What is apoptosis, and why is it important?

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BMJ 2001;322:1536–8

Philosophers have spent many centuries searching for the meaning of life, but in recent decades cell biologists have become even more fascinated by the meaning of death. Apoptosis describes the orchestrated collapse of a cell characterised by membrane blebbing, cell shrinkage, condensation of chromatin, and fragmentation of DNA followed by rapid engulfment of the corpse by neighbouring cells. It is distinguished from death by necrosis by the absence of an associated inflammatory response. These observations were made by Kerr et al as early as 1972,[1] but their importance was underestimated for many years. Today, however, apoptosis is implicated in biological processes ranging from embryogenesis to ageing, from normal tissue homoeostasis to many human diseases, and it has become one of the hottest fields of biomedical research.

Summary points

- Apoptosis is a genetically regulated form of cell death
- It has a role in biological processes, including embryogenesis, ageing, and many diseases
- The molecular mechanisms involved in death signals, genetic regulation, activation of effectors have been identified
- Many existing treatments (such as non-steroidal anti-inflammatories and anticancer treatments) act through apoptosis
- New treatments aimed at modifying apoptosis are being developed and are likely to be used to manage common diseases in the next decade

Biological mechanisms

The term apoptosis is often used interchangeably with programmed cell death. In the strictest sense, programmed cell death may be applied to other forms of cell death that require gene expression without fulfilling some, or all, of the morphological criteria of apoptosis.[2] Whatever the definition, studies clearly show that apoptosis is genetically regulated.

In its simplest model, the stages of apoptosis may be considered as initiation, genetic regulation, and effector mechanisms (figure).[3] Initiators of apoptosis include anticancer drugs, gamma and ultraviolet irradiation, deprivation of survival factors such as interleukin-1, and various other cytokines that activate "death receptors" such as Fas and tumour necrosis factor receptors. Through a variety of pathways, these stimuli in turn generate a characteristic pattern of gene expression.

The bcl-2 family of genes is the best studied and includes at least 20 members; some are pro-apoptotic or "death genes" and some are anti-apoptotic or "survival genes," including bcl-2 itself. The tumour suppressor gene p53 is also a well characterised apoptotic agent. The principal effectors are a family of proteases termed caspases. Studies in the nematode *Caenorhabditis elegens*, the fruitfly *Drosophila*, and the mouse indicate that the molecular machinery of apoptosis is evolutionarily conserved and intrinsic to all metazoan cells.

