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REVIEW

Mitochondrial targeted peptides for cancer therapy

Sadaf Farsinejad[1] · **Zohre Gheisary**[1] · **Sanaz Ebrahimi Samani**[1] · **Ali Mohammad Alizadeh**[1]

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Abstract Mitochondria are a key pharmacological target in all cancer cells, since the structure and function of this organelle is different between healthy and malignant cells. Oxidative damage, disruption of mitochondrial ATP synthesis, calcium dyshomeostasis, mtDNA damage, and induction of the mitochondrial outer membrane permeabilization (MOMP) lead to the mitochondrial dysfunctionality and increase the probability of the programmed cell death or apoptosis. A variety of the signaling pathways have been developed to promote cell death including overexpression of pro-apoptotic members of Bcl-2 family, overloaded calcium, and elevated reactive oxygen species (ROS) play a key role in the promoting mitochondrial cytochrome *c* release through MOMP and eventually leads to cell death. There are a wide range of the therapeutic-based peptide drugs, known mitochondrial targeted peptides (MTPs), which specifically target mitochondrial pathways into death. They have prominent advantages such as low toxicity, high specificity, and easy to synthesis. Some of these therapeutic peptides have shown to increased the clinical activity alone or in combination with other agents. In this review, we will outline the biological properties of MTPs for cancer therapy. Understanding the molecular mechanisms and signaling pathways controlling cell death by MTPs can be critical for the development of the therapeutic strategies for cancer patients that would be valuable for researchers in both fields of molecular and clinical oncology.

Keywords Mitochondria · Peptide · Cancer · Review

✉ Ali Mohammad Alizadeh
aalizadeh@sina.tums.ac.ir

[1] Cancer Research Center, Tehran University of Medical Sciences, Tehran, Iran

Abbreviations

MTP	Mitochondrial targeted peptides
IMM	Inner mitochondrial membrane
IMS	Inner mitochondrial space
Cyt c	Cytochrome *c*
MOMP	Mitochondrial outer membrane permeabilization
AIF	Apoptosis-inducing factor
Smac	Second mitochondria-derived activator of caspase
mPTP	Mitochondrial permeability transition pore
ROS	Reactive oxygen species
VDAC	Voltage-dependent anion channels
ETC	Mitochondrial electron transport chain
BH	Bcl-2 homology
ER	Endoplasmic reticulum
SCLC	Small cell lung cancer
AML	Acute myeloid leukemia
CLL	Chronic lymphocytic leukemia
CML	Chronic myeloid leukemia
ALL	Acute lymphoblastic leukemia
HCC	Hepatocellular carcinoma
IP3	Inositol 1,4,5-trisphosphate

Introduction

Cancer is a major public health problem in many parts of the world and includes a wide spectrum of different diseases that can afflict humans with various etiology and epidemiology. The eruption of this condition is initiated by the uncontrollable reproduction of cells in a specific part of the body and failure in mechanisms of cell death [1–3]. An increasing number of cancer survivors will trend in treatment patterns and costs of care. Surgery, radiation, chemotherapy, and endocrine therapy are the current approaches to cancer therapy. Moreover, new antibodies, anti-angiogenesis, viral therapy, and other small

molecules are new therapies that have made treatments more tumor specific and less toxic [4, 5]. Furthermore, a growing number of anti-cancer drugs are being discovered that lead to cell death by targeting distinctive components of malignant cells [6]. One group of these drugs includes "therapeutic peptides." Peptides are small molecules consisting of several amino acids from 2 to 50, linked with peptide bonds. Additionally, by conjugating them to ligand domain for cancer cell targeting, therapeutic peptides can demonstrate better targetable and deliverable quality. Despite the limitation of peptides, their fast elimination from the systemic circulation due to renal clearance and enzymatic degradation, they have significant advantages such as small size, ease of synthesis and modification, tumor-penetrating ability, and good biocompatibility [7, 8]. They can be an excellent alternative targeting agents for human cancers and can alleviate some of the problems with antibody targeting [9]. Application of peptides in a variety of the therapeutic areas including diabetes, cardiovascular diseases, and cancers is raising rapidly. They can be utilized directly as a cytotoxic agent through various mechanisms or can specifically act as a carrier of cytotoxic agents and radioisotopes by targeting cancer cells. Some peptides, called "mitochondrial targeting peptides" (MTPs), can assemble and form pores disrupting the cell or organelle membranes such as mitochondrion, and induce apoptotic or necrotic death [7, 10]. There are some differences between mitochondrial function in normal and cancerous cells, and may suggest the idea of designing anti-cancer agents that can selectively target malignant mitochondrial component to kill cancer cells [11, 12]. In this review, we will focus on mitochondria as a main organelle in cell death, and discuss the latest studies on the use of MTPs as less toxic drugs to induce death in cancer cells, and provide an encyclopedic compendium on major findings of MTPs that promise for the treatment of human malignancies.

