APOPTOSIS

INTRODUCTION

The word ‘‘apoptosis’’ comes from the ancient Greek apo´pto´sis, meaning the ‘‘falling off of petals from a flower’’ or ‘‘of leaves from a tree in autumn’’. The name was first introduced by John Kerr in 1972 and refers to the morphological feature of formation of ‘‘apoptotic bodies’’ from a cell.

Apoptosis is cell suicide by a built-in self-destruct mechanism consisting of a genetically programmed sequence of biochemical events.

Apoptosis plays an essential role in embryogenesis, helping to shape organs during development by eliminating cells that have become redundant. It is the mechanism that each day unobtrusively removes 10 billion cells from the human body. It is involved in numerous physiological events: the shedding of the intestinal lining, the death of time-expired neutrophils and the turnover of tissues as the newborn infant grows to maturity. It is the basis for the development of self-tolerance in the immune system and acts as a first-line defense against carcinogenic mutations by purging cells with abnormal DNA that could become malignant.

Disturbed apoptosis is also implicated in the pathophysiology of many conditions. Conditions associated with excessive apoptosis include:

- chronic neurodegenerative diseases such as Alzheimer's, multiple sclerosis and Parkinson's disease
- conditions with acute tissue damage or cell loss such as myocardial infarction , stroke and spinal cord injury
- depletion of T cells in HIV infection
- osteoarthritis
- haematological disease such as aplastic anemia.

Examples of defective apoptosis include:

- evasion of the immune response by cancer cells and resistance to cancer chemotherapy

- autoimmune/inflammatory diseases such as myasthenia gravis, rheumatoid arthritis , and bronchial asthma
- viral infections with ineffective eradication of virus-infected cells .

Apoptosis play important role in the regulation of the immune response and in the many conditions in which it is an underlying component. There is recent evidence that T cells have a negative regulatory pathway controlled by surface *programmed cell death receptors* (e.g. the PD-1 receptor), and that there is normally a balance between the stimulatory pathways triggered by antigens and this negative regulatory apoptosis-inducing pathway. The balance is important in the maintenance of peripheral tolerance. A disturbance of this balance is seen in autoimmune disease, in the 'exhaustion' of T cells in chronic viral diseases such as HIV, and possibly in tumour escape from immune destruction .

Apoptosis is *a default response*, i.e. continuous active signalling by tissue-specific trophic factors, cytokines and hormones, and cell-to-cell contact factors (adhesion molecules, integrins, etc.) may be required for cell survival and viability, and the self-destruct mechanism is automatically triggered unless it is actively and continuously inhibited by these anti-apoptotic factors. Different cell types require differing sets of survival factors, which function only locally. If a cell strays or is dislodged from the area where its paracrine survival signals operate, it will die.

Withdrawal of these cell survival factors-which has been termed 'death by neglect'-is not the only pathway to apoptosis. The death machinery can be activated by ligands that stimulate *death receptors* ('death by design') and by DNA damage. But it is generally accepted that cell proliferation processes and apoptosis are tightly connected .

THE MAJOR PLAYERS IN APOPTOSIS

The repertoire of reactions in apoptosis is extremely complex and can vary not only between species but between cell types. Yet it could be that the pivotal reaction(s) that lead to either cell survival or cell death are controlled by a single gene or combination of genes. If so, these genes could be attainable targets in the development of drugs for many proliferative diseases. The use

of gene silencing by RNA interference (RNAi) technology permits very efficient and precise block of gene expression and is being used to identify antiapoptotic genes.

The major players in apoptosis are-

1.Caspases :

Caspases-a family of cysteine proteases present in the cell in inactive form. These undertake delicate protein surgery, selectively cleaving a specific set of target proteins (enzymes, structural components), inactivating some and activating others. A cascade of about nine different caspases takes part in bringing about apoptosis, some functioning as initiators that transmit the initial apoptotic signals, and some being responsible for the final phase of cell death .

The executioner caspases (e.g. caspase 3) cleave and inactivate cell constituents such as the DNA repair enzymes, protein kinase C, and cytoskeletal components. A DNAase is activated and cuts genomic DNA between the nucleosomes, generating DNA fragments of approximately 180 base pairs.

Besides the caspases, another pathway involves a protein termed *apoptotic initiating factor* (AIF) that is released from the mitochondria, enters the nucleus and triggers cell suicide.

Not all caspases are death-mediating enzymes; some have a role in the processing and activating of cytokines (e.g. caspase 8 is active in processing the inflammatory cytokines IL-1 and IL-18).

2.BCL-2 : The family of Bcl-2 proteins During lymphocyte development, these cells change their apoptotic propensity (sometimes referred to as ‘‘apoptotic phenotype’’). The major determinants of the ‘‘apoptotic phenotype’’ in lymphocytes are the levels of expression of Bcl-2, Bcl-xL and of Fas and/or Fas ligand (FasL). In general, developmental stages at which selection occurs are characterized by expression of low levels of Bcl-2 and/or Bcl-xL, whereas stages of proliferation are characterized by high expression levels of Bcl-2 and/or Bcl-xL.

Bcl-2 and Bcl-xL are the two most important anti-apoptotic members of the Bcl-2 family of proteins

Mainly three groups of the Bcl-2 family proteins can be distinguished:

(1) the antiapoptotic proteins (most of which contain a C-terminal membrane anchor and the four BH domains), like Bcl-2 and Bcl-xL

(2) the pro-apoptotic members (which lack some of the four Bcl-2 homology [BH] domains; e.g., Bax, Bak) and

(3) the BH3-only proteins (that, as the name suggests, only contain the 3rdBHdomain, an

amphipathic helical structure, and are all pro-apoptotic; e.g., Bad, Bik, Bid, Bim).

