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Apoptosis: its pathophysiology and monitoring. The role of apoptosis in the radioiodine therapy of hyperthyroidism

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Abstract

The review aims to give an up to date understanding of the mechanisms of apoptosis (programmed cell death), the methods of detecting apoptosis, in particular with regard to imaging such changes non-invasively. Radioiodine (I-131) is a gamma and beta emitting radionuclide and is commonplace in the treatment of hyperthyroidism. I-131 therapy relies on the destruction of thyroid tissue by beta radiation, and such destruction is proposed to be partly as a result of apoptosis. The review undertakes to explore and provoke research into the mechanisms of thyroid cell destruction by I-131, and whether such changes are able to be detected or monitored. Current knowledge concerning apoptosis in the thyroid gland in diseased states (including cancer) are described. The clinical significance of monitoring and modifying apoptosis are emphasized. Furthermore, overt and late destruction of thyroid tissue following I-131 therapy requires elaboration, and the relevance of detecting and modifying thyroid cell apoptosis following I-131 are questioned.
Key words: apoptosis, thyroid, radioiodine (I-131)

Introduction

Apoptosis (or programmed cell death) has been the subject of intense interest and research recently. It is known to occur in all organs as a moderator of cellular growth, development death and in many diseases. The study of apoptosis elaborates the mechanisms of such cellular death, giving an insight as to how this process can be regulated. The regulation of such processes would ultimately result in prospects toward therapy in disease where apoptosis plays a significant role. The ways of monitoring apoptosis give an indication of the extent of cellular death in a given region. Of considerable interest is how cellular death changes during tumour therapy and whether apoptosis is enhanced by oncotherapy, giving a guide to the best treatment modality. The ways to monitor apoptosis (invasive and non-invasive) in various organs and tissues have been described, studied and are a hot topic. Nuclear medicine gives one the possibility of non-invasive monitoring and observation of cellular death. Nuclear medicine, due to radiopharmaceuticals which bind to apoptotic markers (for e xample annexin V labelled with technetium 99m) has become one of the main methods of observing apoptosis in living organisms.

Radioiodine (I-131) therapy is commonplace in nuclear medicine. It is known that I-131 destroys thyroid tissue, and hence reduces overactive thyroid tissue. What is not known, however, is exactly how this tissue is destroyed and whether apoptotic changes are initiated following I-131 therapy.

A study of cellular destruction following beta emitting radioiodine is the next step towards understanding how radioiodine damages thyroid tissue and whether apoptosis plays a significant role during I-131 therapy. Can late hypothyroidism be attributed to an excessive stimulation of programmed cell death? What are the current possibilities and methods of monitoring apoptosis in various organs? What is the current focus toward modifying the mechanisms of programmed cellular death? Does knowledge of such methods give an insight into possible additional therapy to modify cellular death following I-131, ultimately resulting in better prognoses? This review of apoptosis and the methods of monitoring it, attempts to answer these questions.

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The essence of apoptosis

Apoptosis appears to have been first discovered by Carl Vogt in 1842 [1], and is an essential component during cellular devel-

opment, embryogenesis, homeostasis, the pathogenesis of many diseases as a means of removing excess, infected or damaged cells by the activation of an extrinsic or intrinsic suicide program. Such sequenced death is essential for proper embryonic development, organogenesis, and in the regulation of cellular turnover, as an integral component in most disease processes.

"Apoptosis" is derived from an ancient Greek word used to describe the way petals fall off from flowers and trees and was the term originally proposed during the "re-discovery" of programmed cell death by Kerr, Wyllie and Currie in 1972 [2], which plays a complementary but opposite role to mitosis in the regulation of animal cell populations, including the worm, Caenorhabditis elegans, where the biochemistry of apoptotic changes have been extensively studied. There appear to be 14 caspases, 11 of which are known to be present in humans [3, 4, 12]. This form of cell death contrasts with necrosis, which is a much more frequent type of cellular death, being an uncontrolled process resulting from acute injuries, resulting from factors such as radiation (of particular interest to nuclear medicine physicians), ischemia and inflammation. Necrotic cells increase in volume and then rupture, releasing their contents, inducing an inflammatory reaction. These necrotic changes occur rapidly, and have a sharp and swift nature which is extremely difficult to treat or prevent. Oncosis differs from necrosis and refers to the pre-lethal changes preceding necrosis, which are characterized by swelling, in contrast to those of apoptosis, which are characterized by shrinkage [4, 5].

