BCL-2 FAMILY OVEREXPRESSION AND CHEMORESISTANCE IN ACUTE MYELOID LEUKEMIA

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PREKOMERNA EKSPRESIJA I HEMOREZISTENTNOST BCL-2 FAMILIJE U AKUTNOJ MIJELOIDNOJ LEUKEMIJI

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ABSTRACT

The family of Bcl-2 proteins is one of the most responsible for apoptosis pathway, that is a critical process to the maintenance of tissue homeostasis. Bcl-2 is an essential apoptotic regulator belonging to a family of functionally and structurally related proteins known as the Bcl-2 family. Some members of this family act as anti-apoptotic regulators, whereas others act in pro-apoptotic function. The relationship between the pro and anti-apoptotic proteins can regulate whether cells begin the apoptosis or remain its life cycle. Increasing of Bcl-2 expression has been found in some hematologic diseases, such as Acute Myeloid Leukemia (AML) and their effects on responsiveness to anticancer therapy have been recently described. Thus, this review aims to discuss apoptosis and the role of the Bcl-2 family of proteins in chemoresistance when overexpressed in patients committed with Acute Myeloid Leukemia submitted to chemotherapy treatment.

Keywords: Acute Myeloid Leukemia, Apoptosis, Bcl-2 family, Chemotherapy resistance

SAŽETAK

Familija Bcl-2 proteina je jedna najznačajnijih za odvijanje apoptoze, procesa od ključnog značaja za održavanje tkivne homeostaze. Bcl-2 je osnovni regulator apoptoze koji spada u porodicu funkcionalno i strukturno povezanih proteina poznatih kao Bcl-2 familija. Neki članovi

ove familije deluju kao anti-apoptotički regulatori, dok ostali pospešuju apoptozu. Odnos između

pro i anti-apoptotskih proteina određuje da li ćelije započinju apoptozu ili nastavljaju životni ciklus. Povećanje ekspresije Bcl-2 postoji u nekim hematološkim bolestima, kao što je akutna mieloidna leukemija (AML) i nedavno su opisani efekti povećanja ekpresije Bcl-2 na antitumorsku terapiju. Usled toga, ovaj revijski rad ima za cilj da razmotri apoptozu i ulogu hemorezistencije Bcl-2 familije proteina kod pacijenata sa akutnom mijeloidnom leukemijom koji se leče hemoterapijom i kod kojih postoji preterana ekspresija Bcl-2.

Ključne reči: Akutna mieloidna leukemija, apoptoza, Bcl-2 porodica, otpornost na hemoterapiju

INTRODUCTION

The mechanism of cell death is a decisive process to the maintenance of tissues homeostasis. Furthermore, its regulation leads to cell death through intracellular mechanism control (1-3). There are some natural mechanisms of cell death, which are most common seen in the literature such as autophagy, apoptosis, necrosis, and the last one added to this list was the necroptosis, which induce cells to die and disseminate especially carcinogen cells (2,4). These are mechanisms that occur inside the tissues in order to maintain the cell balance into the organisms (5). Apoptosis, was classified by Clarke in 1990 as the programmed cell death (PCD) type I, being an essential, critical and important process characterized by some alterations either biochemical or morphological to the cell structure leading to pack up the cell to be removed via phagocytosis and is triggered to remove diseased, damaged or aged cells (6-10). Apoptosis either can be activated by many oncogenes and the bcl-2 is one of them that play an essential function to support the cell lifespan. In fact, bcl-2 was identified as an important oncogene that drives some ability to activate and execute the apoptosis pathway (11). Moreover, it has been demonstrated that high levels of the Bcl-2 proteins especially the anti-apoptotic members leads to inhibition of apoptosis. The capacity to impairing the cell death confer to these proteins an interesting target for potential drugs used to treat cancers. However, the Bcl-2 family also

have been associated to chemoresistance of many anticancer drugs what put it line highlighted for significance related to prognostic and treatment of many cancer such as Leukemia (12) especially Acute Myeloid Leukemia (AML). Bcl-2 has been frequently associated to reduction of the susceptibility of the current chemotherapies especially due to its ability to impair the apoptosis process (13,14evasion of programmed cell death (apoptosisevasion of programmed cell death (apoptosis). Here we suggest revising the role of bcl-2 family and its influence to trigger the chemoresistance of many current drugs used to treat patients committed with AML.

APOPTOSIS

Kerr and colleagues in 1972 suggested apoptosis as a definition for some pattern related to the morphology of the cell death during the embryonic phase after some observation of how cells were eliminated and also in adult healthy tissue turnover atrophy establishment after withdrawing some essential hormone (15). Apoptosis is a process that excludes undesirable cells with its progression morphological modifications can arise into the cell (16, 17). This process is complex and in human organisms have a close comparison with another organism known as *Caenorhabditis elegans* (18). Its regulation however, is performed by molecular components such as the B-cell lymphoma 2 family (Bcl-2 family) that has been in line highlighted due to their relevant role along of the cell death process [6]. Bcl-2 members are regulatory molecules for apoptosis process. Some components work to induce cells to die, acting as pro-apoptotic members such as Bad, Bak, Bax, Bid, Bim, Bmf, PUMA, and NOXA, whereas other act as anti-apoptotic components impairing the death process, which include the Bcl-2, Bcl-w, Bcl-XL, A1 and Mcl-1 (19).

The mechanisms of apoptosis are divided into two main pathways. The first is regulated by death receptor located on the cell surface known as extrinsic pathway, and the second has its focus on modification of the mitochondrial membrane permeability, known as intrinsic pathway (20). The death mechanisms for cancer cells, especially apoptosis and necrosis are not a simple issue, however (21-23).

Carcinogens cells have a high rate of proliferation, however these cells do not have their lives time longer than normal healthy cells; in fact, their lifespan is reduced (24). This was described on a study performed by Alex Carrel in 1925 who stated, "Malignant cells are sick cells which live shorter". Fisher later confirmed this results in 1937, showing that cells with malignant characteristics are sick and have a short lifetime, indeed (24). Fisher also demonstrated in his study that normal and carcinogens cells behave different when dead. Normal cells remains among the living cells in an inert state for long time, whereas cancer cells immediately are hydrolyzed and demise (25). Cancers are likely to develop in different organs such as into the bone marrow that is likely to arise some types known as leuke-

mia, and one of the most well characterized leukemia is the Acute Myeloid Leukemia (AML) that is a severe and common complication among the individuals (26). AML is the most common manifestation of leukemia, being a genetic disorder identified by alterations of precursor or hematopoietic stem cells, resulting in blockage of their differentiation, as a consequence accelerated proliferation premature myeloid cells into the bone marrow, which leads to infiltration to other organs (27-30). Regarding the different therapeutic target for AML, it has been identified that the Bcl-2 proteins are associated with resistance to chemotherapy and cell survive especially the anti-apoptotic members when overexpressed. Therefore, focus on anti-apoptotic proteins and blockage their function might be an efficient alternative to induce apoptosis and activating pro-apoptotic members of Bcl-2 proteins resulting in the elimination of carcinogens cells (24, 31, 32, 33).