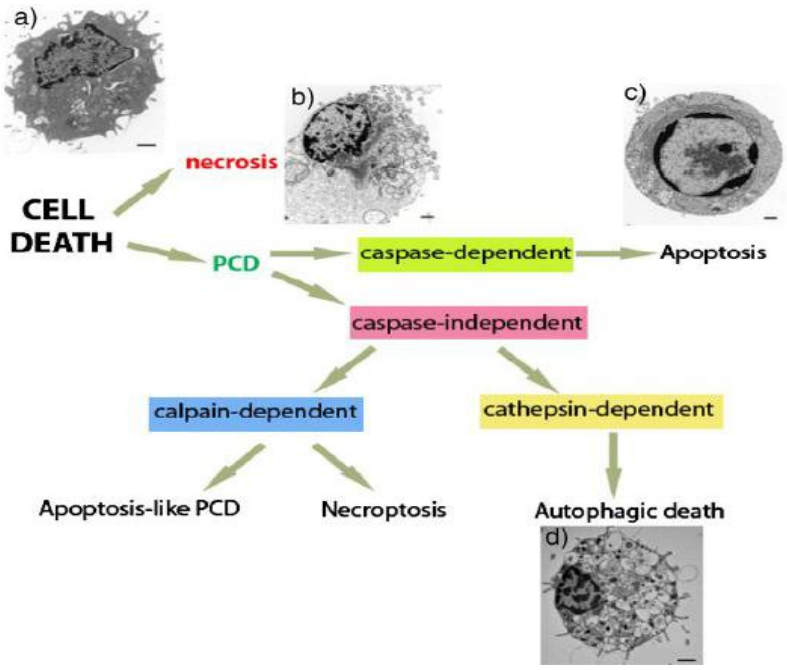
The relative levels of pro- and anti-apoptotic proteins determine a cell's susceptibility to apoptosis. Several members of this protein family are capable of forming deathpromoting or -inhibiting homo- and/or heterodimers. Many death signals converge through BH3-only proteins at the mitochondria.

MOLECULAR MECHANISMS OF APOPTOSIS

Cell death by apoptosis occurs when a specialised intracellular signalling pathway is activated and kills the cell. Apoptosis is the most common way of cells to die *in vivo* but there are other ways (necrosis has been defined as cell death that is not apoptosis; necrosis may in some cases indeed be due to simple physical injuries but there also seems to be at least one signalling pathway that causes necrosis; pyroptosis is when a cell dies as a consequence of the activity of caspase-1, a protease involved in the maturation of cytokines; autophagy has also been linked to cell death. Programmed cell death used to be a term for cell death especially during development, where a cell has the predetermined fate to die. The term is now commonly used to describe any cell death that is the result of intracellular signal transduction (a 'program') and therefore especially encompasses apoptosis).

PATHWAYS TO APOPTOSIS

Apoptosis is triggered by multi-signal pathways and regulated by multi-complicated extrinsic and intrinsic ligands. The process of apoptosis is controlled by diversity cell signals pathway and involved in regulation of cell fate death or survival. There are two major apoptosis pathways distinguished according to whether caspases are involved or not. The mitochondria, as the cross-talk organelles, can connect the different apoptosis pathway.



1. Caspase dependent pathway

Caspase-dependent apoptosis is the classic programmed cell death pathway, the capase-8,caspase-9, caspase-12, caspase-7, caspase-3 cascade usually participate in this type of apoptosis pathway, Variety of receptors take part in this type of apoptosis pathway, such as the TNF-alpha receptor, FasL receptor, TLR, Death receptor and so on. Some iron channels may also be involved in apoptosis pathway. The typical iron channel is the calcium channel,Since calcium's concentration in the cytosol plays an important role in the signal transduction regulation and participates in the cell proliferation and cell death, the cell fate can be controlled by the calcium channel opening or closing. In this part, we will discuss the caspase-dependent apoptosis transduction and review the complex signal crosstalk in the cells.

TNF-alpha induced caspase-8-dependent pathway relies on the TNF-alpha receptor and activates the caspase-8 through the death complex, and then the Bcl-2 protein is activated, Bcl-2 family protein activation may induced the mitochondria membrane changed and stimulates the cytochrome c released. Cytochrome c is the proapoptosis signal molecular which can activates the caspase cascade reaction and induced the apoptosis in the end. Some radiation UV or X ray can make mitochondria depolarization and membrane permeabilization, subsequently, the ROS increased; cytochorme c released, and then trigger caspase-9, caspase-3 activation,In the end, the

substracts will be cleavaged by the activation caspases and the fate of cells will be apoptosis;Some pathogen infection induced the apoptosis may be also have the caspase-8 dependent pathway, The alien pathogens can be recognized by FasL receptor and recruit FADD and caspase-8, for example, the intracellular pathogen herpervirus infection can induced the caspase-8 dependent apoptosis. Beside caspase-8 dependent apoptosis, some pathogens can trigger others caspases dependent apoptosis pathway. For example, Mycobacterium tuberculosis can induce programmed cell death on macrophage, and this apoptosis pathway is the caspase-12 dependent. NO and ROS production, stimulated by ER stress, also take part in apoptosis triggered by Mycobacterium tuberculosis; Exclude bacteria, virus also can induce the apoptosis. The latest research found that an alternative Kaposi's sarcoma-associated herpesvirus replication can trigger host cell apoptosis in caspase dependent manner. Apart 6 Apoptosis and Medicine from the bacteria and virus, we found that many researchers' data show that RNA fragments and DNA can also trigger caspase dependent apoptosis, such as RNA fragment produced by mycobacterium tuberculosis which in the early log-phase growth can trigger caspase-8 dependent apoptosis[3]; In vivo, DNA damage can trigger apoptosis through enhancing ROS level and changing the mitochondria membrane permeability; Many proteins or peptides will also make cell apoptosis, amyloid β peptide cytotoxicity can induce the intracellular calcium disturbance, and then the calpain will be activated by imbalanced calcium storage, While the calpain can activate caspase-12 which can located in ER to inactivate the Bcl-Xl, this is a novel caspase-12 dependent apoptosis pathway. This way can be used by the mitochondria to connect with other cell death pathway.