Programmed cell death is characterised by the maintenance of an intact cellular membrane during the suicide process so as to allow adjacent cells such as phagocytes to engulf the dying cell, without an inflammatory response. The maintenance of an intact membrane is one of the features of apoptosis which is of considerable importance and stresses that this process is essentially intracellular. External membrane changes are the current focus of interest in identifying apoptosis using nuclear medicine techniques. Cells undergoing apoptosis usually exhibit a characteristic morphology by rapidly shrinking (as opposed to the swelling during necrosis), chromatin condensation, nuclear fragmentation, forming small apoptotic bodies, and endolytic cleavage of DNA into small oligonucleosomal fragments which are then phagocytosed by macrophages [3, 4].

There are many signals which can trigger apoptosis, however these signals to remove "unwanted or excessive" cells can be broadly categorized as being either extra cellular or intracellular.

Extracellular signals may either suppress or promote apoptosis, and the same signals may promote survival in one cell type and invoke the suicide program in others. For example, death receptors that are members of the tumour necrosis factor receptor (TNFR) family are present on cell membranes but have an intracellular domain that awaits activation of an intrinsic apoptotic pathway [6, 7]. Conversely, there are other factors, such as the nerve growth factors (NGFs), that bind to cell surface receptors and which act to prevent cell death [8, 9].

Intracellular signals, which also ultimately result in the activation of an intrinsic pathway can result from sub lethal damage caused by factors such as hypoxia, ionizing radiation, chemotherapy, or viral infection [4, 10].

The mechanism of programmed cell death

Two general pathways can be separated. In the extrinsic, or death receptor (DR) pathway, the apoptotic events are initiated by engaging the tumour necrosis factor (TNF) family of receptors (TNFRs), including TNFR1, Fas, DR 3-6. Upon ligand binding, or when over expressed in cells, TNFR family members aggregate, resulting in the recruitment of the adapter proteins, fas associated death domain (FADD) and TNF-related apoptosis inducing ligand (TRAIL) associated death domain (TRADD). These form the death inducing signalling complex (DISC), which then recruits procaspase-8 [7]. This allows proteolytic processing and activation of the receptor-associated procaspase-8 to active caspase 8, thereby initiating the subsequent cascade of additional processing and activation of downstream effector caspases to activate caspase 3 TRAIL or Apo2 ligand (TRAIL/Apo2L) is an apoptosis-inducing member of the TNF gene superfamily [7, 12–15]. Unlike TNF-α and fas ligand (FasL), TRAIL appears to specifically kill transformed and cancer cells while leaving normal cells intact, which is promising with regard to cancer therapy [16, 17].

The second pathway, which is known as the intrinsic or mitochondrial pathway mediates apoptosis induced by diverse stressful stimuli (extrinsic factors) to the cell which cause DNA damage, leading to the activation of p53 mediated mechanisms and other stresses which increase the activity of stress activated protein kinases (Fig. 1).