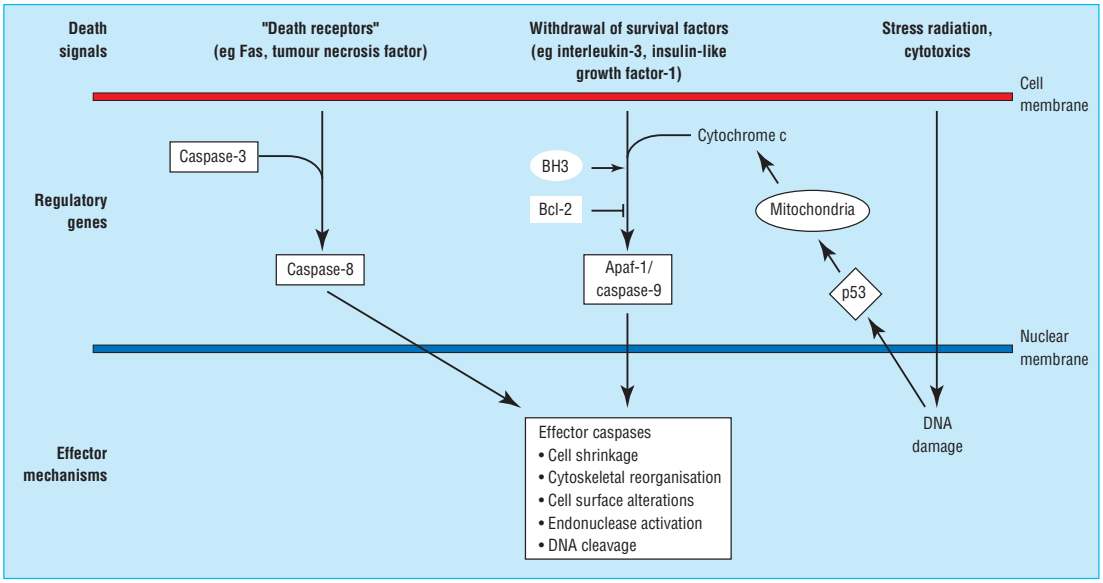
Assessment of apoptosis

Morphological assessment is the standard method for identifying and quantifying apoptosis. Other approaches include the use of fluorescence dyes to stain for condensed nuclei or exposed cell surface phosphatidylserine and the detection of fragmented DNA by terminal transferase mediated dUTP-biotin nick end labelling (TUNEL). Levels of cell death in a tissue are often expressed as an apoptotic index. This approach has serious limitations, mainly because of uncertainty about the duration of apoptosis (which may be less than six hours).[4] Thus, for instance, a small number of apoptotic cells observed in a static analysis may reflect a considerable contribution to cell turnover.

Physiological role

The first role of apoptosis is during intrauterine development. It helps to sculpture organ shape and carve out the interdigital webs of the fingers and toes. Apoptotic mechanisms are important determinants of fetal abnormalities; experiments have shown that wild type p53 mouse embryos will readily abort after radiation induced teratogenesis, whereas p53 null embryos will not.[5] Both the nervous system and the immune system arise through overproduction of cells followed by the apoptotic death of those that fail to establish functional synaptic connections or productive antigen specificities.

Such massacre or altruistic behaviour requires a tightly regulated system. In adulthood, about 10 billion cells die every day simply to keep balance with the



Apoptotic mechanisms in a human cell. Three major pathways are shown, indicating the main levels: death signals, gene regulation, and effector mechanisms. BH3 and bcl-2 represent the pro-apoptotic and anti-apoptotic members of the bcl-2 family. Apaf-1=apoptosis proteases activating factor

numbers of new cells arising from the body's stem cell populations. This normal homoeostasis is not just a passive process but regulated through apoptosis. The same mechanisms serve to "mop up" damaged cells. With ageing, apoptotic responses to DNA damage may be less tightly controlled and exaggerated, contributing to degenerative disease. Alternatively, the apoptotic responses may show reduced sensitivity, contributing to susceptibility to cancer.[6]

Altered apoptosis and disease

There is now a long list of diseases associated with altered cell survival.[7] Increased apoptosis is characteristic of AIDS; neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis; ischaemic injury after myocardial infarction, stroke, and reperfusion; and in autoimmune diseases such as hepatitis and graft versus host disease. Decreased or inhibited apoptosis is a feature of many malignancies, autoimmune disorders such as systemic lupus erythematosus, and some viral infections.

The role of apoptosis in cancer has probably received the greatest research effort.[8] Observations that patterns of spontaneous and induced apoptosis differ between the small and large intestine has led to a plausible explanation for the differences in incidence of cancer between these two sites.[9] Studies in p53 null mice show an increased preponderance of premature tumours and offer strong evidence that such apoptotic related genes are pivotal to development of tumours.

In addition, tumours develop methods to evade elimination by the immune system; one such

Examples of treatments that modulate apoptosis

Existing treatments	
Aspirin, cyclo-oxygenase-2 inhibitors[3]	Evidence that non-steroidal anti-inflammatory drugs protect against colorectal adenomas and cancer. This effect may be due to ability to induce apoptosis through inhibition of of cyclo-oxygenase isoenzyme 2
Anticancer drugs, radiotherapy[3]	Virtually all cytotoxic drugs and radiotherapy induce apoptosis in tumour and normal cells. p53 dependent and independent mechanisms are recognised
New treatments	
bcl-2 antisense[12]	Early clinical studies have shown positive results for some malignancies such as non-Hodgkin's lymphoma
Recombinant TRAIL*[12]	Lung, breast, colon, and kidney cancers are sensitive to TRAIL-stimulated apoptosis, and preclinical data support proof of principle
Caspase inhibitors[12]	Positive preclinical animal models for traumatic brain injury, amyotrophic lateral sclerosis, and Parkinson's disease
Antioxidants[3]	Pyrrolidinedithiocarbamate and 6-hydroxy-2,5,7,8-tetramethylachroman-carboxylic acid (a vitamin E analogue) enhances flurouracil induced apoptosis in colorectal cancer cells
Interleukin-1 receptor antagonists[12]	Reduces ischaemic brain damage in rat models

