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Review Article

Apoptosis (programmed cell death) and its signals - A review

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Abstract

Apoptosis is a sequential order of cell death occurring regularly to ensure a homeostatic balance between the rate of cell formation and cell death. However, a misplaced of this balancing function can contribute to an abnormal cell growth / proliferation or autoimmune disorders etc. Apoptosis is therefore said to be crucial from the point of development of an embryo throughout the growth of an organism contributing to the renewal of tissues and also the getting rid of inflammatory cells. This review seeks to elaborate on the recent overview of the mechanism involved in apoptosis, some element and signal contributing to its function and inhibition together with how their malfunction contribute to a number of cancer related cases.

Keywords: Apoptosis, Caspases, Bcl-2, p53, Inhibitor of Apoptosis (IAP).

Apoptose, morte celular programada e seus sinais: uma revisão

Resumo

A apoptose é uma ordem sequencial de morte celular que ocorre regularmente para garantir um equilíbrio homeostático entre a taxa de formação e a morte celular. No entanto, um desequilíbrio dessa função pode contribuir para um crescimento/proliferação celular anormal ou distúrbios autoimunes. A apoptose é, portanto, considerada crucial do ponto de desenvolvimento de um embrião ao longo do crescimento de um organismo que contribui para a renovação dos tecidos e também a eliminação de células inflamatórias. Esta revisão procura elaborar a recente visão geral do mecanismo envolvido na apoptose, alguns elementos e sinais que contribuem para sua função e inibição, além de como o mau funcionamento deles contribui para vários casos relacionados ao câncer.

Palavras-chave: Apoptose, Caspases, Bcl-2, p53, Inibidor de Apoptose (IAP)

1. Introduction

Apoptosis is a process of sequential order of cell death thus referred to as a programmed cell death that first emerge in 1964 prior to the idea that the cell death does not occur accidentally (Lockshin and Williams, 1964). However, in 1842 Carl Vogt a German scientist and philosopher discovered the phenomenon but did not researched into it much after he noticed that, cells within the notochord disappeared, yet replaced during the process of development. In 1951, Glucksmann hypothesized that cell death was a requisite for the growth and death of an organism. Later on, the phenomenon, and predictions were still noticed by Kerr, Wyllie and Currie in their work, first describing the phenomenon as a morphological sequence of distinct cell death, apoptosis coined from a Greek word "a-po-toe-sis "which literally meant dropping (Kerr, 2002); (Paweletz and Walther, 2001). A number of studies have proven that the process is very crucial for development and homeostasis (Hassan et al. 2014). Though other forms of cell death have been described, the conditions surrounding the phenomenon have also drawn the attention and interest of scientist. Apoptosis may be triggered by uncontrolled cell proliferation, DNA damage or as a result of some diseases and conditions such as Alzheimer's, Parkinson's, spinal muscular atrophy, stress or toxin, demonstrating its importance for the maintenance of cell population and defense against such conditions or disease (Lopez and Tait, 2015; Zaman et al., 2014; Leist and Jaattela, 2001) . Also, it briefs us on the fact that, it's not a single condition triggered phenomenon, but by a number of conditions of which some are even induced personally through the administration of certain therapeutic drugs and hormonal releases. To be certain, apoptosis and necrosis may be used interchangeably but though all can occur at the same time, their means of been triggered is dependent on the level or intensity of the stimuli, toxin, infection, trauma, when caspase fails to do it work or a reduce amount of ATP, based on this conditions apoptosis can be said to be a define process that leads to the activation

of members of cysteine proteases (caspase)to execute a number of process for the cell demise in order to equate the level of cell proliferation for a cell development and the process of homeostasis. (Zeiss, 2003). Taken together, there are number of pathways through which apoptosis can be initiated or inhibited. However, it will be noted that every cell does possess an internal signal which can initiate apoptosis at any point in time (Lavrik et al., 2005; Ziegler and Groscurth, 2004).

