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Role of Bcl-2 in Apoptosis and Mitochondrial Permeability

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ABSTRACT*: Apoptosis is of great importance in various biological systems for functioning of physiological processes in multicellular organisms. It is also required for normal functioning of the body, embryonic development , tissue homeostasis , functioning of immune system , removal of defective cells. Various factors take part in the process of apoptosis , the critical control point being regulated by the Bcl2 family of proteins , which also plays a crucial role in regulation of mitochondrial permeability, calcium homeostasis and endoplasmic reticulum calcium homeostasis. Dysregulation of apoptosis leads to cancer ,autoimmune diseases, viral infections and neurodegenerative disorders , AIDS , ischaemic diseases are enhanced due to excessive apoptosis. In this review we can understand about the anti-apoptotic and non-apoptotic roles of bcl2, inhibitors of bcl2 , malfunctioning of apoptosis more clearly.*

KEYWORDS*: Apoptosis, Bcl-2 proteins , mitochondria, cell death, DNA damage , cancer, auto immune diseases*

I. INTRODUCTION

During embryonic development, metamorphosis and in the functioning of physiological processes of multicellular organisms cell death is very essential (1,2). In the year 1964 the term programmed cell death was framed stating that this process has a definite sequential pattern resulting in temporary localised self-destruction (3). The term 'apoptosis ' was coined in the year 1964 by Kerr, Wyllie and Currie(4) ,which is of greek origin meaning "falling off or dropping off".

Cells which are damaged and the damage is beyond repair apoptosis is seen which is highly cell specific (5). Apoptosis play an essential role in survival of the organisms and is considered to be an imperative component of various processes including normal cell turnover, proper development and functioning of the immune system, multiplication of mutated chromosomes, hormone-dependent atrophy, normal embryonic development, elimination of indisposed cells and maintenance of cell homeostasis (6)(7). A wide variety of pathological cellular insults as well as internal or external physiological stimuli trigger apoptosis, a tightly regulated process of cell death,dependent on the expression of cell-intrinsic suicide mechanism(8). It helps in the removal of cells being generated as excess, that have completed their particular functions, or are toxic to the organism ,tissue renewal, hormone-induced tissue atrophy, and other pathological conditions. BCL-2,the cardinal was initially found as the defining oncogene in follicular lymphomas present at the reciprocal breakpoint of t(14;18)(q32;q21)chromosomal translocation

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Enormous efforts have been pooled in by various scientists and investigators throughout the world after this discovery and has improved our knowledge in basic biology , molecular mechanisms and therapeutic targets in the apoptotic pathway(9). One of the most important reasons which initiate apoptosis are DNA damage and production of the predominant lesions such as cyclobutane pyrimidine dimers (CPDs), 6–4 photoproducts (6–4PPs) and other lesions (10)(11) due to UV radiation (UVR), ionizing radiation (IR), oxidative stress replication or recombination errors from environmental and therapeutic genotoxins.(12)(13)

II. APOPTOSIS- PROGRAMMED CELL DEATH :

Apoptosis which typically means“programmed” cell death is genetically determined elimination of cells (14). Many other types of PCD have been demonstrated and a few other forms is yet to be discovered (15)(16). Intrinsic and extrinsic apoptotic pathways are initiated by the stimuli DNA damage which activates and stabilizes p53 in nucleus and stimulates other proteins (17). Every second in our body 100,000 cells are produced by mitosis and same number of cells die by apoptosis(8)!!!!!!!

Development And Morphogenesis :

A small number of somatic cells die during C.elegans development . In the development of limb separate digits are formed by death of interdigital mesenchymal tissue.

Homeostasis:

A typical example for the involvement of apoptosis is the immune system: millions of B and T Cells are formed and secreted everyday and majority of cells die during maturation(neglect, negative selection or by AICD of peripheral immune cells)

Elimination Of Damaged And Harmful Cells:

Cells with DNA damaged beyond repair are discarded by this process. Auto reactive cells of the immune system and infected cells are eliminated by apoptosis (18)(19).

Morphological Changes:

Cell shrinks, deforms and looses contact to neighbouring cells. Its chromatin condenses and marginates at nuclear membrane, the plasma membrane is blebbing or budding, and in the end cell is fragmented into compact membrane-enclosed structures, called 'apoptotic bodies' that contains cytosol, the condensed chromatin and organelles . Macrophages engulf the apoptotic bodies and they do not cause an inflammatory response while removing from the tissue. These morphological changes result as a consequence of distinguishing molecular and biochemical changes occurring in an apoptotic cell. One of the most significant events to be noted is the activation of proteolytic enzymes which leads to the cleavage of DNA into oligonucleosomal fragments as well as the cleavage of a multitude of specific protein substrates that determines the integrity and shape of the cytoplasm or organelles (20).