Above all, we can give the conclusion that pathogens, RNA or DNA, proteins or peptides,some chemical compounds or native compounds can all trigger caspase dependent apoptosis. They may have the different receptors and induce cell death through different caspase as the transducer to make the downstream signals transduction. The host used this way to defense the harmful factors and maintain the healthy physiological state.

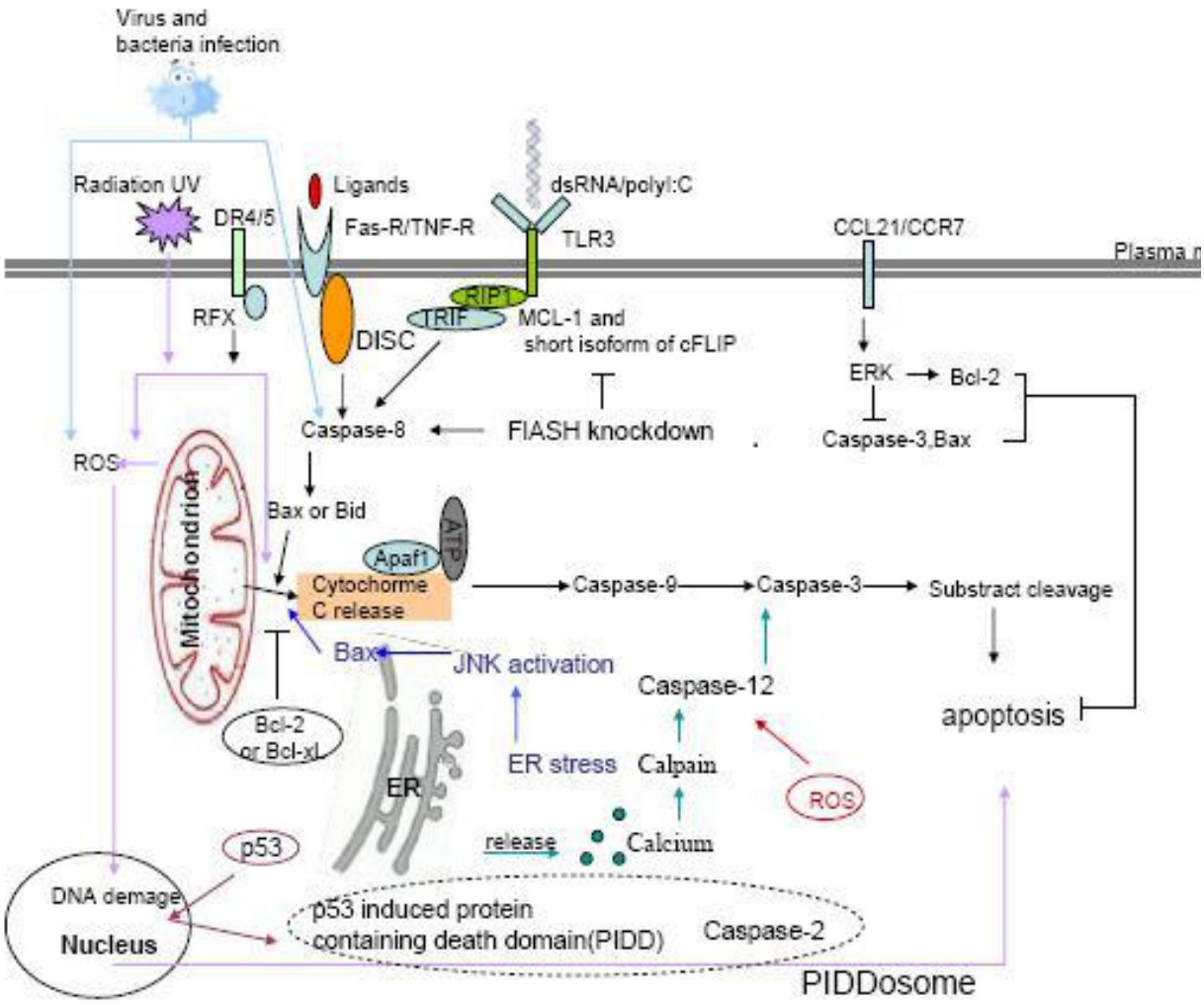


Figure 1. Summarize the caspase dependent apoptosis pathway

In this figure, we can clearly know that mitochondrion and nucleus organelles play the pivotal role in this type cell death and these organelles can connect different signals to induce the caspase activation, in this process period, ROS; Cytochorme C release; mitochondrion membrane potential change; Apart the mitochondrion pathway, some ligands can trigger MAPK pathway, such as activate ERK and then activate the caspase family which can be confluenced with apoptosis pathway.

2. Caspase independent pathway

Apoptosis have many mechanisms. It can be triggered by in vitro and in vivo cell ligands, and have different signal transduction pathways. Now, Apoptosis pathways are classified as caspase dependent pathway and caspase-independent pathway. In the above, we describe and conclude the caspase dependent pathway, which is characterized by the involvement of caspase in this type cell death process. In this paragraph, we will deep research the caspase independent apoptosis pathway. We will know that caspase does not participate in this type of process from the literally meaning. Up to now, this type of apoptosis has been founded by many researchers, and the research data give much information to us, this information can help us to understand the apoptosis complex mechanism, beside the mechanisms, the complicated ligands, which can induce this type of cell death, can also be found by many researchers. In the following, we will give some detailed contents about caspase independent apoptosis.

