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APOPTOSIS (PROGRAMMED CELL DEATH) - A REVIEW

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ABSTRACT

The process of apoptosis or programmed cell death is characterized by distinct morphological characteristics and energy-dependent biochemical mechanisms. It is an intrinsic cell-suicide programme which ensures proper development by maintaining tissue homeostasis and safeguarding the organism by getting rid of damaged or infected cells that may interfere with normal function. Apoptosis is a vital component of various processes including normal cell turnover, hormone-dependent atrophy, proper development and functioning of the immune system, chemical-induced cell death and embryonic development. Dysregulation of apoptotic signalling and inappropriate apoptosis (either too little or too much) is a factor in many diseases including neurodegenerative diseases, autoimmune disorders and many types of cancer. The ability to modulate the life or death of a cell is recognized for its immense therapeutic potential. Therefore, research continues to focus on the elucidation and analysis of the cell cycle machinery and signaling pathways that control cell cycle arrest and apoptosis. The objective of this review is to provide a general overview /comprehensive information of current knowledge on the process of apoptosis including morphology, biochemistry, mechanism, regulation and the role of apoptosis in health and disease, as well as a discussion of potential alternative forms of apoptosis.

KEYWORDS: Apoptosis, programmed cell death, intrinsic/extrinsic pathway, cancer, pathogenesis.

INTRODUCTION

Cell death is an essential part of normal development and continues into adulthood. The human body is composed of approximately 10^{14} cells. Every day billions of cells die in order

to secure the functionality of the whole organism. Thus, the body size remains the same only because cell division exactly balances cell death[1]. Eventually, the term apoptosis had been coined in order to describe the morphological processes leading to controlled cellular self-destruction and was first introduced in a publication by Kerr, Wyllie and Currie[2] Apoptosis is of greek origin, having the meaning "falling off or dropping off", in analogy to leaves falling off trees or petals dropping off flowers. This analogy emphasizes that the death of living matter is an integral and necessary part of the life cycle of organisms. The name was first introduced by John Kerr in 1972 [2] and refers to the morphological feature of formation of ‘‘apoptotic bodies’’ from a cell. Carl Vogt, however, first described the phenomenon more than 100 years earlier in 1842. Over the last 10 years, the number of publications related to apoptosis has increased exponentially to now over 2% of the papers published in the life sciences. A timeline of cell death publication chronology can be found. This great interest in apoptosis arose due to the recognition that many diseases involve too much apoptosis e.g. neuro degenerative diseases, Parkinson’s, Alzheimer’s, spinal muscular atrophy, AIDS or too little apoptosis (e.g., cancer (either by virus infection or by DNA mutations such as p53 and Bcl-2) or autoimmune diseases (diabetes type I, encephalomyelitis). Many toxins and other cellular stresses can also trigger apoptosis (e.g., oxidative stress, alcohol). It should be stressed that apoptosis is a well-defined and possibly the most frequent form of programmed cell death, but that other, non-apoptotic types of cell death also might be of biological significance [3].

Since the mid-nineteenth century, many observations have indicated that cell death plays a considerable role during physiological processes of multicellular organisms, particularly during embryogenesis and metamorphosis[4,5]. The term programmed cell death was introduced in 1964, proposing that cell death during development is not of accidental nature but follows a sequence of controlled steps leading to locally and temporally defined self-destruction[6].

Apoptosis is over 20 times faster than mitosis. Sightings of dying cells in vivo are therefore rare. Apoptotic cells are engulfed and degraded by neighboring cells without a trace. For cell homeostasis to be maintained, a balance between the increase (by differentiation from precursors and by proliferation) and decrease (by further differentiation and cell death) in the number of a cell population has to be neatly balanced.

In the average human adult between 50 and 70 billion cells die every day due to apoptosis. For an average child between the ages of 8 and 14, approximately 20 billion to 30 billion cells die a day[7]. If mitosis proceeded without cell death, an 80-year-old person would have 2 tons of bone marrow and lymph nodes, and a gut 16 km long.

The apoptotic mode of cell death is an active and defined process which plays an important role in the development of multicellular organisms and in the regulation and maintenance of the cell populations in tissues upon physiological and pathological conditions[3].