2. Morphological conditions in apoptosis

Morphologically the presence of apoptosis renders the cell with shrinkage and pyknosis, this is characterized by DNA fragmentation, chromatin condensation and the compacting of the cytoplasm. The blebbing of the plasma membrane is followed by these conditions which causes the nucleus to break (karyorrhexis). This further causes the cells to detach from its surrounding tissues and to be separated into cell fragments with the cytoplasm embedded with tightly packed organelles. Next, this compact bodies referred to as apoptotic bodies assume the process of budding. Finally, the release of cell surface markers (phosphatidylserine) from the cell membrane enhance some cells such macrophages and parenchyma to phagocytized these bodies for further degradation and also to prevent secondary necrosis (Savill and Fadok, 2000; Kurosaka et al., 2003).

3. Biochemical process in apoptosis

From the aforementioned conditions such as protein cleavage, DNA breakdown followed by the phagocytic degradation can all be considered to occur through a biochemical process. During this process the final execution step seem to be solely dependent on the caspase. The caspase which is a cysteine protease responsible for the cleavage of aspartic residue upon its activation, thus a cysteine dependent aspartate-specific protease. These are inactive proenzyme which once activated, it in tend activates other pro-caspase to initiate a protease cascade and the execution of a number of cell death process (Schneider and Tschopp, 2000). Recent reports suggest that, there are about 18 of the caspase which can be group into apoptotic caspase comprising of the initiators (-2,-8,-9,-10) and effector (-3,-6,-7) which are also regarded as the executioners and lastly the inflammatory caspases (-1,-4,-5) that are crucial during inflammatory for cytokine processing (Rai et al., 2005). Aside these caspases are the caspase 11,12,13 and 14 of which each is known to function in a different pattern. The 11 and 12 are proven to function during septic shock by regulating cytokine maturation and cytotoxicity respectively, they are both also involved in apoptosis. Caspase 13 serves as a gene with bovine origin which can execute apoptosis, upon activation by caspase 8, but are also known to be essential for inactivating certain proteins that are crucial for survival, 14 is known to be involved in epidermal differentiation (Nakagawa et al., 2000; Koenig et al., 2001; Kang et al., 2002; Fink and Cookson, 2005). Geertrui et al.implicated that caspase -14 plays a crucial role in the correct processing of (pro) filaggrin during cornification (Geertrui et al., 2008). Furthermore, protein crosslinking serves as biochemical means of accomplishing apoptosis through the expression of tissue transglutaminase, causing an inter nucleosomal DNA cleavage which yields in a number of DNA fragment of about 180-200 base pairs with the visualization of a characteristic DNA ladder on an agarose gel electrophoresis. However, in the state of release of the cell surface markers (phosphatidylserine), the process enhances some cells such macrophages and parenchyma cells for degradation. (McCarthy and Evan, 1998). Finally, these biochemical processes are accomplished through either the intrinsic or extrinsic pathways. The intrinsic can further be categorized into two which is the intrinsic mitochondrial pathway and the intrinsic endoplasmic reticulum pathway.

3.1. Intrinsic mitochondrial pathway

This pathway involves series of intracellular event occurring within the mitochondria. A number of factors such as hormones, growth factors, radiation, toxins, hypoxia, viral infections and hyperthermia leading to the intermembrane permeability of the mitochondria can trigger this pathway. This process further leads to the release of pro-apoptotic proteins through the intermembranes into the cytosol (Saelens et al., 2004; Xu et al., 2015. The presence of cytochrome c in the cytosol binds Apaf-1 and caspase 9 to form a complex called “apoptosome” (Kroemer et al., 2007; Hassan et al., 2014).