In the cell, a lot of ligands can induce mitochondria membrane potential change, the mitochondria damage will be the first step of the apoptosis, then ROS production increase, and ROS may the mainly factor to induce caspase independent apoptosis. For example, Denis Martinvalet found that granzyme A can directly induce the ROS increase and caspase independent mitochondria damage. Then the target of granzyme A, ER associated complex (SET complex), will translocated to nuclear and contributed to apoptosis; AIF has been found the major important caspase-indenpendent pro-apoptosis factor, which can release from the mitochondria and translocate in the nuclear to cleavage the DNA, in the end, if the

DNA damage have not been repaired by cells, the apoptosis will happen. Recently, many researchers found some compounds which can accompany with AIF production and induce cell death, such as simvastatin, staurosporine, cadmium and so on. These factors triggered caspase-independent PCD and fitted for the organism requirement; Beside AIF triggered caspase-independent PCD, ROS also participate in this type cell death. ROS can mediate poly (ADP-ribose) polymerase-1 (PARP-1) activation, and PARP-1 activation is necessary for AIF release from mitochondria. So ROS also involved in this type of cell death networks.However, ROS participated in the caspase dependent apoptosis pathway also, Consequently, ROS might be the important bridge to connect two types of apoptosis in vivo. ROS mainly come from

mitochondria, so mitochondria play important role in apoptosis pathways crosstalk. And the ligands usually trigger complicated reactions, including that AIF nuclear translocation, ROS increase and mitochondrial dysfunctions, these changes can cause to the caspase independent apoptosis pathway. For example, Cyclohexyl analogues of 8 Apoptosis and Medicine Ethylenediamine Dipropanoic Acid, the compound that can induce peripheral blood mononuclear cells apoptosis of both healthy controls and leukemic patients through stimulating many apoptogenic factors activation(such as: AIF nuclear translocation, ROS increase and mitochondrial dysfunction). Referring the ROS, we propose that the GSH ,NO, or other free radical groups may also take part in this type cell death, By this way, the GSH or NO modification proteins may also take part in the apoptosis pathway, GSH and NO can block some active thiol group and make the protein S-glutathionylation or Snitrosylation modification. These types modification may affect the protein's functions and make the cell to apoptosis result. Beside the AIF and ROS, there are many other ligands and signal molecular from the vitro or vivo cells as apoptogenic factors involving in caspase independent apoptosis pathway, such as lysosomal membrane permeabilization; some virus's protein; drugs; p53 suppression tumor factors or many other unknown compounds.Up to now, the caspase independent apoptosis mechanism is still unknown clearly. Although some researchers have found AIF; ROS and other ligands can stimulate this type of PCD, the signal pathway still stay the phenomena stage and the deep mechanism should be dig out by us. No matter either apoptosis form, this type cell death has very important functions and deserves to be researched deeply. We collected a variety of information about this cell death and found that the caspase independent apoptosis existed in a mount of species and played indispensable role in cell growth, proliferation and death. In the figure 2, we give the outline about this type apoptosis pathway and the crosstalk manner between the different pathways, this picture will help us to know this type apoptosis well in the whole level.