THE BCL-2 FAMILY OF PROTEINS

The Bcl-2 proteins are large components of the BCL family, which have an important function modulating process involving cellular death, either by the physiological or pathological pathway. In addition, it can be controlled by the abundance of modification via posttranslational mechanisms (34,35). These proteins family was firstly identified as a translocation problem in the chromosome 18q region 21 (18q21) and in the heavy chain of the gene from immunoglobulin located at the region 14q32, which result in decontrol in transcription of Bcl-2 proteins, this is characterized as a translocation t(14;18), mainly in follicular B-cell lymphomas (36-38). In addition, Bcl-2 family has a crucial role to regulate the apoptosis process in the mitochondria, which regulates the mitochondrial outer membrane (MOM), where the Bcl-2 members are located in (39,40,41). Based on its homology, this family has different subtypes, which are divided in three according to their functions, structure and domains (40,42). The division includes the members that prevent apoptosis or the anti-apoptotic members, whereas the others components stimulate apoptosis, pro-apoptotic proteins, which includes the Bcl-2 only proteins or BH3 pro-apoptotic only proteins (Fig. 1) (43, 44).

BCL-2 FAMILY AND APOPTOSIS REGULATION

The apoptosis regulation via Bcl-2 has been debated for many author along the years, some of them stipulate that Bak and Bax are sequestered by pro-survival proteins, whereas other argued that the main role of these proteins was insulated the BH3 only proteins members (45-50). An experimental study provided that the apoptosis mechanism is inhibited by pro-survival proteins, hence BH3-only proteins are sequestered while Bak and Bax are activated (51).
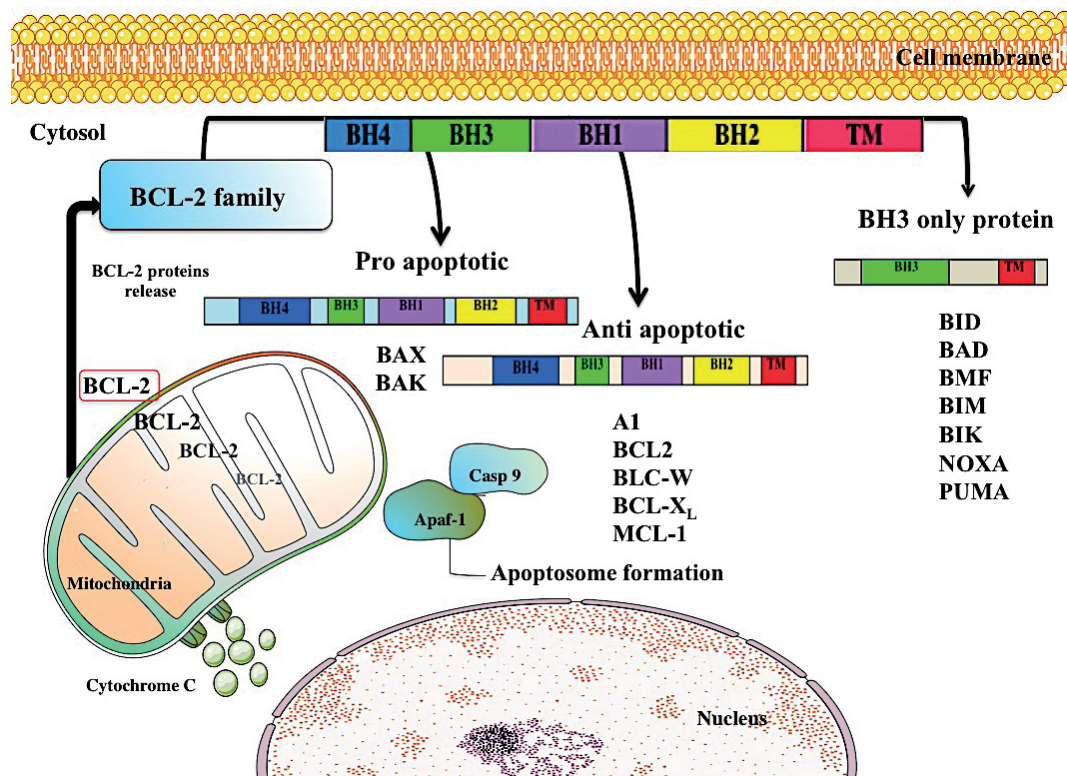
Figure 1. The Bcl-2 family members. The Bcl-2 family proteins are members that share domains and are divided into pro and anti-apoptotic proteins, their domains are known as Bcl-2 homology (BH domains). The figure shows the anti-apoptotic members, which have the function of neutralization, the death process into the cell, and includes A1, Bcl-2, Bcl-w, Bcl-xL and Mcl-1 molecules share BH homology domains from 1 to 4. On the other hand, the pro-apoptotic members are divided in two different groups known as effector and the BH3 only proteins. The members of the effector group are BAX and BAK and share homology domains from 1 to 4, however, the BH3-only proteins members have one BH domain and binding anti-apoptotic proteins including molecules such as BID, BAD, BMF, BIK, NOXA and PUMA.

Another important discovery is related to mitochondria scenery, which argues that the mitochondrial outer membrane (MOM) is extremely important to Bcl-2 family members interaction. In addition, these studies have suggested that such intercommunication among the members play an important role influencing the affinity among each member (47,48,50). Moreover, another character essential for apoptosis control is the tumor suppressor gene p53. It is crucial for apoptosis process due to its capacity to mediate the response to genotoxic stress (52). The apoptosis dependent of p53 activity was firstly observed in thymocytes of irradiated mouse according to Clarke and colleagues and Lowe and collaborators (53). The p53 is an extremely important tumour suppressor gene, mutation as well as inactivation on this gene is crucial for development and spreading of tumours. Indeed, most of the tumor types in humans are related to its mutation. However, when associated to haematological tumor types this arise in 11.1% only for notified cases. These data are according to the International Agency for Research on Cancer (IARC) version R15, that afford information and tools for analyses and studies of mutations related to many human cancers types giving support to investigate their impact in the clinical field (54,55). Furthermore, oncogenes for example adenovirus E1A combined with irradiation is likely to promote the activation p53 resulting in apoptosis, in most of the case for "transcription-dependent effects" (53,56,57). The p53 controls apoptosis through independent and dependent pathways and in addition, some stress signals have been associated with its activity, resulting in accumulation of it inside the cell either in the cytoplasm and nucleus (43, 57, 58).

BCL-2 FAMILY MAKES DECISION IN APOPTOSIS PROCESS

The members of Bcl-2 family regulate whether the cells remain alive or conduct them to apoptosis via some modifications of the mitochondrial membrane and consequent caspases activation. This process result from reciprocal action between anti and pro-apoptotic proteins, as a consequence of this intercommunication cell death process, is conducted to occur in order to maintain the homeostasis of the organism (43, 59, 60). The bcl-2 family has two different functions, being either anti-apoptotic, which avoid mitochondrial outer membrane permeabilization (MOMP) and pro-apoptotic activities or in other words promoting cell death via MOMP process. Furthermore, sharing of homology domains known as Bcl homology or also called BH regions is responsible for controlling the communication among the Bcl molecules

(43, 61, 62). Despite of anti and pro-apoptotic proteins being found in different cellular area, the anti-apoptotic have especial space into the mitochondrial membrane and endoplasmic reticulum, whereas the pro-apoptotic components are located into the cytoskeleton or cytosol (57,63).

which resulting in long life for cells, especially for hematopoietic cell. In addition, studies also showed that only Bax is enough to induce apoptosis process in most of the cases, however, it is extremely necessary to occur a signal that leads to its connection with the activator molecule (39, 66, 61).