III. APOPTOSIS AND NECROSIS:

Morphologically apoptosis is different from that of necrosis and these two processes depend on the types, developmental stages, physiological environment of tissues and the nature of death signal(17). Apoptosis is a synchronised , sequential and energy-dependent mechanism which involves the stimulation of a group of cysteine proteases (caspases) and a complex cascade of events, where as necrosis does not include gene expression and is a passive externally driven mechanism which occurs after cell death in the absence of metabolic self-involvement (22). Apoptosis is morphologically distinct from necrosis in its characteristics features such as cell shrinkage, cytoplasmic condensation, DNA fragmentation, chromatin condensation, nuclear fragmentation, cytoplasmic membrane blebbing and formation of apoptotic bodies where in necrosis involves loss of membrane integrity, cell swelling, formation of cytoplasmic vacuoles, swollen endoplasmic reticulum, distended or ruptured mitochondria, lysosomes, lysis and release of the cytoplasmic contents into the surrounding tissue (23)(24) resulting in an inflammatory reaction which is lacking in apoptotic cells (25) Various analysis and studies of necrotic pathway has paved way for the development of novel notions to destroy apoptosis-resistant tumor cells. In the case of apoptotic cells, loss of membrane integrity, swelling and dis rupture is observed as they suffer a major insult. Where as in the case of necrotic form of cell death, cellular contents are released uncontrolled into the cell's environment damaging surrounding cells and a strong inflammatory response in the corresponding tissue is elicited (21).

Bcl-2 Family Of Proteins:

More than 20 years ago , Bcl-2(B-cell lymphoma-2)was identified due to the up regulation in follicular B-cell lymphoma and (36)that this proto-oncogene inhibits apoptosis instead of promoting proliferation,(37) created a thunder of interest in the role of this protein, and its yet to be discovered related proteins ,involved in cell survival. Nearly 17 human Bcl-2 proteins have been discovered each characterized by up to four regions of sequence homology, ie, the Bcl-2 homology (BH) domains. It's members are categorised into three functional groups (38).The first group includes the prosurvival proteins, Bcl-2, Bcl-xL, Mcl-1, Bcl-w, A1/Bfl-1, and Bcl-B/Bcl-2 L10, containing all four BH domains and responsible for protecting cells from apoptotic stimuli.

The second group contains the BH3-only proteins, Bid, Bim, Puma, Noxa, Bad, Bmf, Hrk, and Bik, whose sequence homology with the other members is restricted to the BH3 domain .The BH3-only proteins are stimulated in response to various cellular stresses like DNA damage, growth factor deprivation, and endoplasmic reticulum stress, initiating apoptosis. Now, the third group, Bax, Bak, and Bok/Mtd, are multidomain proteins, which contains all four BH domains similar to the prosurvival proteins, but these are proapoptotic, with their stimulation downstream of BH3-only proteins , resulting in cell death. Apoptosis manifests the execution of the death program by two major pathways :the caspases pathway and organelle dysfunction where mitochondrial dysfunction is the best understood.(26)(27). Bcl-2 family of proteins play a herculent role in deciding if the cell would live or die ,as they reside upstream of irreversible cellular damage and act at the level of mitochondria. These proteins are broadly classified into anti- and pro- apoptotic

based on their characteristic function and the ratio between theses two decides the susceptibility of cells to a death stimuli (28). These proteins contain 4 homologous BH domain designated as BH1,BH2, BH3,andBH4 corresponding to alpha helical segment (29)(30)(31). The anti-apoptotic members shows sequence conservation in all four domains where as pro apoptotic proteins display less sequence conservation of the first helical segment. Activation of p53 by Death stimulus DNA damage results in the activation of p53 leading to apoptosis by transcriptional activation of pro-apoptotic members of Bcl-2 family of proteins , such as Puma, Noxa, Bim, Bid, Bik, Bak, Bax, Apaf-1, Bmf, Hrk, Pag608, Drs, Fas and Gadd45 (32)(33)(34). This family of proteins contains multi domain pro-apoptotic (e. g. Bax, Bak), anti-apoptotic programmed cell death (PCD apoptotic (e. g. Bcl-2, Bcl-XL, Mcl-1) and a small number of BH3 only (e. g. Puma, Noxa, Bid, etc.) proteins which is responsible for mitochondrial permeability (35).