*Member of tumour necrosis factor superfamily

mechanism involves tumours expressing Fas, which enables them to delete (by apoptosis) antitumour lymphocytes. This phenomenon is known as the "tumour counterattack."[10] There is also increasing evidence that systemic stimuli such as insulin-like growth factor I (anti-apoptotic) and insulin-like growth factor binding protein 3 (pro-apoptotic) may influence the development and progression of many common cancers.[11]

Potential treatments

This brief review has shown that many human diseases may result when cells die that shouldn't or others live that should die. Modulation of apoptotic processes may thus offer valuable methods of treatment. It is now known that many existing drugs (for example, non-steroidal anti-inflammatories) act by altering the levels of apoptosis. Virtually all cytotoxic drugs and radiotherapy programmes induce apoptosis in tumour cells, and resistance to apoptosis is associated with treatment failure. These therapies also induce apoptosis in normal cells, and side effects on bone marrow, gut, and oral mucosa limit the dose that can be used. Many more new treatment strategies are currently in preclinical trials and show promise (box).[3 12] If future clinical studies are fruitful, this translation from basic science to clinical practice will be unique as it will affect not just one, but a broad range of disorders—and many patients will benefit.

Competing interests: None declared.

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How apoptosis is regulated, and what goes wrong in cancer

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BMJ 2001;322:1538–9

Programmed cell death (apoptosis) is an evolutionarily conserved pathway needed for embryonic development and tissue homoeostasis.[1] Apoptosis is the normal physiological response to many stimuli, including irreparable DNA damage. Various diseases evolve because of hyperactivation (neurodegenerative diseases, immunodeficiency, ischaemia-reperfusion injury) or suppression of programmed cell death (cancer, autoimmune disorders).[2]

In cancer, the balance between proliferation and programmed cell death is disturbed, and defects in apoptotic pathways allow cells with genetic abnormalities to survive. Most cytotoxic and hormonal treatments, as well as radiation, ultimately kill cancer cells by causing irreparable cellular damage that triggers apoptosis. Consequently, the efficacy of cancer treatments depends not only on the cellular damage they cause but also on the cell's ability to respond to the damage by inducing apoptotic machinery. Accordingly, mutations in apoptotic pathways may result in resistance to drugs and radiation. Such mutations might serve as predictors of chemoresistance and, most importantly, as new treatment targets.

Mitochondria and cell surface receptors mediate the two main pathways of apoptosis.[1] The mitochondrial pathway is thought to be important in response to cancer treatment and is mediated by bcl-2 family proteins. The final execution of cell death is performed by the caspase cascade, which is triggered by release of cytochrome C from mitochondria.

Apoptotic genes

The most studied genes related to apoptosis are the tumour suppressor gene p53, the anti-apoptotic gene bcl-2, and the pro-apoptotic gene bax. Normal wild type p53 can limit cell proliferation after DNA damage by two mechanisms: arresting the cell cycle or activating apoptosis.[3] p53 has a dual and complex role in chemosensitivity; it can either increase apoptosis or arrest growth and thereby increase drug resistance. This may explain why promising preclinical data indicating that presence of wild type p53 would predict chemosensitivity have translated into more conflicting clinical data.[4 5] Moreover, drugs that do not cause DNA damage, for instance taxanes and vinca alkaloids, may induce apoptosis through pathways that are independent of p53. Heterogeneous clinical data may also have resulted from use of different protein and molecular based methods to determine the p53 status. Sequencing gives the most complete picture of the p53 status,[6] but even functional p53 does not exclude defects somewhere downstream in the apoptotic pathway. The importance of p53 for chemosensitivity, however, is supported by the fact that, currently, the most curable cancers are among the minority of tumours in which p53 is not mutated—that is, some haematopoietic and germ cell tumours.

Overexpression of bcl-2 was first associated with follicular B cell lymphomas. Theoretically, overexpression of bcl-2 could provide a survival advantage for cancer cells, but in vivo, bcl-2 expression has been