PRO-APOPTOTIC MEMBERS

The pro-apoptotic members are the main proteins responsible for MOMP occurrence. These members' activities resulting in caspase activation and consequent release of cytochrome c from mitochondria through pores formation. The two principal members of this group are Bak and Bax (57, 70, 64). This group is also known as effectors proteins and has three BH domains. Bak and Bax molecules are antagonist killer, and associated to X protein respectively and separated promoting MOMP occurrence (57, 64).

Some research performed with mice that had an inadequate quantity of Bak and Bax proteins showed that these proteins are fundamental for apoptosis process in several cell types, especially in lymphocytes, due to their deficiency,

ANTI-APOPTOTIC MEMBERS

The anti-apoptotic components are in contrast with the pro-apoptotic members, responsible for maintenance of MOM integrity preventing the mitochondrial pathway and consequent cell death, remaining the cell life over its life phases (57, 65). This group is basically compounded by different members such as A1 or Bfl-1 (or either the BCL-2 proteins associated to A1 gene), Bcl-B, Bcl-2, Bcl-XL, Bcl-w and Mcl-1 (or related to Myeloid Cell Leukemia) which interact with the BH3 only proteins that belongs to the pro-apoptotic group (66) (Fig. 1) (49). The function of Bcl-2 and Bcl-XL regulate the apoptosis process by binding to pro-apoptotic proteins Bax and Bak inhibiting them in BH3 region as a result of prevention of MOMP (table 1) (57, 44, 62, 66, 67).

Table 1. BCL-2 family members, locations and functions.

Protein	Location	Mechanism of action	Action	References
Bcl-2	Outer mitochondrial membrane, nuclear envelope, endoplasmic reticulum membrane	Preservation of the mitochondrial membrane integrity resulting in apoptosis inhibition	Antiapoptotic	57, 65
Bcl-xL	Transmembrane molecule in the mitochondria	Inhibition of caspase activation cascade by release of cytochrome c from mitochondrial pore formation	Antiapoptotic	57, 69, 64
Bcl-w	Completely on the mitochondria	Cytotoxic conditions reduce cell apoptosis leads to cell survive	Antiapoptotic	67
Mcl-1	Nucleus and mitochondria	Death promoter Bcl-2 associated, half-life reduced, interaction with NOXA, Bak1 and Bcl-2	Antiapoptotic	68, 70
Bax	Cytosol	Caspase cascade activation, release of apoptotic factor such as cytochrome c	Proapoptotic	57, 69, 64
Bak	Membrane of the integral mitochondrial proteins	Induces conformational changes resulting in larger aggregates during apoptosis process	Proapoptotic	57, 60, 65
Bid	Membrane and cytosol	Activate directly Bax protein and induces apoptosis	Proapoptotic	70, 71
Bim	Free Bim in the mitochondria	Bcl-2 or Bcl-xL is binding by free Bim resulting in inactivation of their anti-apoptotic activity, leads to apoptosis through deprivation of cytokines, microtubules perturbation and flux of calcium ions.	Proapoptotic	71, 72
Bad	Free Bad into the mitochondria	Heterodimer with Bcl-2 and Bcl-xL is formed by desphosphorylation of Bad, Inactivation of Bcl-2 and Bcl-xL allow Bak and Bax resulting in apoptosis mechanism	Proapoptotic	69

The Bcl-2 family of proteins is divided into antiapoptotic and proapoptotic members. Each one of them is located in different cell compartment and has different functions when activated. Their mechanism of action is specific, acting in some situation when is required to perform their specific role. Most of them are target to many drugs used in some pathologies such as cancers from different types.

According to Billard (2015), all anti-apoptotic members are able to bind to Bax however; only Mcl-1 and Bcl-XL might interact with Bak through the BH3 domain (fig. 2) (68). In order to develop their function precisely, the anti-apoptotic members require the BH4 domain (69). Despite Bcl-2 functions being located into the mitochondria, some studies have documented their functions either in the endoplasmic reticulum (ER) and nuclear cells membrane. Moreover, its activities have been demonstrated especially for Bcl-2 members through findings of sequence cytochrome b5 or also known as b5-Bcl-2 in the ER. It was showed that this sequence impairing apoptosis promoted by ER agents or expressed by Bax members (70).

BH3 ONLY PROTEINS

In mammalian cells, there are several molecules that compound the Bcl-2 family (fig. 2) (49, 71). The members of this family prevent cells from some cytotoxic damage such as UV and gamma irradiation, cytokine deprivation and drugs for chemotherapy purposes. Members of this family such as Bcl-XL and Bcl-w are the most important molecules that have the cited function. Some other members are identified as the binding proteins and are divided into two distinct groups, one of them compound by a trio of molecules to illustrate Bak, Bax, and Bok, which have a closer similarity to Bcl-2 members either in sequence or structure. The other group has three segments well conserved BH1, BH2, and BH3 or known Bcl-2 homology that form "hydrophobic groove" with anti or pro-apoptotic molecules, These molecules have this description due to their unique BH3 domain (59).

The main members of this subclass include the subtypes Bad, Bid, Bim, Bmf, Hrk, Noxa and Puma (table 1) (64,70,74). The BH3 only protein regulation is controlled by several and distinct mechanisms such as DNA damage, Cytokine deprivation, Tyrosine Kinase inhibitors, proteasome inhibitors, and cytotoxic inducement such as anti-cancer drugs (75, 76). Doerflinger and colleagues defined them as "the sentinels of cellular stress" because when activated, result in apoptosis initiator process (77-79). Their location has been recently discovered into the MOM, especially for the subtypes Bim, Bmf, Bid, Puma, and Noxa. Despite their location have not been prioritized in studies, it is necessary to understand their mechanisms, once this location is crucial to Bax activate Bim, tBid and Puma, and confer the role onto apoptosis process (79).

The activation process is performed by transcriptional and post-transcriptional mechanisms; however, the post-translational signal configures the most important, and once activated the response to death occur translocation in the mitochondria (64). The BH3 only proteins displace the Bak and Bax when apoptosis process initiates, especially Bid, Bim, and Puma are able to bind more efficiently and displace them, which can be bounded previously by anti-apoptotic members (80,81). In addition, BH3 members are divided into two main groups: The activators and sensitizers. The activators members include Bid, Bim, and Puma, due to their capacity to activate direct Bak and Bax, some studies also relate Noxa as an activator as well. On the other hand, the sensitizers' members include Bad and Bik and act releasing the activator, which was connected with anti-apoptotic molecules (81).

LEUKEMIA

Leukemia is defined as a neoplastic and malignant proliferation of the blood cells and the bone marrow. This abnormality affects especially the white blood cell (WBC's). As a consequence of the high proliferation rate, it leads to accumulation and interruption of the WBC's normal function and their production resulting in interruption of the other lineages such as erythrocytes and platelets synthesize characterizing anemia and thrombocytopenia (82,83). According to Hamerschlak, leukemia is divided into different subtypes, four major types are known as Acute Lymphoblastic Leukemia (ALL), and Chronic Lymphocytic Leukemia (CLL), Acute Myeloid Leukemia (AML), Chronic Myeloid Leukemia (CML) (83, 85).