Second Mitochondrial Activator of Caspases/Direct IAP-Binding Protein with Low pI (SMAC/DIABLO) and Ht-A2/Omi then play roles of impeding the apoptotic inhibitory effect of Inhibitor of Apoptosis (IAP) paving way for apoptosis (Figure 1). All these factors are as well controlled and regulated by a class of proteins which belongs to BCL-2 family. These proteins can be categories into either pro-apoptotic protein (Bak, Bcl-10, Bax, Bad, Bid, Bim, Bik, Hrk) which is involved in the regulation of the cytochrome C release through the permeability of the mitochondrial membrane or the anti-apoptotic (Bcl-2, Bcl-x, Bcl-w, Bf-1, Bcl-XL, B-XS, Bcl-w, BAG) which prevent the release action of the cytochrome C, hence very crucial for the occurrence or the non-occurrence of apoptosis. In either case, there should be a balance to ensure normal growth and development of cells. Also, among the BCL-2 family are the Noxa and Puma that are very much involved in the P53 mediated apoptosis (Lomonosova and Chinnadurai, 2008; Elmore, 2007; Schuler and Green, 2001).

Member of the BCL-2 family possess a structural and functional sub groups with a domain known as BH. Members of the anti-apoptotic protein possesses four of the BH domain (BH1-4), the pro-apoptotic members have either three or one of the domains which is (BH1-3) or BH3 (Huang and Strasser, 2000; Elkholi et al., 2011; Kuwana et al., 2005; Certo et al., 2006). However recent studies have suggested the possession of the BH4 domain

by some pro-apoptotic members (Kvansakul et al., 2008). The pro-apoptotic proteins that are said to possess only BH3 domain are capable of binding the anti-apoptotic members to disrupt their inhibitory action and can be grouped into activators (Bid and Bim) for the activation of some effectors (Bax and Bak) and sensitizers /de-repressors (Bad, Bmf, Hrk, Noxa and Puma). Additionally, the (Bid and Bim) are capable of activating other pro-apoptotic protein (Bax and Bak) to further form oligomer that create pores in the mitochondrial membrane to allow permeability. This may be followed by the release of cytochrome C and SMAC to initiate the cell death process (Yu et al., 2015). However, the sensitizers /de-repressors are capable of binding to the BCL-2 anti-apoptotic members to disrupt their activities and also contain other section of members (Bad, Noxa and Bmf) that are notable for binding and inhibition of specific anti-apoptotic proteins (Inohara et al. 1997; Nakano and Vousden, 2001; Oda et al., 2000; Puthalakath et al., 2001; Yang et al., 1995). Studies have proven that puma, Noxa, Bim are capable of restoring the sensitivity of chronic lymphocytic leukemia (CLL) cells to chemotherapeutic agent and also their deletion amount to the increase in tumor burden with a decrease level of Puma, Noxa and Bim being found in humans with colon cancer, small cell lung carcinoma (SCLC) etc. (Gardner and Walter, 2011; Szegezdi et al., 2006; Schroder and Kaufman, 2005).