Significance of apoptosis

Apoptosis is a type of cell death regulated in an orderly way by a series of signal cascades under certain situations. It plays an essential role in regulating growth, development and immune response, and clearing redundant or abnormal cells in organisms. It is also an important way by which organisms can maintain a constant amount of cells in order to live successfully. The induction and execution of apoptosis require the cooperation of a series of molecules including signal molecules, receptors, enzymes and gene regulating proteins. Among them, the caspase-cascade signalling system, regulated by various molecules such as the inhibitor of apoptosis protein (IAP), Bcl-2 family proteins, and calpain, is vital in the process of apoptosis[8]. The development and maintenance of multicellular biological systems depends on a sophisticated interplay between the cells forming the organism, it sometimes even seems to involve an altruistic behaviour of individual cells in favour of the organism as a whole. During development many cells are produced in excess which eventually undergo programmed cell death and thereby contribute to sculpturing many organs and tissues. The term ‘programmed cell death’ has therefore been used primarily to describe the coordinated series of events leading to controlled cell demise in developing organisms. Owing to this early definition, the term ‘physiological cell death’ has been linked to the notion of programmed cell death.

Morphological change in apoptotic cells

Morphologically, it is characterized by compact cytoplasm, vacuoles in the cytoplasm membrane, nuclear chromatin condensation, DNA fragmentation and the formation of apoptotic bodies. At the periphery of the nuclear membrane, chromatin condensation starts, forming a ring like structure. Later on, the nucleus progressively condenses and breaks up (karyorrhexis). The cell detaches from the surrounding tissue and its outlines become convoluted and form extensions. The term budding has been coined for a process whereby

the extensions separate and the plasma membrane seals to form a separate membrane around the detached solid cellular material. These apoptotic bodies are crowded with closely packed cellular organelles and fragments of nucleus. The fine structures, including membranes and mitochondria, are well preserved inside the bodies. The apoptotic bodies are rapidly phagocytosed into neighbouring cells, including macrophages and parenchymal cells. Apoptotic bodies can be recognised inside these cells, but eventually they become degraded. If the fragmented cell is not phagocytosed it will undergo degradation which resembles necrosis and the condition is termed secondary necrosis[9]. Apoptotic shrinkage, disassembly into apoptotic bodies and engulfment of individual cells characteristically occur without associated inflammation, which would be the consequence of releasing intracellular contents into tissues as shown in fig.1.

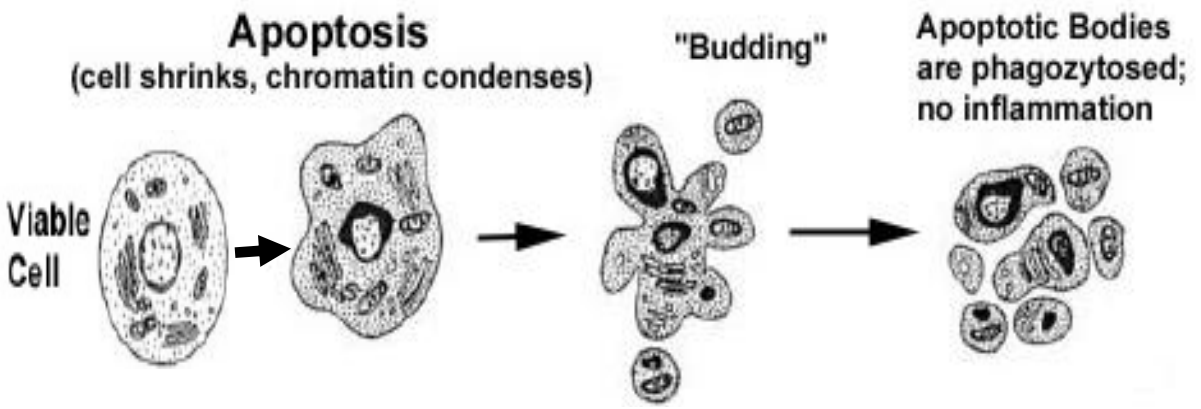

Fig.1.Mophological features of apoptotic cell: Cellular shrinking, chromatin condensation and marginalization, formation of membrane-bound apoptotic bodies, cytosol and nuclear fragmentations.

Biochemical changes in apoptosis

There are three main types of biochemical changes which observed in apoptosis: 1) Activation of caspases, 2) Breakdown of DNA and protein and 3) Membrane changes and recognition by phagocytic cells[10]. Early in apoptosis, there is expression of phosphatidylserine (PS) in the outer layers of the cell membrane, which has been "flipped out" from the inner layers. This allows early recognition of dead cells by macrophages, and results in phagocytosis without the release of pro-inflammatory cellular components[11]. This is followed by a characteristic breakdown of DNA into large 50 to 300 kilobase pieces[12]. Later, there is inter nucleosomal cleavage of DNA into oligonucleosomes in multiples of 180 to 200 base pairs by endonucleases. Although this feature is characteristic of apoptosis, it is not