ACUTE MYELOID LEUKEMIA

Acute Myeloid Leukemia (AML) is an extremely malignant hematological disorder proliferation and results from a clonal, undifferentiated and immature blood cells. AML is a high aggressive disorder that leads to apoptosis resistance and allows their growth and transformation of a hematopoietic precursor known as myeloblasts (84-85,86). Bone marrow and tissues infiltration characterize AML, and the myeloid precursors have abnormal synthesis resulting in their appearance in the blood stream. This abnormality leads to insufficiency of hematopoiesis process (86).
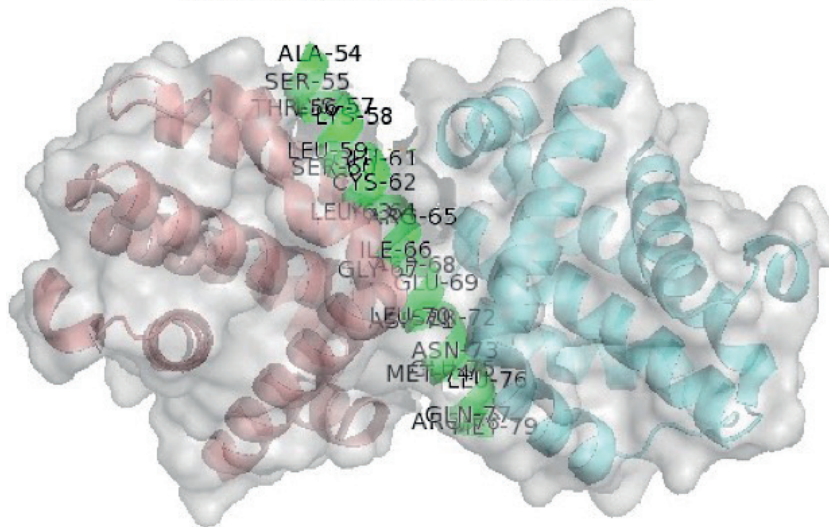
AML diagnosis is based on the presence of more than 30% of blasts cells in the bone marrow according to the French American British system (FAB) (34). On the other hand, according to World Health Organization (WHO), the diagnosis is based on 20% of nucleated cells knows as myeloblasts, however, associated with some molecular and genetics abnormalities located into the chromosomes especially (87,88,89). These myeloblasts were related to express high levels of bcl-2 proteins which have increased the chemotherapy resistance (90, 54).

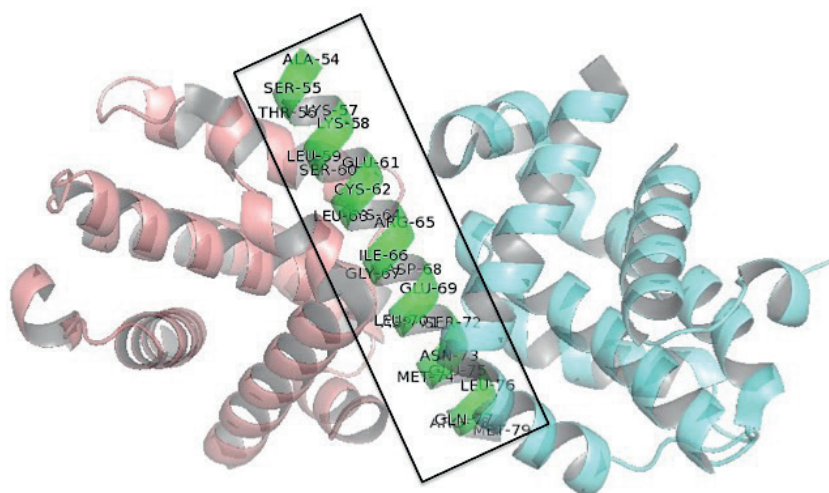## BCL-2 PROTEINS' ROLE IN CHEMOTHERAPY RESISTANCE

According to Tzifi and colleagues, the apoptosis is regulated by caspases, that can be either an initiator such as caspase 9 that activate the effectors caspases 3 and 7 by the cleavage of an internal residues recognized as Asp in their substrates separating small and large unites, and triggered
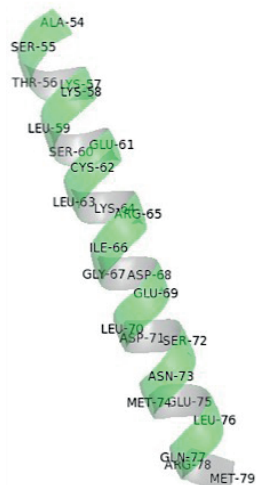
A. Bcl-X$_L$- Bak (salmon and blue),
BH3 domain (green) and protein surface (gray)

ALA-54
SER-55
THR-56 LYS-57
LYS-58
LEU-59 GLU-61
SER-60
CYS-62
LEU-63 ARG-65
ILE-66
GLY-67 68
GLU-69
LEU-70 72
ASN-73
MET-74 LEU-76
ARG-78 79

B. Bcl-X$_L$- Bak (salmon and blue),
BH3 domain (green) with its amino acids

ALA-54
SER-55
THR-56 CYS-57
LYS-58
LEU-59 GLU-61
SER-60
CYS-62
LEU-63 ARG-65
ILE-66
GLY-67 ASP-68
GLU-69
LEU-70 SER-72
ASN-73
MET-74 LYS-75
LEU-76
GLN-77 MET-79

C. BH3 domain (green) evidencing
its amino acids

ALA-54
SER-55
THR-56 LYS-57
LYS-58
LEU-59
SER-60 GLU-61
CYS-62
LEU-63 LYS-64 ARG-65
ILE-66
GLY-67 ASP-68
GLU-69
LEU-70 ASP-71 SER-72
ASN-73
MET-74 GLU-75
LEU-76
GLN-77 ARG-78
MET-79

D. BH3 domain amino acids sequence

54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79

A S T K K L S E C L K R I G D E L D S N M E L Q R M

Figure 2.
Structure of the Bcl-X$_L$ interacting with Bak through the BH3 domain. It can be observed their structures connecting to each other through the BH3 domain (green). The binding groove is formed by some amino acids that give rise to different conformational structure and its organizational form. It is evidenced the structural interaction between the Bcl-XL and Bak. In (**A**) is demonstrated the complex formed by Bcl-X$_L$- Bak showed in salmon and blue, and the BH3 domain in green color, the protein surface is seen in gray. In (**B**) Bcl-XL-Bak (salmon and blue) and the BH3 (green) with the amino acids presence (black square) and (**C**) evidences the BH3 domain with its amino acids and (**D**) shows the BH3 amino acids sequences. Protein Data Bank (PDB) [69] entry for the displayed structure: Bcl-XL–Bak BH3 is 3PL7.

by the Bcl-2 proteins (82,91). These molecules are related to cell survivor, although their activity do not drive any function with the cell proliferation, and it is indeed that the tumor genesis process is linked to the cell survivor and deregulation of Bcl-2 proteins (46). Mcl-1 and Bcl-XL genes are highly expressed in humans diagnosed with AML and multiple myeloma according to recent studies (82).

In addition, it was observed that overexpression Bcl-2 family is related with resistance to cancer treatment (92). Indeed, it has been very common and frequently identified the apoptosis resistance in cancer therapies. Furthermore, when aberrant apoptosis pathway is identified, it is linked to chemotherapy and /or radiotherapy resistance, whereby most of the patients have been attended for many decades (92, 93). Yip and Reed in their study in 2008, argued that overexpression of both Bcl-2 especially the anti-apoptotic members have been associated with chemoresistance, which impair the apoptosis process through some stimuli such as oxidative stress, hypoxia, and deprivation of growth factor (70).