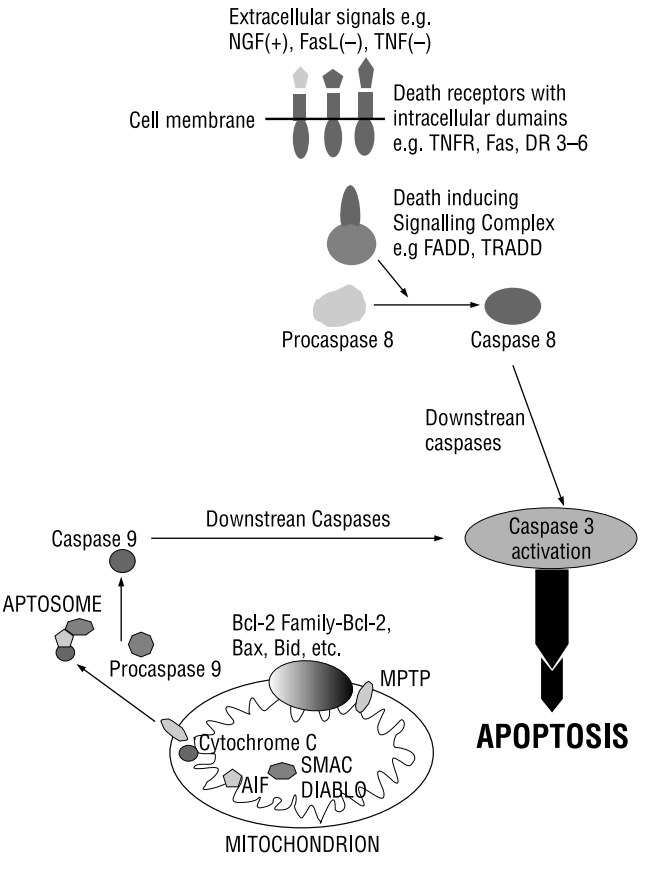

Figure 1. An illustration of the induction of apoptosis by extracellular and mitochondrial pathways. Both pathways converge at caspase 3 activation.

In the mitochondrial pathway, death signals lead to mitochondrial permeability changes induced by cellular stresses and sublethal insults (e.g. beta radiation, ischemia, inflammation), causing the release of pro-apoptotic factors. The factors released include the principal component cytochrome c, apoptosis inducing factor (AIF), and second mitochondria-derived activator of caspase (Smac/DIABLO).This interaction leads to the formation of the „apoptosome," which is a complex composed of cytochrome c, apoptosis protease activating factor (Apaf-1), and procaspase-9. The formation of the apoptosome induces the activation of caspase-9, which then processes and activates other caspases to orchestrate the biochemical execution of cells [18].

The key regulatory proteins of mitochondria-mediated apoptosis are the Bcl-2 family of proteins, which are either anti-apoptotic, and protect cells by inhibiting mitochondrial apoptosis, such as Bcl-2 and Bcl-xl, or pro-apoptotic, which cause mitochondrial apoptosis such as Bax and Bak. Their mechanism of action stills requires elaboration, however, Bcl-2 and Bcl-xl appear to directly or indirectly preserve the integrity of the outer mitochondrial membrane, thus preventing cytochrome c release and mitochondria-mediated cell death initiation, whereas the pro-apoptotic proteins Bax and Bak promote the mitochondrial release of cytochrome c.

The mitochondrial permeability transition pore (MPTP) is known to control the homeostasis of the mitochondrium and its permeablility is regulated by Bcl-2 members. The MPTP is formed by the adenine nucleotide transporter (ANT), the mitochondrial voltage dependent anion channel (VDAC) and cyclophilin D. The MPTP participates in the regulation of matrix Ca^{2+}, pH, mitochondrial membrane potential (m), and volume and functions as a Ca^{2+}–, voltage–, pH–, and a redox gated channel. Bax and Bak have been shown to associate with the MPTP complex, leading to cytochrome c, inducing cell death.

Common ground between the pathways exist, in that both the extrinsic and intrinsic pathways converge at caspase 3 activation. Furthermore Bid, and it's possible activation by caspase 8, and p53 mediated activation of Bax may couple the stress activated mitochondrial damage [7, 12–15, 19–21].

The role of apoptosis in diseases and various conditions

Programmed cell death by nature, is present in all tissues and organs, because life is finite and therefore apoptosis must occur for us to grow old and die. There are, however, many disease states where apoptosis is uncontrolled and excessive, or insufficient. The areas of most interest to nuclear medicine and therapy are.

The central nervous system

Because the CNS is a site of intense apoptosis during its formation during embryogenesis and appears to depend on survival promoting genes such as Bcl-x for survival in adulthood, it may be especially vulnerable to the derangement of apoptotic pathways [22]. Neurodegenerative diseases are, as the term suggests, characterized by a degenerative process. These degenerative diseases, which consist of disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis are generally considered to be the result of neuronal cell death in which apoptosis plays a significant role [23, 24]. Furthermore, interest has extended to the role apoptosis plays in stroke and neonatal hypoxic brain injury [25–27].