3.2. Intrinsic endoplasmic reticulum pathway

Endoplasmic reticulum are intracellular organelles involved in production, modification and ensuring of the folding and translocation of proteins. An impediment in the operation of the ER can result in an interruption in its ability to fold protein. A condition which result from stressed of ER, normally trigger the unfolding protein response (UPR) for the restoration of protein homeostasis. Failure of the UPR action normally result in the occurrence of apoptosis through either pathway, intrinsic or extrinsic pathway. However, the restoration action of the UPR are accomplished by three distinct signaling proteins inositol-requiring protein-1 (IRE1α), protein kinase RNA (IPK-R)-like ER kinase (PERK) and activating transcription factor 6 (ATF6) (Ma and Hendershot, 2004). Intriguingly, another protein known as the binding immunoglobin protein (BiP) serves to bind the ATF6 and PERK making them inactive. On the other hand, the IRE1α exist in it activated mode upon being bounded by an unfolded protein succeeding the accumulation of unfolded protein, BiP's unbounds the ATF6 and PERK to enable the restoration of protein folding. Additionally, the role of the ATF6, PERK and IRE1α are achieved through an induction of a signal transduction event which further causes an increased expressing of ER chaperons and the arrest of mRNA translation process, preventing further release of proteins and also further reverting the movement of unfolded protein in the cytosol for degradation. The ER chaperons is also known to prevent the actions of UPR after stress in the ER subsides (Ron and Hubbard, 2008; Lei and Davis, 2003; Deng et al. 2001). Activation of IRE1α initiate the activation of Apoptotic signaling -1(ASK1) which further activates c-Jun-N- terminal kinase (JNK) and P38 Mitogen Activated Protein Kinase(P38MAPK). Phosphorylation of Bim and Bcl-2 by JNK causes the activation of Bim and the inhibition of Bcl-2 to allow for the apoptotic process. Also, the phosphorylation of C/EBP homologous protein (CHOP) by P38MAPK, activates the CHOP to also enhance and inhibit the expression of Bim and Bcl-2 protein respectively (Puthalakath et al., 2007; Yamaguchi and Wang, 2004; Hollien et al., 2009; Han et al., 2009) (Figure 1). He et al.reports on the triggering effect of P38MAPK and CHOP by the infection with Japanese encephalitis virus and also cell death was discovered to be inhibited by the over-expression of Bcl-2. Similarly, a cytopathic strain of bovine diarrhea virus was capable of inducing apoptosis via CHOP and phosphorylation of PERK and elF2α (He, 2006). ATF6 is translocated upon varying activation from the ER into the Golgi body then finally into the nucleus where they are cleaved by serine protease site-1(S1P) and metalloprotease site-2 protease (S2P). In the nucleus, they bind with some element such as the ATF/CAMP response element and ER stress -response elements to trigger the activation of BiP, Grp94 and CHOP which further execute the action of inhibiting and enhancing of Bcl-2 and Bim respectively. Under stress conditions, the PERK brings about the phosphorylation of eukaryotic translation initiation factor (elf2α) and erythroid 2 P45-related factor 2 (NRF2). In effect, the elf2α allows the activation of activating transcription factor 4(ATF4) which can also be linked to CHOP, growth arrest and DNA damage-inducible (GADD34) and activating transcription factor 3(ATF3) to help in the stress arrest within the ER. Likewise, the activation of NRF2 helps in the induction of heme oxygenase 1 (H0-1) to prevent further oxidative stress (Belmont et al. 2012; Cullinan and Diehl, 2004).

3.3. Extrinsic pathway

Apoptosis is induced by this pathway through a mediated interaction of death ligands with death receptors. Accumulating evidence suggest that these death receptors are members of tumor necrosis factor (TNF) which includes the TNF, Fas ligands (Fas-l) and TNF -related apoptosis inducing ligand (TRAIL) (Goldar et al., 2015; Liu et al., 2017; Shlyakhtina et al., 2017). In the process, the death receptors (Fas receptor, TNF receptors) bind to death ligands (Fas ligands, TNF ligands) to allows the binding of the death domain / adapter Protein (Fas -associated Death Domain (FADD), TNF receptor associated death domain (TRDD)). The binding of the death domain /adapter protein to receptor ligands complex allows the binding of an initiator caspase 8 or 10 through its death effector domain (DED) to form an activated complex called Death inducing signaling complex (DISC). Furthermore, the binding and activation allow the caspase 8 to relay the death signal to an execution caspase to bring about apoptosis. The termination of this process is undertaken by another protein known as the Cellular FLICE Inhibitory Protein (c-FLIP) which is capable of binding to Death Domains/

Adaptor protein thus bringing the action of caspase to a halt (Figure 1). The c-FLIP can therefore be said to regulate the operation of the DISC (Wajant, 2002)

4. Signal alteration

As at 2108, there was an estimation of the rise to about 18.1 million in the global cancer burden and 9.6 million death. However, this sudden increase could be attributed to difficulty involve in diagnosing such conditions especially at the early stage where it could have been easy to be dealt with. An in-depth knowledge into the apoptotic signals will contribute to the means of drug and therapeutic developments to curtail these conditions (WHO, 2018).

4.1. BCL-2

B cell/lymphoma 2 **(**BCL-2) family of proteins are also known to be pivotal in terms of the regulation of apoptosis through activities of their pro-apoptotic and anti-apoptotic proteins by operating independently or dependently, at a point between internal death signals and the cells surface ensuring a balance. BCl-2 was first identified from the B-cell of human lymphomas, this is where it derived it name BCL-2 (b-cell lymphoma2). They are divided into the proapoptotic and anti-apoptotic group base on their function and homology domain. These members possess structural and functional sub groups with a domain known as BH. Members of the Anti-apoptotic protein possesses four of the BH domain (BH1-4), the pro-apoptotic has either three or one of the domains which is (BH1-3) or BH3. However recent studies suggest they been identified to possess the BH4 domain.