More and Letai also stated that most of the cancer cells are habituated to Bcl-2 proteins presence and these oncogenes play an important role inducing their survival and the pro-apoptotic Bcl-2 member's overexpression to oncogenic stimulus in tumor cells do not influence enough to overcome the overexpression of Bcl-2 anti-apoptotic proteins signalization into the cancer cells (62,94-97). Wei and Teh in 2012, argued that increasing of pro-survival members is related to resistance of many cancer therapies as confirmed by Danial, (2007) and Weyhenmeyer and colleagues (2012). In fact, these studies bring us evidences that hematologic disorders especially AML with overexpression of Bcl-2 members have more resistance to chemotherapy (29).

Another members of the Bcl-2 family are the BH3 only proteins, which play an essential and crucial role in chemotherapy resistance. These members modify their capacity to ligate Bcl-2 targets. In addition, Bim neutralizes potentially all apoptotic members, on the other hand, Bad has its function limited to other members such as "Bcl-2, Bcl-x, Bcl-w and Noxa that can bind only to Mcl-1 and A1" (97). Therefore, this role of function leads to predict to the responsiveness of cancer therapy such as chemotherapy to mitochondrial apoptosis both in cancer and normal cells. However, increasing the activity of BH3 only proteins is likely to result in direct activation of Bax and Bak resulting in cell apoptosis (97).

Bcl-2 proteins should occupy the binding site, however, cells that present high levels of BH3 occupied by Bcl-2 proteins are "highly primed" to induce death and measured by its affinity to BH3 members. On the other hand, BH3 member in excess and unoccupied by a Bcl-2 result in "low BH3 priming" as a result of it leads to high cell resistance to mitochondrial depolarization conducted by BH3 only proteins and result in effect of cytotoxic therapeutically methods (97, 98). If a comparison were performed with other pro-survival members activities in AML, BH3 members dislike no previous knowledge of prevailing levels of pro-apoptotic or anti-apoptotic members of Bcl-2 family. This, however, is extremely relevant in cases where Bcl-2 increasing result in stabilization and accumulation of Bim leading to lower level of unoccupied Bcl-2 than expected (98). As Vo and colleagues (2012) stated in their study, "the dominant pro-survival factor in human AML was Bcl-2 compared to Mcl-1 in hematopoietic stem cells with normal conditions" and that the target of the Bcl-2 members is likely to allow the final destruction of carcinogens cells in AML, while the toxicity of normal hematopoietic stem cells would be protected by Mcl-1. In order to overcome these findings some molecules have been designed to target some members of the Bcl-2 family. These compounds, binding to the hydrophobic site of the Bcl-2 anti-apoptotic member and perform its function like BH3-only proteins to induce the apoptosis process (99). Several studies have demonstrated some evidences that apoptosis has an important role in responsiveness to chemotherapy. In addition, was also observed that AML CD 34+ is more resistant to apoptosis than CD34- and this is also correlated to higher expression of bcl-2, Mcl-1, bcl-XL and low levels bax expression. This leads us to conclude that the AML cases that express CD34+ indicate the resistance to apoptosis (90). Some synthetic or even natural molecules have been described as inhibitors of the Bcl-2 family and are know especially as BH3 mimetic molecules (70). These BH3 mimetic components such as ABT-737 have the ability to bind to some blc-2 family molecules mainly to bcl-2, bcl-XL and bcl-w, however not to Mcl-1. This ligation leads to disruption of their synergy with pro-apoptotic members Bak and Bax intensifying the apoptosis, this ligation occurs with high affinity among these proteins (100, 101Bcl-xL and Mcl-1, resulting in resistance to apoptosis and association with poor prognosis. Docetaxel, an antimitotic drug that is the first-line treatment strategy for CRPC, is known to provide a small survival benefit. However, docetaxel chemotherapy alone is not enough to counteract the high levels of Bcl-2/Bcl-xL/Mcl-1 present in CRPC. ABT-737 is a small molecule that binds to Bcl-2/Bcl-xL (but not Mcl-1). ABT-737 is also known as a BAD mimetic that has shown efficacy in some types of the tumor including leukemia. After its ligation and inhibition of the cited molecules it can result in apoptosis of carcinogens cells no affecting the adjacent normal cells. However, in some tumor the resistance mechanism has been associated with overexpression of the Mcl-1 members that is an anti-apoptotic protein that the ABT-737 does not target to (70). As has been suggested by Vo in 2012, if pro-apoptotic members being targeted in AML treatment it might allow a small molecule of the BH3 only proteins with selectivity upon Bcl-2 such as ABT-737, it will target therapeutically, efficient, and safely than most of the drugs and chemotherapies currently available and used for AML treatment (98, 102).

CONCLUSION

The high expression of the bcl-2 have been related to chemotherapy resistance reported in many populations around the world, what can be inferred is that due to their overexpression the induction of apoptosis is affected. Indeed the development of a therapeutic method, which targets the BH3 molecules, might have high and precise activity to treat AML in most of the cases and designing molecules to target especially the members of Bcl-2 family might be an alternative, once they have been found increased in many types of tumor. One of the alternatives to achieve the solution for this problem is decrease these proteins levels via the new emergent technologies. Moreover, another criteria that should have been considered is the patient safety, once receiving the drug this must have a total security regarding its side effect and therapeutic efficiency, when compared to several other drugs and therapies existent and that have been currently released from the pharmaceutical companies worldwide.

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CONFLICT OF INTEREST

Author declare no conflict of interest.

REFERENCES

1. Abdel-Magid A. F. (2015). Inhibitors of the Anti-apoptotic Myeloid Cell Leukemia-1 (Mcl-1) May Provide Effective Treatment for Cancer. ACS Medical Chemistry Letter, 6: 1171–1173. dx.doi.org/10.1021/acsmedchemlett.5b00438

2. AlBakr R. B, Khojah O. T. (2014). Incidence Trend of the Leukemia Reported Cases in the Kingdom of Saudi Arabia, Observational Descriptive Statistic from Saudi Cancer Registry. International Journal Biomedical Research, 5(8).

3. Anderson M. A, Huang D, Robertsa A. 2014. Targeting BCL2 for the Treatment of Lymphoid Malignancies. Seminar of Hematolology, 51(3), 219–227.

4. Marshall K. D & Baines C. P. 2014. Necroptosis: Is there a role for mitochondria? Frontier of Physiology, 5, 323.

5. Asif N, Hassan K. 2013. Acute Myeloid Leukemia amongst Adults. Journal of Islamabad Medical & Dentistry College (JIMDC), 2(4), 58-63.

6. Zhao H, Jaffer T, Eguchi S, Wang Z, Linkermann A, Ma D. (2015). Role of necroptosis in the pathogenesis of solid organ injury. Cell death and disease, 6, 1-10.

7. Gozuacik D, Kimchi A. (2007). Autophagy and Cell Death. Current topics in developmental biology, 78, 217-245. https://doi.org/10.1016/S0070-2153(06)78006-1

8. Martin S. J, Henry C. M, Cullen S.P. (2012). A perspective on mammalian caspases as positive and negative regulators of inflammation. Molecular, 46(4), 387–397. doi: 10.1016/j.molcel.2012.04.026.