The cardiovascular system

Apoptosis plays an essential role during the formation of the heart and its septa. Anomalies in apoptosis may therefore contribute toward illnesses arising from malformations such as congenital heart disease, and disorders of conduction pathways. Programmed cell death is believed to occur in the changes following hypoxic injury and infarction such as reperfusion and remodelling [28, 29]. Moreover, viral and autoimmune myocarditis, cardiomyopathies and vessel wall changes such as aneurism formation and atherosclerotic plaque formation are generally considered to be associated with programmed cell death [30].

Autoimmune disease

The immune system is the source of production of T and B cells, which have an auto-reactive action, and are eliminated by apoptosis. Therefore, it is postulated that unregulated excessive apoptosis may be the cause of various autoimmune diseases that are characterized by an excessive loss of normal or protective cells, such as in multiple sclerosis, type-I diabetes mellitus, Hashimoto's thyroiditis [31], Sjögrens syndrome, arthropathies, systemic lupus erythematosus, rheumatic fever, Kawasaki's disease [30], and certain cancers such as melanoma [32]. Conversely, an inappropriately low rate of apoptosis may promote the survival and accumulation of abnormal cells that can give rise to tumour formation and prolonged autoimmune stimulation such as in cancers and in Grave's disease [33].

Organ transplantation and rejection

In rejected organ transplants, apoptotic cells are frequently encountered, and apoptotic cell death may be part of the mechanism of transplant rejection. High levels of expression of Fas ligand on the allograft correlate with graft acceptance in some models of organ transplantation. Furthermore, some of the immunosuppressive drugs currently in clinical use might exert their activity at least in part through effects on apoptotic pathways [34]. Moreover, apoptosis occurring during rejection has been detected and localized in heart, lung and liver transplantation *in vivo* [35].

Oncology

Malignant tumours represent uncontrolled cellular growth, which may be the result of insufficient or suppressed apoptosis. Mutations of the Bcl-2 and p53 genes have been associated with lymphomas. Additionally, mutations of the p53 gene are a common occurrence in most human cancers (55 to 70%) [36]. Tumour reduction is known to be associated with apoptosis and therefore a measure of the amount of apoptosis in a tumour in response to therapy is of critical importance in assisting oncotherapy [37, 38]. There is currently significant interest in localising and evaluating programmed cell death by techniques involving nuclear medicine, which would predict the chemo/ radiotherapy sensitivity of a given tumour [39, 40].

Monitoring apoptosis by structure and function. A need for non-invasive methods

There are numerous ways in which apoptosis can be detected. The most reliable methods are based on detecting the characteristic morphological changes which require tissue sampling (i.e. *in vitro*). However, due to the very important role apoptosis

plays in disease, methods of detecting and monitoring apoptosis in the living organism (*in vivo*), without the need for tissue sampling are being developed and are an area of intense research.

Detecting apoptosis in vitro

The characteristic structural changes which are characteristic of, and which define apoptosis, are most conclusively detected by morphological viewing using electron microscopy, allowing the early recognition of very subtle intracellular changes. An alternative to electron microscopy is light microscopy, which has a lower resolution although is able to detect membrane blebbing and apoptotic bodies.

TUNEL-Cleavage of genomic DNA during apoptosis may yield double-stranded, low molecular weight DNA fragments (mono- and oligonucleosomes) as well as single strand breaks ("nicks") in high molecular weight DNA. These DNA strand breaks can be identified by labelling free 3´-OH terminals with modified nucleotides (such as biotin or fluorescein) in an enzymatic reaction [41]. This technique relies on the DNA fragmentation that occurs during apoptosis and has become widespread in use; due to the fact that it is simple and sensitive, although its sensitivity has been questioned and has given rise to several variations of the technique [42].

Other methods for the detection of apoptosis include flow cytometry, DNA laddering based on the fragmentation of genomic DNA, Immunohistochemisty, and assays based on the expression of apoptotic factors, namely Fas, FasL, TRAIL, Bcl-2 and caspases (in particular caspases 3 and 8) [43].