Upon the receival of a death signal, these proteins that are localized to the outer membrane change conformation .The pro-apoptotic members become involved in the process to accomplished the cell death as the anti-apoptotic becomes neutralized on the other hand, failure of the pro-apoptotic process, can results in tumorigenesis of a cell and the hindering of the formation of mitochondrial apoptosis induced channel (MAC) thus inhibiting the release of cytochrome C from the mitochondrial membrane . A number of studies have suggested that, the down regulation of Bad has an increasing effect on the tumor size and loss of Bad expression facilitated small cell lung carcinoma (SCLC)

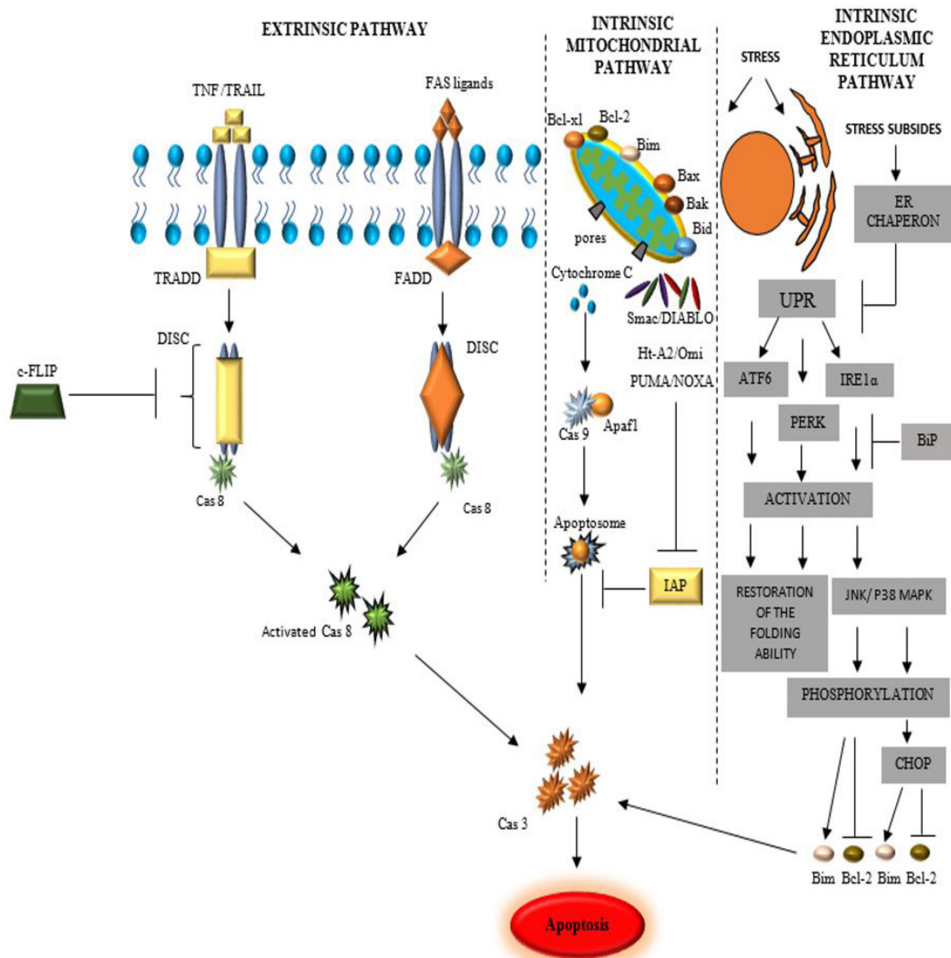

Figure 1. The various pathways of Apoptosis

growth. Also, curcumin was discovered to downregulate Bcl-2 protein in chronic myeloid leukemia cells. Thus, a balance will ensure the normal growth and development of cells (Zhang et al., 2018; Kale et al., 2018).