9. Mohana-Kumaran N, Hill D. S, Allen J. D, Haass N. K. (2014). Targeting the intrinsic apoptosis pathway as a strategy for melanoma therapy. Pigment Cell Melanoma Research, 4, 525-39. doi:10.1111/pcmr.12242. Epub 2014.

10. Hajji N. Joseph B. (2010). Epigenetic regulation of cell life and death decisions and deregulation in cancer. Essays in Biochemistry, 48, 121-146.

11. Hockenbery D. M. (1994). bcl-2 in cancer, development and apoptosis. Journal of Cell Science, Supplement, 18, 51–55.

12. Yip K. W, Reed J. C. (2008). Bcl-2 family proteins and cancer. Oncogene, 27(50), 6398–6406. https://doi.org/10.1038/onc.2008.307

13. Frenzel, A., Grespi, F., Chmelewskij, W. & Villunger. A. (2009). Bcl2 family proteins in carcinogenesis and the treatment of cancer. Apoptosis. 14(4); 584–596. doi:10.1007/s10495-008-0300-z.

14. Kelly P, Strasser A. (2011). The role of Bcl-2 and its pro-survival relatives in tumourigenesis and cancer therapy. Cell Death and Different, 18(10), 1414–1424. https://doi.org/10.1038/cdd.2011.17

15. Susan F. L, Brad C. T. (2005). Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. Infection and immunity, 73(4), 1907-16.

16. Doerflinger M, Glab J. A, Puthalakath H. (2015). BH3-only proteins: a 20-year stock-take. FEBS Journal, 282,1006–1016. doi:10.1111/febs.13190

17. Dewson, G, Kluck R. M. (2009). Mechanisms by which Bak and Bax permeabilise mitochondria during apoptosis. Journal of Cell Science, 122, 2801-8.

18. Ghatage D D, Gosavi S R, Ganvir S. M, Hazarey V. K. (2012). Apoptosis: Molecular mechanism. Journal Orofacial Science, 4 (2).

19. Barak Y, Juven T, Haffner R. (1993). mdm2 expression is induced by wild type p53 activity. EMBO Journal, 12, 461–468.

20. Belizário J, Cordeiro L. V, Enns S. (2015). Necroptotic Cell Death Signaling and Execution Pathway: Lessons from Knockout Mice. Hindawi Publishing Corporation Mediators of Inflammation, 15.

21. Bensi L, Longo R, Vecchi A, Messora C, Garagnani L, Bernardi M. S, Tamassia G, Sacchi S. (1995). BCL-2 Oncoprotein Expression in Acute Myeloid Leukemia. Haematology, 80, 98-102.

22. Billard C. (2015). Apoptosis as a Therapeutic Target in Chronic Lymphocytic Leukemia. Lymphocytitc and Chonic Lymphocytic Leukemia, 5, 11–15. doi:10.4137/LCLL.S13718.

23. Blau O. (2015). Gene Mutations in Acute Myeloid Leukemia-Incidence, Prognostic Influence, and Association with Other Molecular Markers. INTECH, 75-100.

24. Blatt N. B, Glick G. D. (2001). Signaling pathways and effector mechanisms pre-programmed cell death. Bioorganic and Medical Chemistry, 9(6), 1371-84.

25. Fisher A. (1937). The theory of the developmental physiology of malignant tumor. The American journal of cancer , 31(10).

26. Breckenridge D. G, Germain M, Mathai J. P, Nguyen M, Shore G. C. (2003) Regulation of apoptosis by endoplasmic reticulum pathways. Oncogene, 22, 8608–8618. doi:10.1038/sj.onc.1207108

27. Bruin E. C, Medema J. P. (2008). Apoptosis and non-apoptotic deaths in cancer development and treatment response. Cancer Treatment Reviews, 34(8), 737-749. doi: http://dx.doi.org/10.1016/j.ctrv.2008.07.001

28. Brunelle J. K, Letai A. (2009). Control of mitochondrial apoptosis by the Bcl-2 family. J Cell Science, 122, 437-441.

29. Chaabane W, User S. D, El-Gazzah M, Jaksik R, Sajjadi E, Rzeszowska-Wolny J, Łos M. J. (2013). Autophagy, Apoptosis, Mitoptosis and Necrosis: Interdependence Between Those Pathways and Effects on Cancer. Archive of Immunology Therapy and Experimental, 61, 43–58, (2013). DOI 10.1007/s00005-012-0205-y

30. Chipuk J. E. (2015). BCL-2 proteins: melanoma lives on the edge. Oncoscience, 34(7), 857-67.

31. Chonghaile T. N, Letai A. (2009). Mimicking the BH3 domain to kill cancer cells. Oncogene, 27, 149–157. doi:10.1038/onc.2009.52

32. Thorburn A. (2008). Apoptosis and autophagy: regulatory connections between two supposedly different processes. Apoptosis, 13(1), 1-9. Doi: 10.1007/s10495-007-0154-9

33. Kelly P, Strasser A. (2011). The role of Bcl-2 and its pro-survival relatives in tumourigenesis and cancer therapy. Cell Death and Differentiation, 18(10), 1414–1424. https://doi.org/10.1038/cdd.2011.17

34. Palai T. K, Mishra S. R. (2015). Caspases: An apoptosis mediator. Journal of Advanced Veerinary and Animal Research, 2(1), 18-22. doi: 10.5455/javar.2015.b52

35. Shimizu S, Yoshida T, Tsujioka M, Arakawa S. (2014). Autophagic Cell Death and Cancer. International Journal of Molecular Science, 15, 3145-3153. doi:10.3390/ijms15023145

36. Siddiqui W. A, Ahad A, Ahsan H. (2015). The mystery of BCL2 family: Bcl-2 proteins and apoptosis: an update. Archives of Toxicology, 89, 289-317.

37. Singh L, Pushker N, Saini N, Sen S, Sharma A, Bakhshi S, Chawla B, Kashyap S. (2015). Expression of pro-apoptotic Bax and anti-apoptotic Bcl-2 proteins in human retinoblastoma. Clinical Experimental Ophthopedic, 43, 259–267. doi: 10.1111/ceo.1239

38. Su Z, Yang Z, Xu Y, Chen Y, Qiang Y. Q. (2015). Apoptosis, autophagy, necroptosis, and cancer metastasis. Molecular Cancer, 14(48). Doi:10.1186/s12943-015-0321-5

39. Guicciardi M. E, Gores G. J. (2009). Life and death by death receptors. FASEBJ, 23(6), 1625-37. doi: 10.1096/fj.08-111005.

40. Marsden V. S, Ekert P. G, Delft M. V, Vaux D. L, Adams J. M, Strasser A. (2004). Bcl-2–regulated apoptosis and cytochrome c release can occur independently of both caspase-2 and caspase-9. The Journal of Cell Biology, 165(6),775–780. http://www.jcb.org/cgi/doi/10.1083/jcb.200312030

41. Mason K, Vandenberga C. J, Scotta C. L, Wei A. H, Corya S, Huanga D. (2008). In vivo ef cacy of the Bcl-2 antagonist ABT-737 against aggressive Myc-driven lymphomas. The Proceedings of the National Academy of Sciences, 105, 17961-17966.