Detecting apoptosis in vivo

Magnetic resonance imaging. Apoptosis causes cellular shrinkage, which consequently causes a change in water resonance and diffusion, which MRI techniques are able to detect. Such shrinkage is observed as a darkened area on an MRI study. Changes in tissue water diffusion detected by diffusion weighted MRI (DW-MRI) are considered to have a potential role in the in vivo monitoring of apoptosis. For example, the volume reduction of a tumour in response to chemotherapy can be measured, but simple MRI would detect such changes too late to be clinically of use. Furthermore, some tumours respond to certain chemotherapeutic agents by swelling, which would give a false result. Magnetic resonance spectroscopy (MRS), based on the principle of differences in the frequency of 1H, 31P, 13C is tissue dependant and has been shown to be of use in detecting apoptotic changes [23, 25, 26].

A promising technique using MRI is using a protein as a contrast agent, namely the C2 domain of synaptotagmin I, which binds to anionic phospholipids in cell membranes, such as phosphatidylserine (PS). PS lies on the cytoplasmic side of mammalian cells and is absent on the surface of normal cells. This externalization is an active process and requires the deactivation of translocase and floppase, and the activation of scramblase, which are enzymes which maintain an asymmetric distribution of anionic and cationic phospholipids. The externalization of PS occurs rapidly, within 30–60 mins following the onset of apoptosis, before blebbing, nuclear and cytoplasmic shrinkage. One drawback of mapping PS, is that externalisation is not believed to be purely specific for apoptosis [44, 45].

Using radioisotopes. Radioisotopes have, for a long while, been used in determining possible areas of cell death-such radioisotopes include Tc99m-pyrophosphate, Indium 111-labelled antimyosin and Tc-99m-glucaric acid. All these radiotracers rely on the loss of sarcolemma integrity in order to localize regions of necrosis [46]. With respect to non-invasive imaging of myocardial damage, it has recently been proposed that Tc-99m glucaric acid has only an affinity for the oncotic myocardium, whereas indium antimyosin has an affinity for both the oncotic and apoptotic myocardium [47]. PS, the most significant molecule known to change location following cellular damage, has now become the main target for detecting apoptosis [45].

Annexin V is a member of the calcium and phospholipid binding family of proteins which also has a vascular anticoagulant activity. Annexin V is largely found on the cytosolic side of plasma membranes and is known to have a high affinity (10-9M) for PS in the presence of physiological concentrations of calcium. Hence, due to this fact it is the main substance of concern for localizing PS. Annexin V can be used to stain tissues and is a useful property in the staining of apoptotic cells arising from the fact that it can bind to many sites on cell surfaces, and therefore results in a very intense signal. Due to its tracing abilities, annexin V was first used successfully to identify thrombi in in-vitro models [48].

There have been several attempts to couple or modify annexin V using various substrates. The labelling of annexin V with fluoroscein isothiocyanate has been used as a marker of apoptosis. Biotin labelled annexin V has also been used to detect cellular death and apoptotic changes [46].

The use of nuclear medicine techniques to demonstrate the externalization of PS that occurs during apoptosis in vivo was first used when Tc-99m-labelled annexin V, where the annexin V had been conjugated with hydrazinonicotinamide (HYNIC) was used in 3 animal models of apoptosis [35]. Since then, several studies have been conducted with regards to tumours and chemotherapy induced apoptosis using labelled annexin V [49–51].

There are many possibilities in using radiolabelled annexin V — an additional role worthy of note is possible applications in the monitoring of graft-host disease [30, 34].

Theoretically, annexin V can and has been used with other radioisotopes to study and identify regions of cellular death, mainly using I-123 and I-124 and 18F using PET [52–54].

Apoptosis in the thyroid

Whilst little is known concerning apoptosis of a normal thyroid, an extensive study has been carried out to elaborate apoptotic pathways in autoimmune diseases such as Hashimoto's and Graves' disease (GD) as well as in cancer. Increasing evidence suggests that apoptosis plays an important role in the pathogenesis of autoimmune and proliferative thyroid diseases, and that the apoptotic pathways involved are complex and highly regulated. It is known that thyrocytes from patients with Hashimoto's thyroiditis are destroyed as a result of apoptosis, however it is still uncertain whether such cellular death is caused by intrathyroidal lymphocytes or the thyrocytes themselves [55, 56].