specific as the typical DNA ladder in agarose gel electrophoresis can be seen in necrotic cells as well[13]. Another specific feature of apoptosis is the activation of a group of enzymes belonging to the cysteine protease family named caspases. The "c" of "caspase" refers to a cysteine protease, while the "aspase" refers to the enzyme's unique property to cleave after aspartic acid residues[14]. Activated caspases cleave many vital cellular proteins and break up the nuclear scaffold and cytoskeleton. They also activate DNAase, which further degrade nuclear DNA. Although the biochemical changes explain in part some of the morphological changes in apoptosis, it is important to note that biochemical analyses of DNA fragmentation or caspase activation should not be used to define apoptosis, as apoptosis can occur without oligonucleosomal DNA fragmentation and can be caspase-independent. While many biochemical assays and experiments have been used in the detection of apoptosis, the Nomenclature Committee on Cell Death (NCCD) has proposed that the classification of cell death modalities should rely purely on morphological criteria because there is no clear-cut equivalence between ultrastructural changes and biochemical cell death characteristics.

Mechanisms of apoptosis

Apoptosis is a tightly regulated and efficient cell death program involving multiple factors. Every cell contains an intrinsic mechanism which signals death or survival and any imbalance in these signals can result in apoptosis[15]. Understanding the mechanisms of apoptosis is crucial and helps in the understanding of the pathogenesis of conditions as a result of disordered apoptosis. This in turn, may help in the development of drugs that target certain apoptotic genes or pathways. Caspases take major and a central role in apoptotic mechanism. The term caspases is derived from cysteine-dependent aspartate-specific proteases[15]. Caspases are central to the mechanism of apoptosis as they are both the initiators and executioners. There are three pathways by which caspases can be activated. The two commonly described initiation pathways are the intrinsic or mitochondrial and extrinsic or death receptor pathways of apoptosis. Both pathways eventually lead to a common pathway or the execution phase of apoptosis. A third less well-known initiation pathway is known as intrinsic endoplasmic reticulum pathway (Rebbeca, 2011).

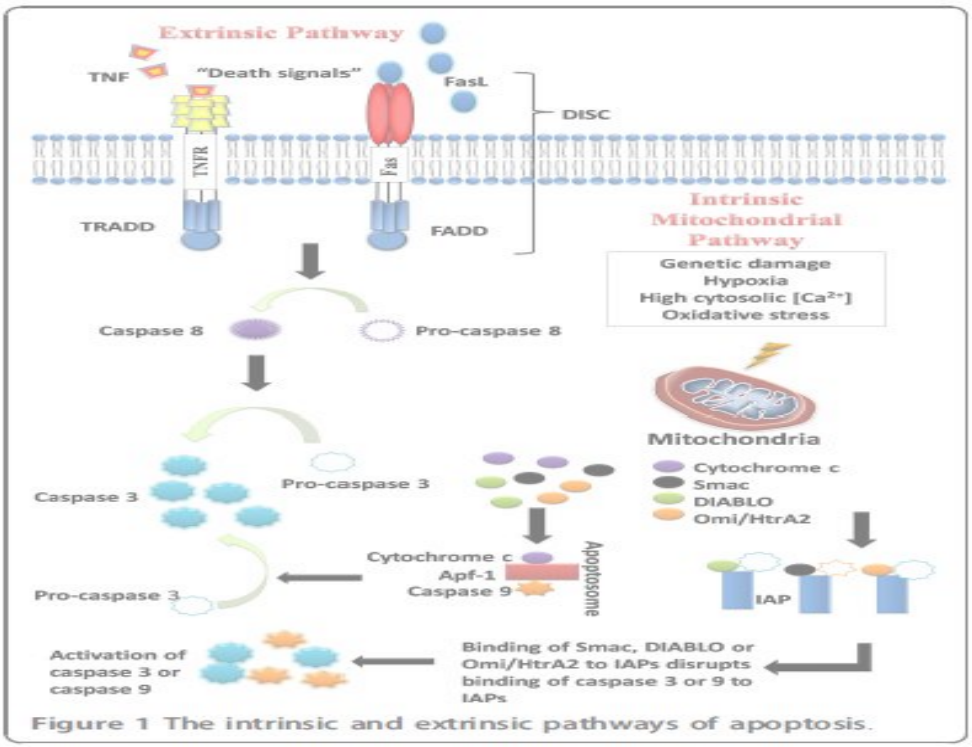

Figure 2: The intrinsic and extrinsic pathways of apoptosis [17].

The extrinsic death receptor pathway

The extrinsic death receptor pathway begins when death ligands bind to a death receptor. Cell surface receptors transmit apoptotic signals after ligation with specific ligands[15]. Although several death receptors have been described, the best known death receptors is the type 1 TNF receptor (TNFR1) and a related protein called Fas (CD95) and their ligands are called TNF and Fas ligand (FasL) respectively. These death receptors have an intracellular death domain that recruits adapter proteins such as TNF receptor-associated death domain (TRADD) and Fas-associated death domain (FADD), as well as cysteine proteases like caspase 8 [16]. Binding of the death ligand to the death receptor results in the formation of a binding site for an adaptor protein and the whole ligand-receptor-adaptor protein complex is known as the death-inducing signalling complex (DISC). The DISC initiates the assembly and activates pro caspase -8. Active caspase-8 then processes downstream effector caspases which subsequently cleave specific substrates resulting in cell death[17].