Bcl-2 In Mitochondrial Permeability :

Mitochondria is the powerhouse of the cell as it is the major site for electron transport, production of ATP and also cells survival regulation.during PCD, mitochondria releases apoptotic mediators ,by the rupture of outer mitochondrial membrane ,which is involved in the extrinsic pathway of apoptosis i.e in the activation of caspases and DNA degradation(48). This makes the regulation of outer mitochondrial membrane integrity an essential critical point in the apoptotic form of cell death. The characteristic features of the Bcl-2 family of proteins is clear but the mechanism by which they regulate cell survival is yet to be clearly understood(46).Many metabolites are permeable through the outer mitochondrial membrane which is been mediated by the Bcl-2 family of proteins through the voltages dependent anion channel (VDAC)(49). Moreover a few pro-apoptotic proteins form multi metric channels for releasing apoptosis initiating proteins from inner mitochondrial space. Apart from this , theses proteins are responsible for the outer and inner mitochondrial permeability through permeability transition pore(PT)(47). Various studies have showed that alterations in cellular metabolism results in pro-apoptotic changes like changes in intracellular pH, redox potential and ion transport. Bcl-2 proteins are also involved in mitochondrial energy generation and regulate cellular bio energetics(39). Some anti-apoptotic Bcl-2 proteins prevent matrix swelling, ROS damage, release of cytochrome c and results in loss of membrane potential associated with apoptosis.whereas apoptotic family of proteins cause mitochondrial transport problems leading to matrix swelling (40)(41)(42)(43).These proteins respond to an apoptotic stimulus by showing conformational changes and trans locate to the mitochondrial membrane (44). One of the diverse effects of Bcl-2 group of proteins involves it's influence on mitochondrial fission and fusion, but it's exact mechanism is still unclear. But it is also believed that it is mediated by direct interactions between members of Bcl-2 family of proteins and the components of fusion/fission machinery such as Drp 1 and Mfn2. Hence it is important to note that Bcl-2 proteins act as regulatory elements of this mechanism similar to the pattern in which anti- apoptotic Bcl-2 proteins interact with Beclin-1 to regulate autophagy mechanism (45).

IV. NON-APOPTOTIC ROLES OF BCL-2:

Role In Mitochondrial Calcium Homeostasis:

Bcl-2 family of proteins regulates calcium homeostasis directly at the mitochondria which can be understood based on the fact that these proteins control apoptosis to a large extent (50). Initially the relationship between Bcl-2 proteins and mitochondrial calcium homeostasis was based on the study on neural cells. This study proved that expression of Bcl-2 enables mitochondria to uptake more calcium without causing any mitochondrial respiratory impairment conclUding that Bcl-2 can protect mitochondria from calcium overload (51). Similar conclusions were made confirming that Bcl-2 improves capacity of mitochondria to store calcium by two groups (52,53,54). These studies further expressed that Bcl-2 induces reduction of mitochondrial Na+/Ca2+ exchange activity causing decreased Ca2+ efflux from the mitochondria(55). But some other analysis have proved that Bcl-2 had no effect on calcium uptake by the mitochondria(58) though excessive level of these proteins can be detrimental for mitochondria(59).

Cell cycle:

The expression of Bcl-2 in cell cycle was emphasised after it's discovery as in the absence of IL-3. Bcl-2 over expressing cells are arrested in G1 phase itself(57). Diverse studies have specified the importance of Bcl-2 in cell cycle control and Bcl-2 deficient cells accelerate cell cycle and it's over expression retards G0/S transition (58).

Neural Plasticity:

Neural plasticity,also called as neuro plasticity, is the changes in structure , function and organization of neuronal network(60). Bcl-2 and Bcl-xL are expressed in the central nervous system ,during early embryonic development, when axons elongate and it's expression gradually declines as neurons will lose their ability to

produce new axons(62). But with ageing Bcl-2 expression is reduced in CNS , sometimes even restricted to only peripheral nervous system whereas Bcl-xL expression is increased (61).

Also, Bcl-2 enhances neurite growth and regeneration making it a critical control component in neuro plasticity. Mitochondria and ER alter their intracellular calcium which results in the inhibition of neurite growth which is mediated by Bcl-2 proteins in spiral ganglion neuron (63).