4.2. P53

P53 is a tumor suppressor protein existing as a tetramer with protein size of about 53 kDa thus the name p53. They are involved in cell cycle regulation and development, induction of members of the BCL-2, involved in the mitochondrial outer-membrane permeabilization (MOMP), the mediating of oxidative stress and endoplasmic stress, receptor signaling pathways etc. (Bai and Zhu, 2006). However, conditions such chemicals, viruses' example Human papillomavirus (HPV) infection can render the p53 inactive or reduce it activity thus reducing the intensity of suppression of proliferation.

Upon its activation they either enable the cell cycle arrest in the G-phase or an irreparable DNA damage causes them to trigger the activation of some apoptotic inducing proteins such as (Bak, Bcl-10, Bax, Bad, Bid, Bim, Bik, Hrk) and at the same time inhibit anti-apoptotic proteins(Bcl-x, Bcl-w, Bf-1, Bcl-XL, B-XS, Bcl-w, BAG). Additionally, the inability for this process to occur allows tumorigenesis, recent studies have proven that flavonoids were able to induce apoptosis in breast cancer cell through the downregulation of some downstream molecules of p53 (Bcl-2 and Bcl-xl) .Also the alteration of p53 or loss in it activity can downregulate Bax /Noxa /Puma paving way for the upregulation of Bcl-2 thus preventing the MAC formation and also Meng et al.suggest that, the negative regulation of p53 by mouse double minute 2 homolog MMD2 is capable of impeding it action. (Kang and Rosenwaks, 2018; Liu et al., 2015; Meng et al., 2015).

4.3. IAP

IAP is an eight-member family of caspase inhibitors possessing a novel domain of about 70- 80 amino acids known as the baculoviral IAP repeat (BIR). They include the X-linked inhibitors of apoptosis Protein (XIAP), IAP-like protein 2 (ILP-2), BIR Containing Ubiquitin-Conjugating Enzyme (BRUCE)/(Apollon), livin, Survivin, Cellular IAP1(cIAP1), Cellular IAP 2(cIAP2) and Neuronal Apoptosis Inhibitory Protein (NAIP) (Vucic and Fairbrother, 2007; Silke and Meier, 2013). IAP's are normally involved in the direct inhibition of some caspase like caspase 3, and are able to provide protection from Fas/caspase 8 inducing apoptosis through the arrest of the proteolysis cascade, they do so by binding their conserved domain (BIR) to the targeted caspase. XIAP, c-IAP1 and c-IAP2 have been found in the direct inhibition of caspase -3,7 and -9. XIAP for example is involved in the protection of cells from self-destruction and its overexpression was noticed in the developing stage of prostate cancer. CIAP is also found to mediate ubiquitination and degradation of caspase. (Dohi et al., 2004; Werner et al., 2018; Worthey et al., 2011).

Survivin has also been found to interact with XIAP for the inhibition of caspase 9 further preventing the recruitment of Apaf1. They are also regarded among the top 5 cancer related genes and have been found to be efficient in the proliferation of cancer cells through the upregulation of human telomerase reverse transcriptase. Livin is also known to possess a single BIR for the inhibition of apoptosis and has been found to be heavily expressed in melanoma. However, all the IAP's are known to be upregulated in cancer cells and therefore a number of studies have also put it up, affirming the strong relation between IAPS and cancer (Furuya et al., 2009)

5. Methods of Detection of Apoptosis

Apoptosis is characterized by a number of morphological changes which include shrinkage, blebbing etc. Necrosis is also marked by morphological changes, but both are being triggered by different conditions and processes, (Zhivotovsky et al., 1996). Focusing on apoptosis, their mode of detection has developed over time due to how the whole knowledge of apoptosis have also developed with time. Earlier research began with the morphological studies followed by the DNA degradation process, detection of some biochemical changes, flow cytometric and nuclease assays for structural and functional changes (Muppidi et al., 2004).