The intrinsic mitochondrial pathway

As its name implies, the intrinsic pathway is initiated within the cell. Factors like hypoxia, genetic damage, high concentration of cytosolic calcium ions, extreme oxidative stress trigger the initiation of the mitochondrial pathway resulting in increased mitochondrial

permeability[15]. The release of apoptogenic factors from the mitochondrial intermembrane space to the cytosol, such as cytochrome C is triggered by a poptotic stimuli. This pathway is closely regulated by a group of proteins belonging to the Bcl-2 family, named after the BCL2 gene originally observed at the chromosomal breakpoint of the translocation of chromosome 18 to 14 in follicular non-Hodgkin lymphoma[18]. There are two main groups of the Bcl-2 proteins, namely the pro-apoptotic proteins (e.g. Bax, Bak, Bad, Bcl-Xs, Bid, Bik, Bim and Hrk) and the anti-apoptotic proteins (e.g. Bcl-2, Bcl-X_L, Bcl-W, Bfl-1 and Mcl-1)[19]. While the anti-apoptotic proteins regulate apoptosis by blocking the mitochondrial release of cytochrome-c, the pro-apoptotic proteins act by promoting such release. It is not the absolute quantity but rather the balance between the pro- and anti-apoptotic proteins that determines whether apoptosis would be initiated[19]. Other apoptotic factors that are released from the mitochondrial intermembrane space into the cytoplasm include apoptosis inducing factor (AIF), second mitochondria-derived activator of caspase (Smac), direct IAP Binding protein with Low pI (DIABLO) and Omi/high temperature requirement protein A (HtrA2)[17]. Cytoplasmic release of cytochrome c activates caspase 3 via the formation of a complex known as apoptosome which is made up of cytochrome c, Apaf-1 and caspase 9 [20]. On the other hand, Smac/DIABLO or Omi/HtrA2 promotes caspase activation by binding to inhibitor of apoptosis proteins (IAPs) which subsequently leads to disruption in the interaction of IAPs with caspase-3 or -9 [20].

The common pathway

A series of caspases are activated in the execution phase of apoptosis. Caspase 9 is the upstream caspase for the intrinsic pathway while caspase 8 is that of the extrinsic pathway. The intrinsic and extrinsic pathways converge to caspase 3. Caspase 3 then cleaves the inhibitor of the caspase-activated deoxyribonuclease, which is responsible for nuclear apoptosis[21]. In addition, downstream caspases induce cleavage of protein kinases, cytoskeletal proteins, DNA repair proteins and inhibitory subunits of endonucleases family. They also exert an effect on the cytoskeleton, cell cycle and signalling pathways, which together contribute to the typical morphological changes in apoptosis[21].

The intrinsic endoplasmic reticulum pathway

Endoplasmic reticulum (ER) is known for synthesis and folding of different types of protein. Survival of cells and maintanance of its activity largely depends on healthy and functioning ER. If function of ER is impaired, aggregations of unfolded proteins take place. In stressed

condition, its protein folding capacity interrupts. To restore its normal functions transmembrane receptors detect the onset of stress and try to bring the normal ER function back by initiating various protecting mechanisms, collectively called as Unfold Protein Response (UPR)[22]. If there is failure of adaptive response or when stress is prolonged, apoptosis take place.

The complex cellular process leading to apoptosis is mediated through three ER transmembrane receptors, namely pancreatic ER kinase (PKR) like ER kinase (PERK), activating transcription factor-6 (ATF-6) and Inositol Requiring Enzyme-1(IRE-1)[22]. When the cells are in rest, all three ER stress receptors are maintained in an inactive state through their association with the Glucose Regulated Protein 78 (GRP78) and ER chaperone. Due to prolong stress when unfolded protein accumulates, dissociation of GRP78 occurs which activate the UPR, a prosurvival response to restore normal function of ER by reducing the accumulation of unfolded proteins[22,23]. After UPR activation, if stress does not restore or protein aggregation persist, prosurvival signalling switches to pro apoptotic signalling. The UPR-mediated signals might elicit apoptosis by three distinct phases namely initiation, commitment and execution. When cells are in rest, the pro-apoptotic Bax and Bak (Bax/Bak) remain inactive by interaction with BCL2 both on the mitochondrial as well as endoplasmic reticulum (ER) membranes, whereas Bim (BH3) is inhibited by binding to cytoskeletal dynein[22].