42. Mérino D, Khaw S. L, Glaser S. P, Anderson D. J, Belmont L. D, Wong C. (2012). Bcl-2, Bcl-xL, and Bcl-w are not equivalent targets of ABT-737 and navitoclax (ABT-263 in lymphoid and leukemic cells. Blood, 119, 5807-5816.

43. Mehdipour P, Santoro F, Minucci S.(2015). Epigenetic alterations in acute myeloid leukemias. FEBS Journal, 282, 1786–1800. doi:10.1111/febs.13142

44. Momand J, Zambetti G. P, Olson D. C. (1991). The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. Cell, 69, 1237–1245.

45. Kang M. H, Reynolds C. P. (2009). Bcl-2 Inhibitors: Targeting Mitochondrial Apoptotic Pathways in Cancer Therapy. Clinical Cancer Research, 15(4). doi:10.1158/1078-0432.CCR-08-0144

46. Koff J. L, Ramachandiran S, Bernal-Mizrachi L. (2015). A Time to Kill: Targeting Apoptosis in Cancer. International Journal Molecular Science, 16, 2942-2955. doi:10.3390/ijms16022942

47. Kontny U, Lissat A. (2015). Apoptosis and drug resistance in malignant bone tumors. Primary bone tumours. Doi: 10.1016/B978-0-12-416721-6.00036-4

48. Lavrik I. N. (2014). Systems biology of death receptor networks: live and let die. Cell Death and Disease, 5. doi:10.1038/cddis.2014.160

49. Leber B, Lin J, Andrews D. W. (2010). Still embedded together binding to membranes regulates Bcl-2 protein interactions. Oncogene, 29, 5221-30.

50. Letai A, Sorcinelli M. D, Beard C, Korsmeyer S. J. (2002). Antiapoptotic BCL-2 is required for maintenance of a model leukemia. Cancer Cell, 6, 241–9.

51. Le´veille F, Papadia S, Fricker M, Bell K. F. S, Soriano F. X, Martel M, Puddifoot C, Habel M, Wyllie D. J, Ikonomidou C, Tolkovsky A. M, Hardingham G. E. (2010). Suppression of the Intrinsic Apoptosis Pathway by Synaptic Activity. The Journal of Neurology, 30(7), 2623–2635.

52. Liu B, Bhatt D, Oltvai Z. N, Greenberger J. S, Bahar I. (2014). Significance of p53 dynamics in regulating apoptosis in response to ionizing radiation, and poly-pharmacological strategies. Scientific Reports, 4, 6245. Doi: 10.1038/srep06245

53. Li M. X, Dewson G. (2015). Mitochondria and apoptosis: emerging concepts. F1000Prime Reports, 7(42). doi:10.12703/P7-42.

54. Abramowitz J, Neuman T, Perlman R, Ben-Yehuda D. (2017). Gene and protein analysis reveals that p53 pathway is functionally inactivated in cytogenetically normal Acute Myeloid Leukemia and Acute Promyelocytic Leukemia. BMC Medical Genomics, 10(1),18. https://doi.org/10.1186/s12920-017-0249-2

55. Petitjean A, Mathe E, Kato S, Ishioka C, Tavtigian S. V, Hainaut P, Olivier M. (2007). Impact of Mutant p53 Functional Properties on TP53 Mutation Patterns and Tumor Phenotype: Lessons from Recent Developments in the IARC TP53 Database. Human mutation, 28(6), 622-629.

56. Llambi F, Moldoveanu T, Tait Stephen W. G, Bouchier-Hayes L, Temirov J, McCormick L. L, Dillon C. P, Green D. R. (2011). A unified model of mammalian BCL-2 protein family interactions at the mitochondria. Molecular Cell, 44, 517-31.

57. Fulda S, Debatin K. M. (2006). Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. Oncogene, 25(34), 4798-811.

58. Löwenberg B, Rowe J. M. (2015). Introduction to the review series on advances in acute myeloid leukemia (AML). Blood, 127(1). doi:10.1182/blood-2015-10-66268

59. Mongiat M, Ligresti G, Marastoni S, Lorenzon E, Doliana R, Alfonso C. Regulation of the Extrinsic Apoptotic Pathway by the Extracellular Matrix Glycoprotein EMILIN2. (2007). Molecular Cell Biology, 27(20), 7176–7187. doi:10.1128/MCB.00696-07

60. Moore V. D. G, Letai A. (2012). BH3 profiling – measuring integrated function of the mitochondrial apoptotic pathway to predict cell fate decisions. Cancer Letter, 332(2), 202–205. doi:10.1016/j.canlet.2011.12.021.

61. Moore D. G. V, Brown J. R, Certo M. (2007). Chronic lymphocytic leukemia requires BCL2 to sequester prodeath BIM, explaining sensitivity to BCL2 antagonist ABT-737. Journal of Clinical Investiment, 117, 112–21.

62. Naseri H. M, Mahdavi M, Davoodi J, Tackallou S. H, Goudarzvand M, Neishabouri S. H. (2015). Up regulation of Bax and down regulation of Bcl2 during 3-NC mediated apoptosis in human cancer cells. Cancer Cell International, 15(55). Doi: 10.1186/s12935-015-0204-2

63. Ng S. Y, Davids M. S. (2014). Selective Bcl-2 inhibition to treat chronic lymphocytic leukemia and non-Hodgkin lymphoma. Clinical Advanced Hematology Oncology, 12(4), 224-9.

64. Noguchi M, Hirata N, Edamura T, Ishigaki S, Suizu F. (2015). Intersection of Apoptosis and Autophagy Cell Death Pathways. Austin Journal of Molecular & Cell Biology, 2(1), 1004.

65. Oltersdorf T, Steven W, Elmore S. W, Shoemaker A. R, Armstrong R. C, Augeri D. J, Belli B. A. (2005). An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature, 435, 677-681.

67. Gibson L, Holmgreen S. P, Huang D. C, Bernard O, Copeland N. G, Jenkins N. A, Sutherland G. R, Baker E, Adams J. M, Cory S. (1996). Bcl-w, a novel member of the bcl-2 family, promotes cell survival. Oncology, 13(4), 665-75.

88. Zhong Q, Gao W, Du F, Wang X. (2005). Mule/ARF-BP1, a BH3-only E3 ubiquitin ligase, catalyzes the polyubiquiti- nation of Mcl-1 and regulates apoptosis. Cell, 121, 1085–1095.

69. Czabotar P. E, Lee E. F, Delft M. F, Day C. L, Smith B. J, Huang D. C. S, Fairlie W. D, Hinds M. G, Colman P.M. (2007). Structural insights into the degradation of Mcl-1 induced by BH3 domain. Proceedings of the National Academy of Sciences, 104 (15) 6217-6222. DOI:10.1073/pnas.0701297104

70. Huang D. C. S, Strasser A. (2000). BH3-Only Proteins—Essential Initiators of Apoptotic Cell Death. Cell, 103, 839–842.

71. Shamas-Din A, Kale J, Leber B, Andrews D. W. (2013). Mechanisms of Action of Bcl-2 Family Proteins. Cold Spring Harbor Perspective in Biology. doi: 10.1101/cshperspect.a008714

72. Belka C, Budach W. (2002). Anti-apoptotic Bcl-2 proteins: structure, function and relevance for radiation biology. International journal of radiation Biology, 78(8), 643-658. Doi: 10.1080/0955300021013768 0

73. Bouillet P, Metcalf D, Huang D. C, Tarlinton D. M, Kay T. W, Kontgen F, Adams J. M, Strasser A. (1999). Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity. Science, 286, 1735–1738.