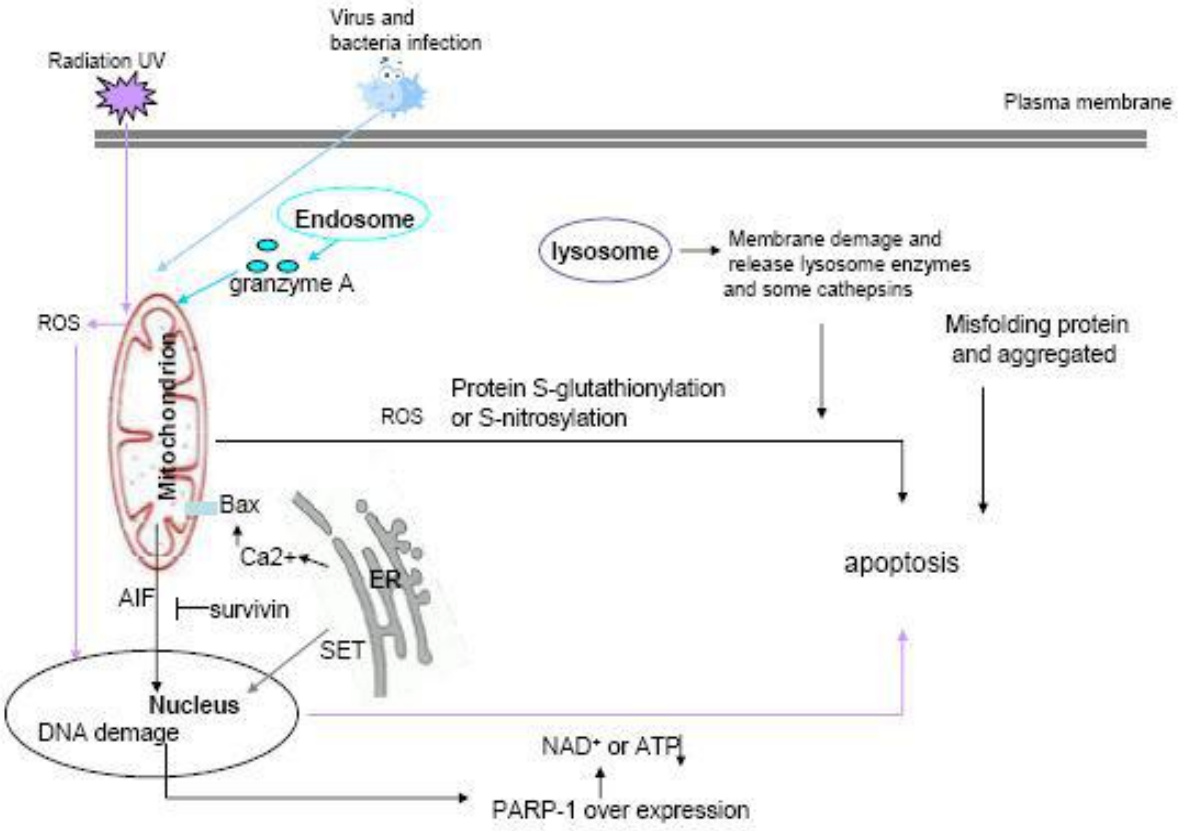


Figure 2. Caspase independent apoptosis pathway

Figure 2. In this type apoptosis, caspase family members did not involve in this cell death, and can not be inhibited by the caspase inhibitor {such as: the z-VAD-FMK; quinolyl valyl-o methylaspartyl[-2,6-difluorophenoxy]-methyl ketone(Q-VD-OPH);Ac-DEVD-FMK and so on}. In the cells, some components and events, such as the AIF; ROS; Ca2+; NAD+ and ATP; protein misfolding and modification, can trigger the caspase independent apoptosis.

3. Mitochondria dynamics and apoptosis

Mitochondria, as a semiautonomous organelle in cells, apart from containing their own genetic material, play an important role in the energy metabolism. It can produce ATP to maintain the cell life activity and be known as an energy company in cells. Beside this major role, mitochondria are the places that the lot of biological reaction processes happened, such as ROS production, apoptosis, and regulation of aging and so on. Mitochondria's dysfunction has the relation with many diseases (Alzheimer's disease; Parkinson's disease,cancer, diabetes). These

diseases have been identified to have some relation with the apoptosis; ROS produced by mitochondria have been regarded as one of important factors for apoptosis. Currently, many researches found the ROS produced increased when some pathogen infected, ROS can trigger apoptosis, through apoptosis, the pathogens may lost the perfect living environment, the host may defense the pathogen's diffusion by this manner. Due to these roles, mitochondria may be used as a proper therapeutic target to cure diseases related with this type cell death.

As a dynamic organelle, mitochondria can change their shape and structure constantly to respond to the different stimuli and metabolic demands of cells. According to the latest researches about biochemistry and cell biology, the mitochondrial shape changes between fusion and fission play a very important role in the regulation of apoptosis. There were some debates about the opinion that apoptosis occurred relating with the mitochondria fission. These debates focused on which process happened firstly, either apoptosis is the result of the mitochondria fission and fragmentation, or reversely, as a following up affair, the mitochondria's fission and fragmentation happened at the downstream of apoptosis. While, we can sure that mitochondria shape dynamic changes must be connected with apoptosis according to the follow latest researches: Calcium irons act as the upstream stimulus which can activate the cellular mitochondrial fission, the mitochondrial became fragmentation rapidly depended by the increased calcium level in intracellular. If the calcium level increased protractedly, mitochondria's fragmentation will be non-reversible and lead to apoptosis. So Jennifer R. Hom group regarded that the calcium involved in mitochondria morphology and participated in the apoptosis processing;some mitochondria membrane proteins also have been found to control mitochondrial morphology, for example, the Bcl-2 protein resides in the outer mitochondrial membrane, and this protein acts as a central regulator of the intrinsic apoptotic cascade; while some other toxins or proteins can also regulate the mitochondrial fission/fusion, and these variation shapes of mitochondria was found to have relation with some diseases, For instance, the PD(Parkinson's disease) have been found with the 10 Apoptosis and Medicine mitochondrial morphology changes, there were two toxin proteins parkin and PINK1 which were detected to play a role in maintaining mitochondrial homeostasis through targeting the mitochondria and regulating the mitochondrial dynamics; Fusion and fission often occur swiftly and are found to have relation with the mitochondria membrane potential changes .there are DRP1-dependent division and FZO1-dependent fusion reaction in mammalian cells, and division of mitochondria

accompany with apoptosis.,If mitochondrial fission/fusion dynamics losses balance, it will lead to some neurodegeneration diseases. So in brief, mitochondria act as the important role to keepcell healthy.

Mitochondrial iron transporter cytochrome C plays an classic role in apoptosis. As a bridge, it connect with caspase cascade reaction. When cytochrome C is released to the cytoplasm from the mitochondria as a result of response to some intrinsic or extrinsic ligands, it can trigger the downstream caspases and induce the intrinsic apoptosis. So mitochondria are not only the energy company of cell, but also have the ability to control the intrinsic apoptosis pathway through either cytochrome C, calcium, morphology changes(fission/fusion) or some membrane proteins expression imbalance(Bcl-2).

PATHOPHYSIOLOGICAL IMPLICATIONS

As mentioned above, cell proliferation and apoptosis are involved in many physiological and pathological processes. These are:

- the growth of tissues and organs in the embryo and later during childhood
- the replenishment of lost or time-expired cells such as leukocytes, gut epithelium and uterine endometrium
- immunological responses, including development of immunological tolerance to host proteins
- repair and healing after injury or inflammation
- the hyperplasia (increase in cell number and in connective tissue) associated with chronic inflammatory, hypersensitivity and autoimmune diseases
- the growth, invasion and metastasis of tumours
- regeneration of tissues.

The role of cell proliferation and apoptosis in the first two processes listed is self evident and needs no further comment, and their involvement in immune tolerance is discussed briefly above. But the other processes need further comment.

