochondria with

... Contd. p. 2

the release of cytochrome C.

(vii) Breakage of cells into small fragments

(viii) Engulfment of cell fragments by phagocytic cells.

(ix) Activation of nucleases and synthesis of RNA and proteins

Genetic regulation of Apoptosis :

(i) Some apoptosis genes have been identified which are responsible for switching on or off apoptosis. These include ICE (Interleukin-1b Converting Enzyme) and P53.

(ii) There are other factors that also regulate the process of apoptosis. One of them is signal protein which is released either due to some cell injury or through cytokine mediated pathways.

(iii) There are some critical proteins or modulating factors which determine whether a cell will be repaired or undergo death. These genes or factors may initiate some stimuli for cell death or induce cellular susceptibility to apoptosis or initiate some effector mechanisms for apoptosis.

List of Genes/Factors :

(a) Initiating Stimuli	Function
Tumor Necrosis Factor: α} receptor family (TNF)	Death signal
ceramide	Gives signal for apoptosis induction
FAS/Apo-1	Death signal like TNF; For peripheral deletion of T lymphocytes
Nur 77 (Zn finger containing steroid receptor)	Death signal in thymocytes

.... Contd. p.3

(b) Inducing Cellular Susceptibility

c-myc — Produces myc protein which gives cell susceptibility for apoptosis

Rb-1 — Deficiency of Rb-1 gives susceptibility; Rb protein may inhibit P53 mediated apoptosis

E2F1 — Induces susceptibility

P53 — Apoptosis in response to cell injury is dependent on P53

(c) Modulating Factors

DAD 1 gene — Gives signal for cell death

BCl-2 gene family — Some members inhibit cell death such as BCl-2, BCl-x. Members which promote death like bax, bid and bad.

(d) Effector mechanisms

Caspases, ICE, Ich-1 — Genes encoding cysteine proteases which are involved in the effector pathway of apoptosis.

Mechanism of Apoptosis:

There are generally three different mechanisms for apoptosis. These are:

1. Triggered by internal signals, i.e., signals arising within the cell
2. Triggered by external signals
3. By Apoptosis-Inducing Factor (AIF)

1. By Internal Signals:

(i) In a normal cell, the protein (BCl-2) produced from a gene BCl-2 remains on the

... Contd. p. 4

outer surface of mitochondria.

(ii) The protein BCl-2 holds the apoptotic protease activating factor-1 (Apaf-1).

- But when the damage occurs in the cell internally due to some reactive oxygen, the Apaf-1 factor is released from BCl-2-Apaf-1 complex. This allows the protein Bax to penetrate the mitochondrial membrane causing a leakage of cytochrome C from the mitochondria.

- Then the released cytochrome C and Apaf-1 bind to molecules of caspase-9.

(iii) The complex containing cytochrome C, Apaf-1, Caspase 9 and ATP is called Apoptosome.

(iv) Caspase 9 is actually one form of protease which cleaves proteins at Aspartic acid residues.

- Caspase 9 activates other caspases creating a cascade of proteolytic activity which leads to the lysis of cell through digestion of structural proteins of the cytoplasm and degradation of chromosomal DNA.

2. By External Signals:

(i) Some receptor proteins (FAS and TNF) and other molecules residing on the surface of the cell are responsible for apoptosis.

(ii) When cytotoxic T cells containing complementary factor FASL bind to the target cell, FASL binds with the FAS of the target cell leading to the death of the cell by apoptosis.

3. By Apoptosis - Inducing Factor (AIF):

(i) AIF is a protein located in

.... Contd. p. 5

the intermembrane space of mitochondria.

(ii) When the cell receives the signal for its death, AIF is released from the mitochondria to the cytoplasm.

— AIF then migrates to the nucleus and binds to DNA causing destruction of DNA and finally death of the cell.

(iii) In case of cancer, there are some viruses like Human Papiloma Virus (HPV), Epstein-Barr Virus (EBV) which produce a special type of protein E6 or BCl-2 which inactivate apoptosis promoter P53, leading to the proliferation of cancer.

— Cancer cells without the intervention of viruses also have some techniques to inactivate apoptosis.

— Some B-cell leukemias, Melanoma (a type of skin cancer), lung cancer cells, colon cancer cells, etc. produce some proteins or factors like BCl-2, "decoy" molecules, FAS-L, which can avoid apoptosis by inhibiting Apaf-1, or binding to FAS leading to proliferation of cancer.

Density-dependent Inhibition:

(i) Normal cells show density-dependent inhibition of cell division in culture but cancer cells continue to proliferate independent of cell density.

(ii) Proliferation of normal cells continues until they reach a finite cell density. When they reach a finite density, they enter the G1 state of the cell cycle. But cancer cells continue to divide to high cell density.

Cellular Characteristics:

(i) Cancer cells have a high nucleus/

...Contd. p. 6

cytoplasm ratio, prominent nucleoli, many mitoses, and relatively little specialized structure.

(ii) Normal cells have a cytoskeleton consisting of microtubules and micro-filaments.

— However, the cytoskeleton of cancer cells undergo depolymerization and the microtubules disaggregate.

Chromosomal Change:

(i) Normal cells contain normal chromosome number, but chromosomes in cancer cells undergo structural and numerical changes.

— Aneuploidy is commonly observed in cancer cells.

(ii) Occurrence of any aneuploid cells in a particular tissue may have the possibility to become cancerous cell.

Interactions with immune system:

(i) A few normal cells may be transformed into pre-cancer cells every-day in response to radiation, certain viruses or chemical carcinogens in the environment.

(ii) If pre-cancer cells are destroyed by the immune system, some transformed cancer cells show that their surface proteins are not so changed. Such cancer cells may remain antigenetically similar to normal cells. As a result, the immune system cells may fail to distinguish the cancer cells from normal cells.

— In such case, cancer cells can stimulate B cells to produce IgE antibodies that combine with antigens on the surface of the cancer cells. These blocking antibodies may block the T cells so that they are unable to adhere to the surface of cancer cells and destroy them.