Severe ER stress leads to activation of c-Jun N-terminal kinase (JNK) and induction of C/EBP homologous protein (CHOP) in the initiation phase[22,24]. Anti-apoptotic effect of BCL2is eliminated by both JNK and CHOP; along with CHOP blocks expression of BCL2, whereas JNK phosphorylates it. Bim is also phosphorylated by JNK. As JNK phosphorylates Bim, it is released from the cytoskeleton and become activated. It happens in commitment phase.

Together, all these changes allow activation of Bax and Bak, transmission of the signal from the ER to the mitochondria as well as death. It has been proposed that Caspase 12 is a key mediator of ER stress-induced apoptosis[22]. Due to these changes this phase is called execution phase.

Regulation of apoptosis

Apoptosis involves a cascade of complex events which include the delivery of external signals through defined receptor complexes, the well-regulated expression of a number of genes, and the execution of apoptosis by proteases and endonucleases.A large number of genes and proteins have been implicated in the control of apoptosis. These can be categorized by their activities at discrete steps in the apoptotic pathway as well as their relationships to specific disease states (Figure 2). Adverse assortment of triggers activate the cascade, which is subject to tight homeostatic regulation by a number of regulators or modulators of the death pathway. The „point of no return" in apoptosis is reached when caspases become enzymatically active in cleaving target proteins (the „executioners" of apoptosis). When there is disruption in the balance of anti-apoptotic and pro-apoptotic members of the Bcl-2 family, the result is dysregulated apoptosis in the affected cells. This can be due to an overexpression of one or more anti-apoptotic proteins or an under expression of one or more pro-apoptotic proteins or a combination of both.

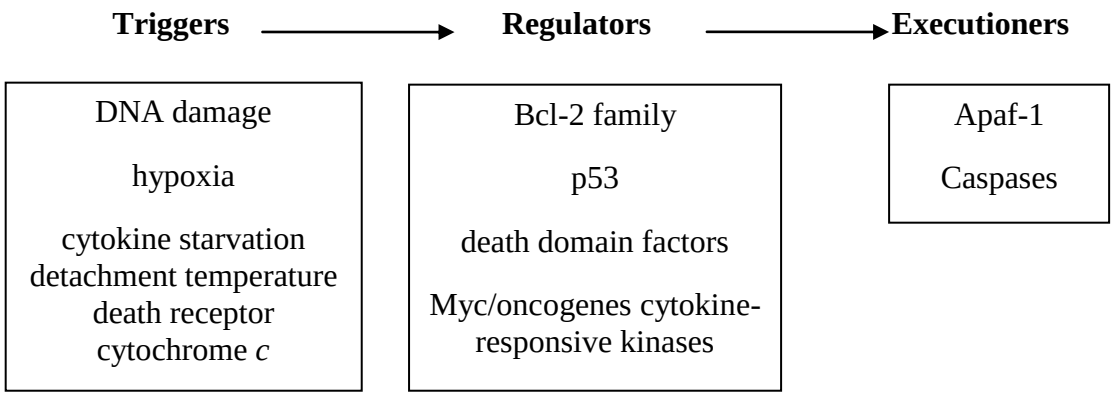

Figure.3. The apoptosis cascade: triggers, regulators and effectors (executioners). A variety of triggers both pathological and physiological (e.g. during normal development) can activate apoptosis. Numerous regulators include factors which can dampen or amplify the apoptotic signal, as well as intermediates which are essential participants in a specific apoptotic pathway (e.g. p53). Executioners are activated as downstream effectors. Their activation represents a point of no return in the life or death of a cell.

FACTORS OF APOPTOSIS

The Bcl-2 family of proteins

The Bcl-2 family of proteins comprises of pro-apoptotic and anti-apoptotic proteins which play a pivotal role in the regulation of apoptosis, especially via the intrinsic pathway as they reside upstream of irreversible cellular damage and act mainly at the mitochondria level[25]. Bcl-2 encoded by the BCL2 gene was the first protein of this family to be identified more than 20 years ago, which derives its name from B-cell lymphoma 2, the second member of a

range of proteins found in human B-cell lymphomas with the t (14; 18) chromosomal translocation[18].

Location of all the Bcl-2 members are on the outer mitochondrial membrane. They are dimmers which are responsible for membrane permeability either in the form of an ion channel or through the creation of pores[26]. Based on their function and the homology (BH) domains the Bcl-2 family members are further divided into three groups. The first group are the anti-apoptotic proteins containing all four BH domains and they protect the cell from apoptotic stimuli. Some examples are Bcl-2, Bcl-xL, Mcl-1, Bcl-w, A1/Bfl-1, and Bcl-B/Bcl2L10. The second group is made up of the BH-3 only proteins, so named because in comparison to the other members, they are restricted to the BH3 domain. Examples include Bid, Bim, Puma, Noxa, Bad, Bmf, Hrk, and Bik. In times of cellular stresses such as DNA damage, growth factor deprivation and endoplasmic reticulum stress, the BH3-only proteins, which are initiators of apoptosis, are activated. Therefore, they are pro-apoptotic. Members of the third group contain all four BH domains and they are also pro-apoptotic. Some examples are Bax, Bak, and Bok/Mtd.