REPAIR AND HEALING

Repair occurs when there has been damage or loss of tissue; it is also implicated in the resolution of the local inflammatory reaction to a pathogen or chemical irritant. In some instances, damage or tissue loss can lead to regeneration, which is quite different to repair and is considered separately below.

In repair and healing, there is an ordered series of events involving cell migration, angiogenesis, proliferation of connective tissue cells, synthesis of extracellular matrix and finally remodelling-all coordinated by the growth factors and cytokines that are relevant for the particular tissue involved. TGF-β is a key cytokine in several of these processes.

There is considerable overlap between the inflammatory reaction and repair in terms of the cells and mechanisms activated.

HYPERPLASIA

Hyperplasia (cell proliferation and matrix expansion) are hallmarks of chronic inflammatory, hypersensitivity and autoimmune diseases such as rheumatoid arthritis , psoriasis, chronic ulcers, chronic obstructive lung disease, the processes underlying the bronchial hyperreactivity of chronic asthma and glomerular nephritis.

Cell proliferation and apoptotic events are also implicated in atherosclerosis, restenosis and myocardial repair after infarction .

THE GROWTH, INVASION AND METASTASIS OF TUMOURS

Perturbations in the growth factor signalling pathways, the antiapoptotic pathways and the function of the cell cycle controllers have an important role in the pathogenesis of malignancy. New understanding of this is leading to novel approaches to the treatment of cancer.

Repair, healing and regeneration

- Repair and healing occur when there has been damage or loss of tissue and are also implicated in the resolution of the local inflammatory reaction to a pathogen or chemical irritant. It involves the activation and proliferation of connective tissue cells, white blood cells and blood vessels.

- Regeneration is the replacement of the tissue or organ that has been damaged or lost. It involves the activation of primitive stem cells that have the potential to develop into any cell in the body. Regeneration of a tissue or organ is rare in mammals. If a mammal is injured or has its tissue removed, repair processes-often with subsequent scarring-usually make good the damage.
- It may be that repair (with rapid closure of the defect after tissue loss) is an evolutionary trade-off in mammals for the lost power of regeneration. But recent work has suggested that it might be possible to activate in mammals the original regenerative pathways-at least to some extent and in some organs.

STEM CELLS AND REGENERATION

Regeneration after damage or tissue loss implies restitution or replacement of the area so that it is identical to what was there before.

Many animals (e.g. amphibians and other lower orders) have an impressive power to regenerate their tissues, even to regrow an organ such as a limb. The essential process is the activation of *stem cells*-undifferentiated cells that have the potential to develop into any or most of the specialised cells in the body. Amphibians have a plentiful supply of these primitive cells in their organs and, furthermore, many of their specialised cells can dedifferentiate to become stem cells. These stem cells then multiply and retrace the pathways that generated the organ (e.g. a limb) during fetal life, proliferating again and again and eventually differentiating into the various cell types needed to replace the missing part.

However, during evolution, mammals have lost this ability and now have regenerative capacity in only a few tissues. Blood cells, intestinal epithelium and the outer layers of the skin are replaced continuously throughout life. Of the more discrete organs, there is a low degree of turnover and replacement of cells in such organs as liver, kidney and bone. This is in essence physiological renewal and is effected by local tissue-specific stem cells.

There is an account of liver regeneration in Greek myths. Prometheus stole the secret of fire from Zeus and gave it to mankind. To punish him, Zeus had him shackled to a crag in the Caucasus, and every day an eagle tore at his flesh and devoured much of his liver.

> But during the night, it regenerated and in the morning was whole again. The legend doesn't say whether the requisite 25% was left after the eagle had had its fill, and the regeneration described is unphysiologically speedy-rat liver takes 2 weeks or more to get back to the original size after 66% hepatectomy.

Almost alone, the liver has significant ability to replace itself if much of it is removed. It can regenerate to its original size in a remarkably short time, provided that at least 25% has been left intact. And the mature parenchymal liver cells participate in this process as well as all the other cellular components of the liver.

Although stem cells are known to exist in most tissues in adult mammals, they are very few in number, the vast majority of cells in most tissues being irreversibly differentiated. If a mammal is injured or its tissue is removed, repair processes-often with subsequent scarring-usually make good the damage. It seems that rapid closure of the defect after tissue loss (which is much more speedily accomplished by repair mechanisms) takes priority over regeneration.

Until recently, it was assumed that this was an unalterable situation, except for a few examples, some mentioned above. But recent work has suggested that it might be possible to activate in mammals the original regenerative pathways-at least to some extent and in some organs. Regeneration of a lost limb as happens in amphibians is manifestly not possible in humans, but regeneration of limited areas of a tissue or of a small part of an organ may well be feasible. For this to happen, it would be necessary to encourage some stem cells to proliferate, develop and differentiate at the relevant sites. Or-and this is a rather more remote prospect in humans-to persuade some local specialised cells to dedifferentiate. This can occur in some mammals under special circumstances. However, it may be that repair is the Janus face of regeneration, repair being an evolutionary trade-off in mammals for the lost power of regeneration.

Where are the relevant stem cells that could be coaxed into regenerative service? Various possibilities are being vigorously investigated and in some cases tested clinically. These include:

- embryonic stem cells (limited availability and serious ethical issues)
- bone marrow-derived mesenchymal stem cells
- muscle-derived stem cells
- human-induced pluripotent stem cells

- tissue-residing progenitor cells.

For a tissue such as the liver to regenerate, local tissue-specific stem cells must be stimulated by growth factors to enter the cell cycle and continue to proliferate. Other essential processes are:

- angiogenesis to supply the necessary blood vessels
- activation of MMPs and growth factors to replace the matrix in which the new cells are embedded
- interaction between matrix and integrins and fibronectin to link the new elements together.

Concomitant replacement of components of the lost connective tissue (fibroblasts, macrophages, etc.) would also be necessary.