74. Polčic P, Jaká P, Mentel M. (2015). Yeast as a tool for studying proteins of the Bcl-2 family. Microbiology Cell, 2(3), 74-87. doi: 10.15698/mic2015.03.193

75. Sáez G. A. J. (2012). The secrets of the Bcl-2 family. Cell Death Differentiation, 11, 1733-40. doi: 10.1038/cdd.2012.105.

76. Scaffidi C, Fulda S, Srinivasan A, Friesen C, Li F, Tomaselli K. J, Debatin K. M, Krammer P. H, Peter M. E. (1998). Two CD95 (APO-1/Fas) signaling pathways. The EMBO Journal, 17(6), 1675–1687.

77. Schnerch D, Yalcintepe J, Schmidts A, Becker H, Follo M, Engelhardt M, Wäsch R. (2012). Cell cycle control in acute myeloid leukemia. American Journal of Cancer Research, 2(5), 508-528.

78. Scandura J. M, Boccuni P, Cammenga J, Nimer S. D. (2002). Transcription factor fusions in acute leukemia: variations on a theme. Oncogene, 21, 3422-3444. Doi: 10.1038/sj/ onc/1205315

79. Shimizu S, Yoshida T, Tsujioka M, Arakawa S. (2014). Autophagic Cell Death and Cancer. International Journal of Molecular Science, 15, 3145-3153. doi:10.3390/ijms15023145

80. Smaili S. S, Hsu Y. T, Carvalho A. C. P, Rosenstock T. R, Sharpe J. C, Youle, R. J. (2003). Mitochondria, calcium and pro-apoptotic proteins as mediators in cell death signaling. Brazilian Journal of Medical Biology Research. 36(2), 183-190.

81. Strasser A. (2005). The role of BH3-only proteins in the immune system. Nature Reviews Immunology, 5, 189-200.doi:10.1038/nri1568

82. Sun Z, Cheng Z, Taylor C. A, McConkey B, Thompson J. E. (2010). Apoptosis Induction by eIF5A1 Involves Activation of the Intrinsic Mitochondrial Pathway. Journal of Cell Physiology, 223, 798–809. Doi: 10.1002/jcp.22100

83. Tian K.Y, Liu X. J, Xu J.D, Deng L. J, Wang G. (2015). Propofol inhibits burn injury-induced hyperpermeability through an apoptotic signal pathway in microvascular endothelial cells. Brazilian Journal of Medical Biology Research, 48(5), 401-407. http://dx.doi.org/10.1590/1414-431X20144107

84. Trump B. F, Berezesky I. K, Chang, S. H, Phelps P. C. (1997). The Pathways of Cell Death: Oncosis, Apoptosis, and Necrosis. Toxicologic Pathology, 25(1), 82-8. Doi: 10.1177/019262339702500116

85. Tiwari M, Sharma L. K, Saxena A. K, Godbole M. M. (2015). Interaction Between Mitochondria and Caspases: Apoptotic and Non-Apoptotic Roles. Cell Biology, 3(2), 22-30. doi: 10.11648/j.cb.s.2015030201.14

86. Tzifi F, Economopoulou C, Gourgiotis D, Ardavanis A, Papageorgiou S, Scorilas A. (2012). The Role of BCL2 Family of Apoptosis Regulator Proteins in Acute and Chronic Leukemias. Hindawi Public Corporation Advanced Hematology, doi:10.1155/2012/524308.

87. Vela L, Gonzalo O, Naval J, Marzo I. (2013). Revealed by fluorescence complementation. Journal of Biological Chemistry, doi:10.1074/jbc.M112.422204

88. Verbrugge I, Johnstone R. W, Smyth M. J. (2010). SnapShot: Extrinsic Apoptosis Pathways. Cell, 143(7), 1192. DOI 10.1016/j.cell.2010.12.004

89. Vo T. T, Ryan J, Carrasco R, Neuberg D, Rossi D. J, Stone R. M, Letai A. (2012). Relative mitochondrial priming of myeloblasts and normal HSCs determines chemotherapeutic success in AML. Cell, 151(2), 344-355.

90. El-Shakankiry N. H, El-Sayed G. M, El-Maghraby S, Moneer M. M. (2009). Bcl-2 protein expression in egyptian acute myeloid leukemia. Journal of Egypt Natural Cancer Institute, 21(1), 71–6.

91. Shi Y. (2002). Mechanisms of caspase activation and inhibition during apoptosis. Molecular Cell. https://doi.org/10.1016/S1097-2765(02)00482-3

92. Wang R. A, Li Z. S, Yan Q. Q, Bian X. W, Ding Y. Q, Xiang D. X, Sun B. C, Yun-Tian S. Y. T, Xiang-Hong Zhang X. H. (2014). Resistance to apoptosis should not be taken as a hallmark of cancer. Chinese Journal of Cancer, 33(2).

93. Wei A. Teh T. C. (2012). Primed for the kill: occupying Bcl-2 to target death in acute myeloid leukaemia. BioDiscovery, 6(1). doi:10.7750/BioDiscovery.2012.6

94. Weyhenmeyer B, Murphy A. C, Prehn J. H. M, Murphy B. M. (2012). Targeting the anti-apoptotic BCL-2 family members for the treatment of cancer. Experimental Oncology, 34, 192–199.

95. Woess C, Tuzlak S, Labi V, Drach M, Bertele D, Schneider P, Villunger A. (2015). Combined loss of the BH3-only proteins Bim and Bmf restores B-cell development and function in TACI-Ig transgenic mice. Cell Death Differentiation, 22, 1477–1488. doi: 10.1038/cdd.2015.8

96. Wu M, Ding H. F, Fisher D. E. Apoptosis: Molecular Mechanisms. Encyclopedia of Life Science 2001.

97. Yip K. W, Reed J. C. (2008). Bcl-2 family proteins and cancer. Oncology, 27, 6398–6406.

98. Yohe S. (2015). Molecular Genetic Markers in Acute Myeloid Leukemia. Journal of Clinical Medicine, 4, 460-478 (2015). doi:10.3390/jcm4030460

99. Youle R. J, Strasser A. (2005). The BCL-2 protein family: opposing activities that mediate cell death. Nature Reviews of Molecular and Cell Biology, 9, 47–59.

100. Perez-Stable C, Parrondo R, De Las Pozas A, Reiner T. (2013). ABT-737, a small molecule Bcl-2/Bcl-xL antagonist, increases antimitotic- mediated apoptosis in human prostate cancer cells. Biochemistry, biophysics and molecular biology, https://doi.org/10.7717/peerj.144

101. Zhao G, Zhu Y, Eno C. O, Liu Y, DeLeeuw L, Joseph A, Burlison J. A, Chaires J. B, Trent J. O, Li C. (2014). Activation of the Proapoptotic Bcl-2 Protein Bax by a Small Molecule Induces Tumor Cell Apoptosis. Molecular Cell Biology, 34(7), 1198 –1207. doi:10.1128/MCB.00996-13

102. Zong W. X, Lindsten T, Ross A. J, MacGregor G. R, Thompson C. B. (2001). BH3-only proteins that bind pro-survival Bcl-2 family members fail to induce apoptosis in the absence of Bax and Bak. Gene & Development, 15, 1481–1486.