V. BCL-2 IN APOPTOTIC PATHWAY:

There has been quite some debate about how the Bcl-2 family controls apoptosis: one model proposes that Bcl-2 members might directly control caspase activation (64),whereas another model claims that they mainly act by guarding mitochondrial integrity (65). In support of the first model, the worm Bcl-2 orthologue ced-9 binds to the Apaf-1-like adaptor protein ced-4 and prevents it from activating the caspase ced-3 unless the BH3-only protein Egl-1 displaces ced-4 as shown in (66). In contrast, the mammalian ced-4 homologue Apaf-1 obviously does not interact with Bcl-2-like proteins (67) but is activated by cytosolic cytochrome c and it is the release of cytochrome c from the mitochondria that can be controlled by Bcl-2 (68)(69). Therefore it appears likely, that the central function of mammalian Bcl-2 family members is to guard mitochondrial integrity and to control the release of mitochondrial proteins into the cytoplasm (35) .How then is mitochondrial integrity affected by proapoptotic Bcl-2 family members? Central to this question are Bax and Bak, even though inactivation of the Bax gene alone affected apoptosis only slightly and disruption of Bak alone did not show any effect. However, the double knockout of Bax and Bak resulted in dramatic impairment of apoptosis during development in many tissues with superfluous cells accumulating in the hematopoietic system and in the brain. Additionally, cells derived from those Bax -/- Bak -/- mice are insensitive to treatment with e.g. etoposide or irradiation (70)(71). Bax is a cytosolic monomer in viable cells but during apoptosis changes its conformation, integrates into the outer mitochondrial membrane and oligomerizes (72).

Although the mechanism is controversial, Bax and Bak oligomers are believed to provoke or contribute to the permeabilization of the outer mitochondrial membrane (PT), either by forming channels by themselves (73) or by interacting with components of the PT pore such as VDAC (74). In contrast, antiapoptotic Bcl-2 members sequester proapoptotic Bcl-2 members by bindig to their BH3 domains and thereby ultimately prevent Bax or Bak activation/ oligomerization and consequently inhibit mitochondrial proapoptotic events: overexpression of Bcl-2 or Bcl-XL potently inhibits apoptosis in response to many cytotoxic insults, among others by suppressing the generation of ROS, stabilizing $\Delta\psi$, preventing PT and consequently blocking the release of e.g. cytochrome c (75).

Besides eliciting its antiapoptotic effects on the mitochondrial level by indirectly controlling the activation of the apoptosome, Bcl-2 also appears to inhibit apoptotic pathways that are independent of Apaf-1/caspase-9 and which might depend on caspase-7 as a central effector (76) .In this context one might even expect the existence of another but up to now unidentified Apaf-1 homologue that can be directly controlled by Bcl-2/Bcl-XL (77) . Regulation of apoptosis by the Bcl-2 family. In a viable cell, the proapoptotic Bcl-2 famil members Bax, Bak, and BH3-only proteins are antagonized by antiapoptotic members such a Bcl-2. In response to an apoptotic stimulus, BH3-only members are activated by transcriptional upregulation (Bax, Noxa, Puma), subcellular relocalization (Bim, Bmf), dephosphorylation (Bad), or proteolysis (Bid). Activated BH3-only proteins prevent antiapoptotic Bcl-2 members from inhibiting proapoptotic members.

In addition, they might directly induce a conformational change o f Bax and Bak which subsequently oligomerize and insert into the mitochondrial membrane where they form pores either by themselves or by associating with the permeability transition pore complex. In consequence, proapoptotic factors are released from the inner mitochondria l membrane into the cytosol, such as cytochrome c which contributes to the formation of the apoptosome and the subsequent activation of the caspase cascade Whereas Bax and Bak represent the central core of a proapoptotic Bcl-2 death machinery that is held in check by the pro-survival members Bcl-2 and Bcl-XL, members of the BH3-only subfamily are required for the activation of proapoptotic Bax/Bak function (78). On the other hand, the killing effect of BH3-only members depends on Bax/Bak, since cells double-deficient for Bax and Bak do not die upon overexpression of BH3-only proteins as it would be the case in wildtype cells, indicating that BH3-only members function upstream of Bax and Bak (70,71). Importantly, just as the proapoptotic activity of multidomain proteins Bax and Bak is controlled by their interaction with the antiapoptotic guardians Bcl-2/Bcl-XL, also most BH3-only members display a strong binding preference to antiapoptotic Bcl-2/Bcl-XL and in this way are kept under control (79). In general, BH3-only proteins are thought to interfere with the fine-tuned balance of homo- or heterooligomerization between proapoptotic multidomain members Bax/Bak and antiapoptotic members Bcl-