When the balance of anti-apoptotic and pro-apoptotic members of the Bcl-2 family is disrupted, it results in deregulated apoptosis in the affected cells. This can be due to an overexpression of one or more anti-apoptotic proteins or an under expression of one or more pro-apoptotic proteins or a combination of both. For example, Raffo *et al* showed that the over expression of Bcl-2 protected prostate cancer cells from apoptosis[1], while Fulda *et al* reported Bcl-2 over expression led to inhibition of TRAIL-induced apoptosis in neuroblastoma, glioblastoma and breast carcinoma cells[27]. Over expression of Bcl-xL has also been reported to confer a multi-drug resistance phenotype in tumour cells and prevent them from undergoing apoptosis[28]. In colorectal cancers with microsatellite instability, on the other hand, mutations in the *bax* gene are very common. Impaired apoptosis resulting from *bax* (G) 8 frameshift mutations could contribute to resistance of colorectal cancer cells to anticancer treatments is demonstrated by Miquel *et al*[29]. In case of chronic lymphocytic leukaemia (CLL), the malignant cells have an anti-apoptotic phenotype with high levels of anti-apoptotic Bcl-2 and low levels of pro-apoptotic proteins such as Bax *in vivo*. Leukaemogenesis in CLL is due to reduced apoptosis rather than increased proliferation *in vivo*[30]. According to Pepper *et al* B-lymphocytes in CLL showed an increased Bcl-2/Bax ratio

in patients with CLL and that when these cells were cultured *in vitro*, drug-induced apoptosis in B-CLL cells was inversely related to Bcl-2/Bax ratios[31].

The p53

The p53 protein is also known as tumour protein 53 (or TP 53). It is one of the best known tumour suppressor proteins encoded by the tumour suppressor gene *TP53* located at the short arm of chromosome 17. It is named after its molecular weights, i.e., 53 kDa. It was first identified in 1979 as a transformation-related protein and a cellular protein accumulated in the nuclei of cancer cells which bind tightly to the simian virus 40 (SV40) large T antigen. Initially, it was found to be weakly-oncogenic. It was later discovered that the oncogenic property was due to a p53 mutation, or what was later called a "gain of oncogenic function" [32]. Many studies have looked into its function and its role in cancer since its discovery. It is involved in the induction of apoptosis and also plays a key role in cell cycle regulation, development, differentiation, gene amplification, DNA recombination, chromosomal segregation and cellular senescence and hence called the "guardian of the genome".

More than 50% of human cancers have been linked to defects in the p53 tumour suppressor gene[32]. Recently, it is reported that some target genes of p53 involved in apoptosis and cell cycle regulation are aberrantly expressed in melanoma cells, which lead to abnormal activity of p53 and contribute to the proliferation of these cells. In a mouse model with an *N*-terminal deletion mutant of p53 (Δ122p53) that corresponds to Δ133p53, Slatter *et al* demonstrated that these mice had decreased survival, a different and more aggressive tumor spectrum, a marked proliferative advantage on cells, reduced apoptosis and a profound pro-inflammatory phenotype[33]. It has also been found that when the p53 mutant was silenced, such down-regulation of mutant p53 expression resulted in reduced cellular colony growth in human cancer cells, which was found to be due to the induction of apoptosis.

Inhibitor of apoptosis proteins (IAPs)

Apoptosis, cytokinesis and signal transduction are regulated by a group of structurally and functionally similar proteins which are the inhibitors of apoptosis. They are characterised by the presence of a baculovirus IAP repeat (BIR) protein domain. To date eight IAPs have been identified, named as, NAIP (BIRC1), c-IAP1 (BIRC2), c-IAP2 (BIRC3), X-linked IAP (XIAP, BIRC4), Survivin (BIRC5), Apollon (BRUCE, BIRC6), Livin/ML-IAP (BIRC7) and IAP-like protein 2 (BIRC8) [34]. IAPs are endogenous inhibitors of caspases and by binding their conserved BIR domains to the active sites of caspases, they can inhibit caspase activity

by promoting degradation of active caspases or by keeping the caspases away from their substrates.