5.1. Morphological Analysis

Conditions such as shrinkages, blebbing etc. at the initial stages of apoptosis can somewhat be evident, these changes can be monitored with varying forms of microscopy which is directly dependent on the nature of cells or tissues. Among these, are the Light Microscopy, Fluorescence Microscopy, Electron Microscopy, Phase Contrast Microscopy etc. (Bánfalvi, 2009). Light Microscopy requires staining with hematoxylin and Geismar for the determination of both cellular and nuclear morphological changes. In some cases, Hematoxylin and Eosin can both be used to examine the cytonuclear morphological changes. Although this method is quite fast and cheap, it is known to possess some short comings on its quantitative measurement objective (Johnson et al., 2000). Fluorescence Microscopy is used to ascertain condense nuclear DNA morphologically related changes for both live and dead cells, small dyes such as DAPI, Hoechst 33342 or 33258 and Calcein-M are used for the internal DNA labelling and larger dyes such Propidium iodide (PI), used for lysed or death cells. This approach is costly compared to light microscopy. (Cursio et al., 2008).

Electron Microscopy, also allows a clear view of morphological changes of cellular and nuclear membrane in either their live or dead state, phagocytose apoptotic cells are much observed by using this approach (Asher et al., 1995). The Phase Contrast Microscopy is suitable for observing some apoptotic bodies and membrane blebbing's in apoptotic cells observed in cell culture flask or plates. Normally within flasks or plates, differentiation between cells undergoing mitosis, apoptosis or even necrosis becomes difficult, but by using this approach it allows the distinguishing between mitotic cells and apoptotic

cells. (Ulukaya et al., 2011). Other approaches used are the Confocal Laser Scanning Microscopy for observing macro molecular morphological changes and localization and the Video Time-lapse Microscopy which allows the continuous viewing of cells in their microscopic state for over a period of time providing varying information of different apoptotic events. (Smith et al., 1991).

5.2. Biochemical Analysis

This include DNA fragmentation, changes in some organelles, cell membrane and other conditions such as the activation of caspase. All these changes can be analyzed by using an immunohistochemistry, immunological or array-based approaches.

Analyzation of the DNA fragmentation: This phenomenon is mostly examined by Agarose gel electrophoresis. DNA fragment cleaved into an inter base pairs of about 180-200, are observed as ladder upon electrophoresis. Normally, high molecular weight allows easy detection, although some report advices the relative synchronous nature of apoptosis, since the exact state of DNA might not be known until analysis. However, it is a perfect approach, which help in distinguishing between apoptosis and necrosis, since results from necrosis proves to be a smear on the gel rather than a ladder (Rainaldi et al., 2008). This method can be divided into Conventional gel electrophoresis, Pulse field gel electrophoresis, Field inversion gel electrophoresis and Single cell gel electrophoresis (McNair et al., 1997).

Also, Terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) is another means of determining DNA fragments as an in-situ labelling of 3-OH ends and allows analysis of frozen and fixed sections. This approach involves the terminal labelling of DNA fragments with certain nucleotides such as Biotin or DIG and fluorescein. TUNEL is known to be a good method but yet, it can be time consuming. Other approaches require the labelling with *E-coli* polymerase. Live and necrotic cells could prove false positive under conditions such as contamination. Lastly in cell cultures, inter-nucleosomal DNA fragmentation can be analyzed by enzyme-linked immunosorbent assay (ELISA) (Hegyi et al., 1997; Kockx et al., 1998).

Changes in the Organelles and Cell Membrane: Changes such as alteration of membrane permeability in the mitochondria and externalization of phosphatidylserine serves as a hallmark of changes peculiar to apoptosis (Koopman et al., 1994). Annexin V. FITC assay allows the use of Annexin V accompanied by fluorescent labelling example FITC and a fluorescence microscopy detection or an augmentation with other method such as flow cytometry following the labelling process. Fluorescence microscopy can give false positives, it can therefore be replaced with the flow cytometry (Martin et al., 1995). This makes the flow cytometry a promising tool in the analysis of apoptosis, allowing the accessibility of results within a short time.