Autoimmune thyroid diseases such as GD and Hashimoto's thyroiditis have been associated with differential expression of Fas and TRAIL receptor-mediated apoptosis and Fas is known to play

a considerable role in thyroid disease [31]. Thyroid cell destruction characteristic of autoimmune thyroiditis can be seen as the consequence of inappropriate expression of Fas or TRAIL death pathway molecules and down-regulation of the apoptosis controlling protein Bcl-2, which may be induced by cytokines released locally by infiltrating lymphocytes. In contrast, GD thyrocytes are protected from apoptotic death possibly by the anti-apoptotic action of thyrotrophin receptor antibodies or soluble Fas and/or the over expression of Fas ligand, which all create an anti-apoptotic potential for the thyroid cells and favour apoptosis of the infiltrating lymphocytes. Moreover, has been demonstrated that GD thyrocytes express less Fas/FasL than HT thyrocytes, and that GD thyrocytes express increased levels of anti apoptotic Bcl-2.

Thyroid hormones have been shown to induce apoptosis in lymphocytes, reduce the expression of bcl-2, and induce the intracellular formation of free radicals , whereas an excess of Iodine is believed to induce Fas mediated apoptosis [57, 58]. An imbalance between thyroid cell proliferation and cell death would appear to be a prerequisite for goitre formation or cancer development and progression. In human thyroid goitre, Fas-mediated apoptosis is suppressed, leading to thyroid cell hyperplasia. With regard to malignancies of the thyroid, malignant cells may avoid an immune response by over expressing Fas L and by inducing apoptosis in the invading immune cells.

Radioiodine and apoptosis — future possibilities

It is known that radiation induces apoptosis in various tissues by causing sub lethal damage and activating the mitochondrial pathway by the release of p53 in response to DNA damage. Radiation is also thought to activate mitochondrial apoptosis by induction of bax and down-regulation of anti-apoptotic genes, such as bcl-2 are also thought to play a role. Recent concepts have been introduced that radiation-induced apoptosis can be divided into pre-mitotic and post-mitotic apoptosis, pre-mitotic apoptosis being associated with a prompt activation of caspase 3, and post-mitotic, which occurs after cell division, does not require such a rapid activation of caspase 3 [59, 60].

Radioiodine (I-131) has been used for the treatment of hyperthyroidism since the 1940's to treat conditions such as Graves' disease and struma nodosa. Several long term studies have shown I-131 therapy to be safe, with little, if any long term risks. Some studies have shown a slight association with thyroid cancer, but the reasoning for the association has been to put to question. I-131 therapy has now replaced surgery as the non-conservative method of treating hyperthyroidism, and is the preferred mode of therapy for Grave's disease in the U.S.

I-131 therapy relies on the fact that this isotope of iodine emits beta radiation, and is selectively accumulated by hyper-functioning thyroid tissue, where such beta emission induces cell death [61].

High doses which patients receive during I-131 therapy immediately destroy thyroid tissue by simple necrosis. However, it is reasonable to assume that neighbouring tissue which survives and receives sub lethal damage would undergo apoptotic changes by activating mitochondrial apoptosis. Such tissue destruction by apoptosis would occur later, after the activation of apoptotic pathways. Our department for treating hyperthyroid patients monitors patients for at least 6 months following I-131 therapy, and up to 12 months, such monitoring being necessary because experience has shown that renewed hyperthyroidism or hypothyroidism may present relatively late.

A question which has been posed is why some patients have such differing outcomes, considering that prior to therapy, they had similar thyroid masses, scans, uptakes, biological half life, as well as having received an identical dose? A hypothesis is that late destruction of thyroid tissue, leading to late hypothyroidism is caused by excessive apoptosis, stimulated by radioiodine.

Apoptosis is known to be present in thyroid disease states but its role in healthy thyroid is still in question, whilst beta emission is known to stimulate apoptosis. It is obvious that both healthy and diseased thyroids exhibit apoptosis and such patients who quickly become overtly hypothyroid may have a state of apoptosis in their thyroids, which is already excessive and vulnerable to over stimulation by radioiodine.

The questions this short review poses are how exactly radioiodine affects apoptosis in the thyroid gland? To what degree does radioiodine stimulate apoptosis and how can apoptosis be monitored or visualized to improve patient care?

Our department is currently working towards ways to assess the degree of apoptosis in the thyroid in order to understand the effects of radioiodine on thyroid tissue in order to answer these questions [61].

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