In many cancers there is report of deregulated IAP expression. For example, Lopes *et al.* demonstrated abnormal expression of the IAP family in pancreatic cancer cells and resistance to chemotherapy is due to this abnormal expression. Among the IAPs tested, the study concluded that drug resistance correlated most significantly with the expression of cIAP-2 in pancreatic cells[35]. On the other hand, Livin was demonstrated to be highly expressed in lymphoma and melanoma[34,36]. While Apollon, was found to be upregulated in gliomas and was responsible for cisplatin and camptothecin resistance[37]. Over expression of another IAP, Survivin, has been reported in various cancers. Small *et al*. observed that transgenic mice that over expressed survivin in haematopoietic cells were at an increased risk of haematological malignancies and that haematopoietic cells engineered to overexpress Survivin were less susceptible to apoptosis [38]., Together with XIAP, Survivin was also found to be overexpressed in non-small cell lung carcinomas (NSCLCs) and the study concluded that the over expression of Survivin in the majority of NSCLCs together with the abundant or upregulated expression of XIAP suggested that these tumours were endowed with resistance against a variety of apoptosis-inducing conditions[39].

Reduced capsase activity

The caspases are classified into two groups: 1) those related to caspase 1 (e.g. caspase-1, -4, -5, -13, and -14) and are mainly involved in cytokine processing during inflammatory processes and 2) those that play a central role in apoptosis (e.g. caspase-2, -3. -6, -7,-8, -9 and -10). The second group can be further classified into 1) initiator caspases e.g. caspase-2, -8, -9 and -10 which are primarily responsible for the initiation of the apoptotic pathway and 2) effector caspases (caspase-3, -6 and -7) which are responsible in the actual cleavage of cellular components during apoptosis[40]. Caspases play an important role in the initiation and execution of apoptosis. It is therefore reasonable to believe that low levels of caspases or impairment in caspase function may lead to a decrease in apoptosis.

In one study, downregulation of caspase-9 was found to be a frequent event in patients with stage II colorectal cancer and correlates with poor clinical outcome[41]. In another study, Devarajan *et al* observed that caspases-3 mRNA levels in commercially available total RNA samples from breast, ovarian, and cervical tumuors were either undetectable (breast and cervical) or substantially decreased (ovarian) and that the sensitivity of caspase-3-deficient

breast cancer (MCF-7) cells to undergo apoptosis in response to anticancer drug or other stimuli of apoptosis could be enhanced by restoring caspase-3 expression, suggesting that the loss of caspases-3 expression and function could contribute to breast cancer cell survival[42]. In some instances, more than one caspase can be downregulated, contributing to tumour cell growth and development. In a cDNA array differential expression study, Fong *et al* observed a co-downregulation of both capase-8 and -10 and postulated that it may contribute to the pathogenesis of choriocarcinoma[43].

Impaired death receptor signaling

Death receptors and ligands of the death receptors are key players in the extrinsic pathway of apoptosis. Other than TNFR1 (also known as DR 1) and Fas (also known as DR2, CD95 or APO-1) mentioned in Section 2.3, examples of death receptors include DR3 (or APO-3), DR4 (or TNF-related apoptosis inducing ligand receptor 1 (TRAIL-1) or (APO-2), DR5 (or TRAIL-2), DR 6, ectodysplasin A receptor (EDAR) and nerve growth factor receptor (NGFR). These receptors posses a death domain and when triggered by a death signal, a number of molecules are attracted to the death domain, resulting in the activation of a signalling cascade. However, death ligands can also bind to decoy death receptors that do not posess a death domain and the latter fail to form signaling complexes and initiate the signaling cascade[44].

Several abnormalities in the death signaling pathways that can lead to evasion of the extrinsic pathway of apoptosis have been identified. Such abnormalities include downregulation of the receptor or impairment of receptor function regardless of the mechanism or type of defects, as well as a reduced level in the death signals, all of which contribute to impaired signaling and hence a reduction of apoptosis. For instance, down regulation of receptor surface expression has been indicated in some studies as a mechanism of acquired drug resistance. A reduced expression of CD95 was found to play a role in treatment-resistant leukaemia[45] or neuroblastoma cells[46]. Reduced membrane expression of death receptors and abnormal expression of decoy receptors have also been reported to play a role in the evasion of the death signaling pathways in various cancers[27]. In a study carried out to examine if changes in death ligand and death receptor expression during different stages of cervical carcinogenesis were related to an imbalance between proliferation and apoptosis, Reesink-Peters *et al* concluded that the loss of Fas and the deregulation of FasL, DR4, DR5, and tumor necrosis

factor-related apoptosis-inducing ligand (TRAIL) in the cervical intraepithelial neoplasia (CIN)-cervical cancer sequence might be responsible for cervical carcinogenesis[47].