Western Blotting also ascertains the presence of apoptosis related proteins e.g. the Bcl-2 family protein with other apoptosis related proteins, the state and translocation of some molecules such as the cytochrome from the mitochondria to the cytoplasm. This process involves the analysis and identification of cytochrome in the cytoplasmic fraction instead of mitochondria using cytochrome antibody to depict the presence of apoptosis. (Li et al., 1997). Aside the Western blotting process the cytochrome C can also be detected by ELISA. Also changes in the mitochondria membrane which results in reduced oxidative and transmembrane potential may requires the use of different dyes such as 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbe nzimidazolcarbocyanine iodide (JC-1) rhodamine-123. (Cossarizza et al., 1993).

Caspase Activity: This can be analyzed using either an immunology or immunohistochemical method. Implementation of the western blotting approach here, may require the detection of the molecules they target examples Poly ADP-ribose polymerase (PARP) and also, tissues of epithelia origin which expresses cytokeratin 18 (M30 antigen) epitope break down as result of caspase activation (Ueno et al., 2005; Soni et al., 2011).Also, among these assays are the colometric detection base method which involves the use of chromophore p-nitroaniline labelled substrate with the spectrophotometric measurement of the absorbance at 405nm. Additionally, enzyme activation assay is another assay which is solely base on the buffer or salt composition, optimal pH, ionic strength for effective results. Luminometric caspase activity assay also employ a number of substrates with luciferase enzymes upon cleavage, caspase activity is determined as light emission. Other method in the detection process is DNA micro-array method which involves gene expression (mRNA) but is somewhat considered to be cost effective (Green, 2000).

6. Conclusion

Apoptosis being a programmed cell death will have to do with the approaches on how cell get proliferated and with a detailed study of the elements and signals involved will help to provide an in-depth knowledge for further development of cancer related drugs. Apoptosis can therefore be said to be a significant condition which help in the normal development and growth of cells. This review seeks to enlighten us on the recent conditions occurring in the processes of apoptosis together with its signals, triggering, inhibition elements and methods of detection. Further studies will seek to help in the curtailing of some conditions that results in abnormal proliferation of cell in cancer related cases.

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Abbreviations: small cell lung carcinoma SCLC, mitochondrial apoptosis induced channel (MAC), Human papillomavirus (HPV), Mitochondrial Outer-membrane Permeabilization (MOMP), BCL-2 (B-cell lymphoma 2), Death Effector Domain (DED), Death Inducing Signaling Complex (DISC), Fas -Associated Death Domain (FADD), TNF Receptor Associated death domain (TRDD), Fas Ligands (Fas-l), TNF -Related Apoptosis Inducing Ligand (TRAIL), Tumor Necrosis Factor (TNF), C/EBP Homologous protein (CHOP), Growth Arrest and DNA Damage-inducible (GADD34) Heme Oxygenase 1 (HO-1), Activating Transcription Factor 4 (ATF4), Activating Transcription Factor 3 (ATF3), erythroid 2 P45-related factor 2 (NRF2), Eukaryotic Translation Initiation Factor (elf2α), c-Jun N-terminal kinase (JNK), Inositol-Requiring protein-1 (IRE1α), Unfolding Protein Response (UPR), Endoplasmic Reticulum (ER), Protein kinase RNA (IPK-R)-like ER kinase (PERK), Apoptotic Signaling -1 (ASK1), Chronic Lymphocytic Leukemia (CLL),: Cellular FLICE Inhibitory Protein (c-FLIP), P38 Mitogen Activated Protein Kinase (p38 MAPK), Neuronal Apoptosis Inhibitory Protein (NAIP), BIR Containing Ubiquitin-Conjugating Enzyme (BRUCE), Second Mitochondrial Activator of Caspases/Direct IAP-Binding Protein With Low pI (SMAC/DIABLO), Cellular IAP1 (cIAP1), Cellular IAP 2 (cIAP2), Binding immunoglobin protein (BiP).Terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL), Enzyme-linked immunosorbent assay (ELISA), Poly ADP-ribose polymerase (PARP).