Because most tissues do not regenerate spontaneously, mechanisms that could awaken the lost regenerative ability could be of immense value in numerous diseases. Two areas where recent progress has been reported include the regeneration of heart muscle after an infarction and replacement of insulin-secreting cells for the treatment of type I diabetes mellitus.

HEART MUSCLE

Until recently it was assumed that cardiac muscle had no power to regenerate. But in a particular strain of mouse, when part of the heart is damaged by freezing, repair processes do not start up; instead, the area is replaced by regeneration within a few months. Regeneration of heart tissue also occurs in dogs after acute heart failure. Mitosis of myocytes is seen in the normal human heart, and proliferation of myocytes immediately after infarction has been reported. Indeed, the sequence of events described above has been shown to occur during the process of remodelling after myocardial infarction in rodents.

More recently, stem cell therapy has been shown to improve ventricular function in the failing heart and to reduce infarct size and end systolic function in patients with myocardial infarction .

INSULIN-SECRETING CELLS

The results of ongoing clinical trials in patients with type I diabetes suggest that haemopoietic stem cell transplantation can remove the need for daily insulin injections.

THERAPEUTIC PROSPECTS

Considerable effort is being expended on finding compounds that will inhibit or modify the processes described in this chapter. So far there are few in clinical use, the main examples being those mentioned earlier, but it is likely that such agents will figure strongly in the pharmacology of the next decade, much work being aimed at developing new drugs for cancer therapy. Theoretically, all the processes could constitute targets for new drug development. Here we concentrate on those approaches that are proving or are likely to prove fruitful.

APOPTOTIC MECHANISMS

Compounds that could modify apoptosis are being intensively investigated. Here we can only outline some of the more important approaches.

Drugs that promote apoptosis by various mechanisms were heralded as a potential new approach to cancer treatment, and are actively being studied, though none has yet been approved for clinical use. Potential proapoptotic therapeutic approaches need to be targeted precisely to the diseased tissue to avoid the obvious risks of damaging other tissues. Examples include the following:

- An antisense compound against Bcl-2 (**oblimersen**) is in phase III trial for chronic lymphocytic leukaemia.
- **Obatoclax**, a small molecule inhibitor of Bcl-2 action, is in Phase I/II trial for haematological malignancies.
- MicroRNA technology could also be used to promote apoptosis .
- Two monoclonal agonist antibodies to the death receptor ligand TRAIL (**mapatumumab** and **lexatumumab**) are in Phase I/II trial against solid tumours and lymphomas.
- A new drug, **bortezomib**, which inhibits the proteasome, is available for the treatment of selected cancers. It causes the build-up of Bax, an apoptotic promoter protein of the Bcl-2 family that acts by inhibiting antiapoptotic Bcl-2. Bortezomib acts partly by inhibiting NFκB action.
- An endogenous caspase inhibitor, *survivin*, occurs in high concentration in certain tumours, its gene being one of the most cancer-specific genes in the genome. A small molecule suppressor of survivin is in clinical trial, the object being to free caspases to induce cancer cell suicide.

Despite the appeal of inhibiting apoptosis as a means of preventing or treating a wide range of common degenerative disorders, success in developing inhibitors for clinical use has so far proved elusive, and a number of such compounds have been found to lack efficacy in clinical trials:

- The use of a blocking antibody to the PD-1 death receptor is a potentially fruitful new avenue to explore for the treatment of HIV, hepatitis B and hepatitis C infections, as well as other chronic infections and some cancers that express the ligand for PD-1 (Williams & Bevan, 2006).
- Several caspase inhibitors are under investigation for use in the treatment of myocardial infarction, stroke, liver disease, organ transplantation and sepsis. **Emricasan** (IDN-6556) is undergoing trials in patients needing liver transplants.

ANGIOGENESIS AND METALLOPROTEINASES

Metalloproteinases and angiogenesis have critical roles in physiological (e.g. growth, repair) and pathological processes (e.g. tumour growth, chronic inflammatory conditions). The search for clinically useful MMP inhibitors is continuing, but has not so far been successful. At present, only one new drug has been approved for use in cancer treatment: the antiangiogenesis compound **bevacizumab**, a monoclonal antibody that acts against VEGF (see above) which is also used to treat age-related macular degeneration, a disease of the retina associated with excessive proliferation of retinal blood vessels.

CELL CYCLE REGULATION

The main endogenous positive regulators of the cell cycle are the cdks. Several small molecules that inhibit cdks by targeting the ATP-binding sites of these kinases have been developed; an example is **flavopiridol**, currently in clinical trials, which inhibits all the cdks, causing arrest of the cell cycle; it also promotes apoptosis, has antiangiogenic ability and can induce differentiation .

Some compounds affect upstream pathways for cdk activation and may find uses in cancer treatment. Examples are **perifosine** (currently in development for cancer treatment) and **lovastatin** (a cholesterol-lowering drug, which may also have anticancer properties).

Bortezomib, a boronate compound, covalently binds the proteasome, inhibiting the degradation of proapoptotic proteins. It is used in treating multiple myeloma.

Of the various components of the growth factor signalling pathway, receptor tyrosine kinases, the Ras protein and cytoplasmic kinases have been the subjects of most interest. Kinase inhibitors recently introduced for cancer treatment include **imatinib**, **gefitinib** and **erlotinib**.