2/Bcl-XL . It has been proposed that Bid and Bim possess BH3 domains (Bidlike BH3 domain) which can directly mediate Bax/Bak oligomerization, whereas Bad and Bik possess Bad-like BH3 domains which do not directly act on Bax/Bak but preferentially interact with antiapoptotic Bcl-2/Bcl-XL. As a consequence, activated Bad/Bik might be able to displace Bid/Bim from the binding pocket of antiapoptotic Bcl-2/Bcl-XL, and - in this way released - Bid/Bim might provoke Bax/Bak oligomerization and cytochrome c release even at subliminal levels (80). In summary, a current model of how Bcl-2 family members regulate apoptosis can be descibed as follows :specific apoptotic stress signals trigger the activation of particular BH3-only proteins which then interact with antiapoptotic members on the outer mitochondrial (but also nuclear/ER) membrane, resulting in the release of Bax-like proapoptotic factors. Bax-like factors undergo a conformational change (possibly assisted by some BH3-only proteins), insert into the outer mitochondrial membrane where they provoke PT and the release of apoptogenic factors (81).

VI. DYSREGULATION OF APOPTOSIS:

Malfunctioning of individual apoptotic machinery may cause several human diseases like cancer, neurodegenerative as well as several types of autoimmune disorder (82,83,84) .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 (85) and in brain tissues of Alzheimer's as well as Parkinson's disease patients (86,87,88). 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 (89). 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) (90)

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Three autosomal dominant diseases such as Muckle Wells syndrome, familial cold auto-inflammatory syndrome and chronic infantile neurological cutaneous and articular (CINCA) syndrome caused by missense mutations in the NACHT domain of NALP3 protein are closely related to autoinflammatory syndromes distinguished by periodic fever, skin rashes, amyloidosis and development of neurological complications (91,92,93). It has been suggested that loss of caspase-14 expression is associated with progression of ovarian cancer (94) and the mutation in p53 gene may cause neoplastic diseases (95). Thus it seems that apoptotic pathway is associated with several biological processes and plays a vital role in regulating various diseases. Recently, potent apoptosis-inducing compounds associated with human health have been recorded that prevent tumor promotion, progression, and the occurrence of cellular inflammatory responses. Certain photosensitizing drugs are being employed in photodynamic therapy to induce apoptosis for the treatment of cancer and non-cancerous cells. Several recent studies have revealed a number of natural as well as synthetic anticancer drugs that act through the induction of apoptosis to prevent tumor promotion, progression, and the occurrence of cellular inflammatory responses other than necrosis (96). Anticancer therapy may cause both apoptosis as well as autophagic cell death, however, there is little information about the connection between autophagy and apoptosis. Presently, large numbers of synthetic as well as natural compounds have been found that are pharmacologically highly effective against certain diseases through inducing the apoptosis of target cells such as cancerous cells. These compounds may promote the development of novel remedy based on the inflection of apoptosis.

VII. CONCLUSION

Bcl-2 family members are critical determinants of cell survival in essentially all cells, both during development and in mature differentiated cells. They are the major regulators of stress-induced apoptosis, and in certain cases also regulate the apoptosis induced by death receptors. The current understanding of each of the Bcl-2 family members, their interactions, and final activation of Bak and Bax to permeabilize mitochondria, is now allowing the development of agents that target different steps in the pathway. Success in this strategy promises the ability to treat a range of pathologies, including the removal of harmful cells such as autoreactive lymphocytes or those with damaged DNA. A prominent example is the successful preclinical and Phase I clinical trials of small-molecule BH3-mimetics in certain cancers. Our ongoing challenges include obtaining a better understanding of the molecular control of apoptosis and so improve the specificity and efficacy of agents that target Bcl-2 proteins (97). It also seems feasible to develop small molecules that specifically block apoptosis. Clearly, it is crucial to develop means of targeting specific cells, be they transformed lymphocytes in a lymphoma, persistent granulocytes in an asthmatic airway, or β-cells in a glucose-bathed pancreas. In some cases, acute administration may effectively initiate or block apoptosis in the target cell with limited effect on

other cells. Targeting would also be improved by the development of a simple method of profiling the Bcl-2 proteins in each cell type, including in each cancer.

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