Malfunctioning of apoptosis and pathogenesis

Improper apoptosis or malfunctioning of individual apoptotic machinery may cause several human diseases like cancer, neurodegenerative as well as several types of autoimmune disorder [48,49,50] (Figure 4). It has been found that unnecessary cell death and unsound regulation of caspase activity are associated with certain diseases such as Alzheimer's disease, Parkinson's disease and Huntington's disease. Augmented activities of caspases-8 and -9 have been observed in peripheral blood mononuclear cells of Alzheimer's disease patients[51] and in brain tissues of Alzheimer's as well as Parkinson's disease patients[52,53]. Huntington's disease, a neurodegenerative disorder, has also been found to be caused by increased activity of caspase-10 in a manner similar to caspase-8. Mutations on Fas and Fas ligand (Fas-L) in humans may cause a complicated immune disorder like autoimmune lymphoproliferative syndrome (ALPS), a semblance of murine lymphoproliferation (lpr) and generalized lymphoproliferative disorder (gld)[54].

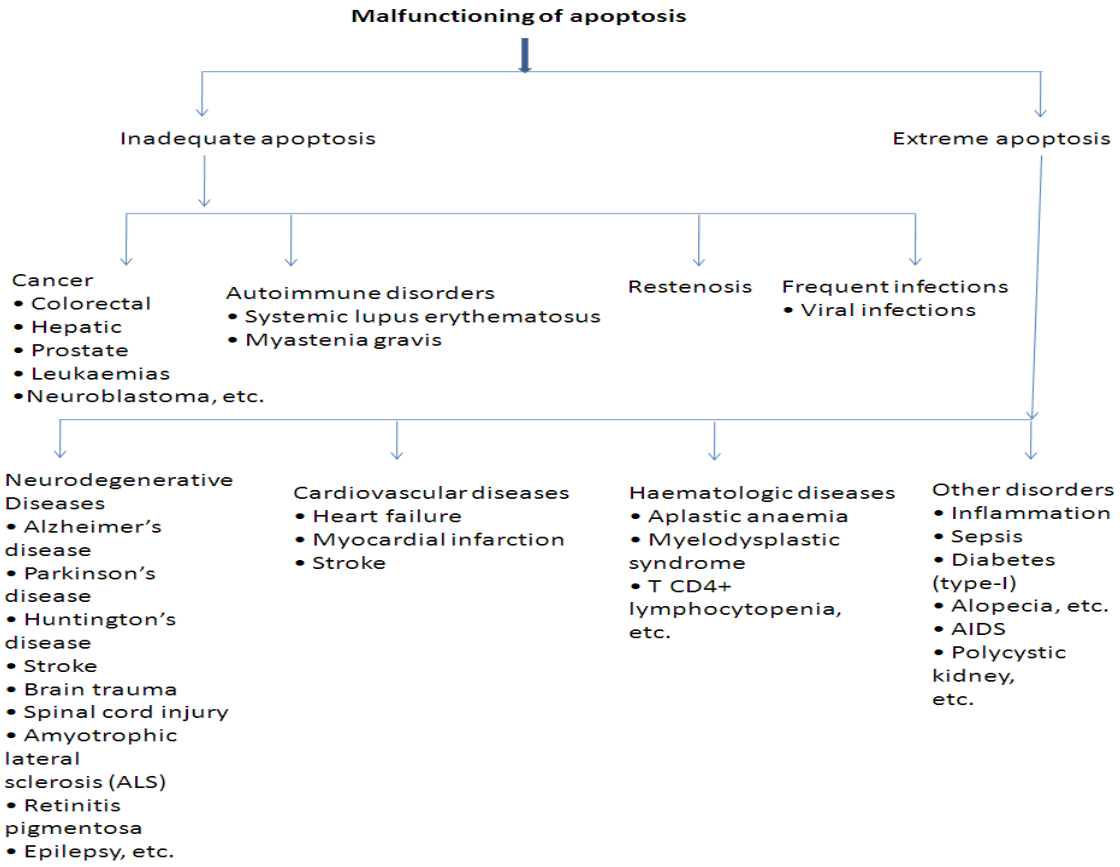

Figure 4: Some common diseases associated with malfunctioning of apoptosis or PCD.

CONCLUSION

Apoptosis is an exceedingly complicated phenomenon, with energy-dependent flow of molecular events accomplished by two types of pathways such as intrinsic and extrinsic that involves the activation of a set of cysteine proteases known as “caspases”. The process of cell death by means of apoptosis (PCD) is accompanied by a number of distinctive morphologic and metabolic changes. Apoptosis plays a significant role in survival by maintaining the homeostasis in multicellular organisms as well as in the management of many diseases, since malfunctioning of apoptotic pathway may cause several human diseases like cancer, neurodegenerative as well as several types of autoimmune disorders.

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