AGONIST AND ANTAGONIST

Table 1 Inhibitor of apoptosis protein (IAP) family members

Abbreviation	Full name	Discoverer and time	Expression tissue or stage
Baculovirus			
cp-IAP	*Cydia pomonella* MNPV IAP	Crook *et al*, 1993	Entire virus
op-IAP	*Orgyia pseudotsugata* MNPV IAP	Birnbaum *et al*, 1994	
ac-IAP	*Autographa californica* MNPV IAP	Clem *et al*, 1994	
bm-IAP	*Bombyx mori* IAP	Huang *et al*, 2001	
*Hycu*IAP	*Hyphantria cunea* NPV IAP	Ikeda *et al*, 2004	
Yeast			
ScIAP/Bir1p	*Saccharomyces cerevisiae* IAP	Uren *et al*, 1999 Yoon *et al*, 1999	Most parts of yeast
SpIAP/Bir1p	*Schizosaccharomyces pombe* IAP		
Plant (*Arabidopsis thaliana*)			
AtILP1	*A. thaliana* IAP-like protein 1	Higashi *et al*, 2005	Most plant tissue
AtILP2	*A. thaliana* IAP-like protein 2		
Nematode (*Caenorhabditis elegan*)			
CeIAP1/BIR-1	*C. elegans* BIRPs, BIR-1	Fraser *et al*, 1999	Highly expressed in embryo
CeIAP2/BIR-2	*C. elegans* BIRPs, BIR-2		Only in adults and embryos

Nematode (*Caenorhabditis elegan*)			
CeIAP1/BIR-1	*C. elegans* BIRPs, BIR-1	Fraser *et al*, 1999	Highly expressed in embryoger
CeIAP2/BIR-2	*C. elegans* BIRPs, BIR-2		Only in adults and embryos
Fly (*Drosophila melanogaster*)			
DIAP1	*Drosophila* IAP 1	Hay *et al*, 1995	Most *Drosophila* tissue
DIAP2	*Drosophila* IAP 2		Most *Drosophila* tissue
Deterin	Deterin	Jones *et al*, 2000	Early stage embryos
Amphibian (*Xenopus*)			
xEIAP/XLX	*Xenopus* embryonic IAP	Tsuchiya *et al*, 2005	Highly expressed in oocyte and
xXIAP	*Xenopus* ortholog of XIAP		Oocyte and egg
Mammal			
XIAP/ILP-1/MIHA/BIRC4	XIAP	Duckett *et al*, 1996	Most human tissues
c-IAP1/hIAP1/MIHB/BIRC2	Cellular IAP 1	Rothe *et al*, 1995	Most human tissues; highly ex in adult thymus, testis, ovary
c-IAP2/hIAP2/MIHC/BIRC3	Cellular IAP 2	Rothe *et al*, 1995	Most human tissues; highly ex in adult spleen, thymus
ILP2/BIRC8/TS-IAP	IAP-like protein 2	Richter *et al*, 2001 Lagace *et al*, 2001	Adult testis

Apoptosis-inducing agents

The BCL-2 family as a target for new drugs

The BCL-2 protein is oncogenic because it inhibits apoptosis and increase resistance to cancer chemotherapy; other antiapoptotic member of the BCL-2 family are BCL-x_L and MCL-1. These are all current targets for anticancer drugs.

Some other inducing agents are:

1 Nutlin-3

2 RITA

3 ETA

4 IKA

5 PRIMA

Table 2. Examples of anticancer agents reported to induce apoptosis in cultured cells.

Drug	Cell lines tested
Amsacrine	Thymocytes
Aphidicoline	CHO strain AA8
	CHO strains AA8, UV 41
1-β-D-Arabinofuranosyl-cystosine	HL60, KGIA
BCNU	CCRF/CEM C7, F89, Molt-4-F, EB1, EB2-3945
Camptothecin	HL60, KGIA
Cisplatin	CHO strains AA8, UV 41
	HL60, KGIA
	L1210/0
Etoposide	Thymocytes
	CHO strains, AA8, UV 41
	HL60, KGIA
	CHO
	Chronic lymphocytic leukemia
5-Fluorodeoxyuridine	CHO strains AA8, UV 41
5-Fluorouracil	CHO strains AA8 UV 41

DIFFERENCES BETWEEN NECROSIS AND APOPTOSIS

Necrosis	Apoptosis
Morphological features	
● Loss of membrane integrity	● Membrane blebbing, but no loss of integrity
	● Aggregation of chromatin at the nuclear membrane
● Begins with swelling of cytoplasm and mitochondria	● Begins with shrinking of cytoplasm and condensation of nucleus
● Ends with total cell lysis	● Ends with fragmentation of cell into smaller bodies
● No vesicle formation, complete lysis	● Formation of membrane bound vesicles (apoptotic bodies)
● Disintegration (swelling) of organelles	● Mitochondria become leaky due to pore formation involving proteins of the bcl-2 family.
Biochemical features	
● Loss of regulation of ion homeostasis	● Tightly regulated process involving activation and enzymatic steps
● No energy requirement (passive process, also occurs at 4°C)	● Energy (ATP)-dependent (active process, does not occur at 4°C)
● Random digestion of DNA (smear of DNA after agarose gel electrophoresis)	● Non-random mono- and oligonucleosomal length fragmentation of DNA (Ladder pattern after agarose gel electrophoresis)
● Postlytic DNA fragmentation (= late event of death)	● Prelytic DNA fragmentation
	● Release of various factors (cytochrome C, AIF) into cytoplasm by mitochondria
	● Activation of caspase cascade
	● Alterations in membrane asymmetry (i.e., translocation of phosphatidylserine from the cytoplasmic to the extracellular side of the membrane)

Physiological significance	
Affects groups of contiguous cells	Affects individual cells
Evoked by non-physiological disturbances (complement attack, lytic viruses, hypothermia, hypoxia, ischemica, metabolic poisons)	Induced by physiological stimuli (lack of growth factors, changes in hormonal environment)
Phagocytosis by macrophages	Phagocytosis by adjacent cells or macrophages
Significant inflammatory response	No inflammatory response

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