Mitochondria

Mitochondria are cell energy sources, necessary for cell survival, and the most important regulator of cell death [11]. The respiratory chain of the inner mitochondrial membrane (IMM) (complexes I–IV) consists of enzymes and redox intermediates that transport electrons from substrates to molecular oxygen and uses the released energy to produce ATP. This energy is high enough to extrude protons from the mitochondrial matrix to the intermembrane space. Created electrochemical proton gradient can act as a driving force for the back flow of protons by ATP synthase complex [13]. In addition, mitochondria are crucial for cell differentiation, innate immune system, oxygen sensing, and calcium metabolism. Disruption to these processes causes a range of human pathologies, making mitochondria a potentially great as a therapeutic target [14]. They also are crucial to apoptotic cell death [15]. It is now clear that impaired apoptosis leads to tumor development and metastasis [16]. Cell death is mostly classified into either subtypes of stepwise regulated programmed cell death (apoptosis) or a passive disintegration cell death (necrosis) [17, 18], which both are regulated by different but overlapping pathways. The critical mitochondrial event in apoptosis is mitochondrial outer membrane permeabilization (MOMP), which allows release of the pro-apoptotic factors, including cytochrome *c* (Cyt c) to activate caspases and apoptosis-inducing factor (AIF), second mitochondria-derived activator of caspase (Smac), endonucleases, and a serine protease HTRA2 [19]. In contrast, the key mitochondrial event in primary necrosis is early opening of the mitochondrial permeability transition pore (mPTP) in IMM followed by fast decreasing of the electrical potential difference across IMM without releasing of Cyt c. These events lead to interruption of ATP synthesis, and huge water and small solutes influx to matrix along their electrochemical gradient results in severe osmotic swelling of mitochondria [12, 20, 21]. Therefore, loss of ionic homeostasis leads to necrotic cell death. As mentioned above, mitochondria can emerge as a novel therapeutic target, especially for cancer therapy. Interestingly, a group of agents with anti-cancer activity that induce apoptosis by mitochondrial destabilization were considered in recent investigations. Among these agents, therapeutic peptides which specifically target mitochondria have advantages and recently received much attention. These peptides can be classified into four groups according to their mechanisms of action including those which targeting Bcl-2 family, elevated ROS, overloaded calcium, and voltage-dependent anion channels (VDAC). In each section, we will introduce and discuss on the efficacy and result of some main MTPs for cancer therapy.

Bcl-2 family as a member of MTPs for cancer therapy

Bcl-2 family is the key regulatory proteins of apoptosis intrinsic pathway and cell death. MOMP can be achieved by pore formation via pro-apoptotic Bcl-2 family proteins remarkably Bax and Bak, and followed membrane ruptures as a result of mitochondrial swelling (Fig. 1). The Bcl-2 protein family consists of 25 pro- and anti-apoptotic member, all contain characteristic regions of homology termed as Bcl-2 homology (BH) domains [22]. Tumor cell survival is highly dependent on expression of certain pro-survival Bcl-2 family proteins. The ratio of pro- and anti-apoptotic Bcl-2 family members is important to determine the cell death or life. Targeting the anti-apoptotic Bcl-2 proteins can promote cell death. All of the BH3 proteins (e.g., PUMA, NOXA, Bad, Bim, and Bid) are classified as pro-apoptotic, while Bcl-2 proteins with four regions of high sequence similarity (BH1, BH2, BH3, and BH4)

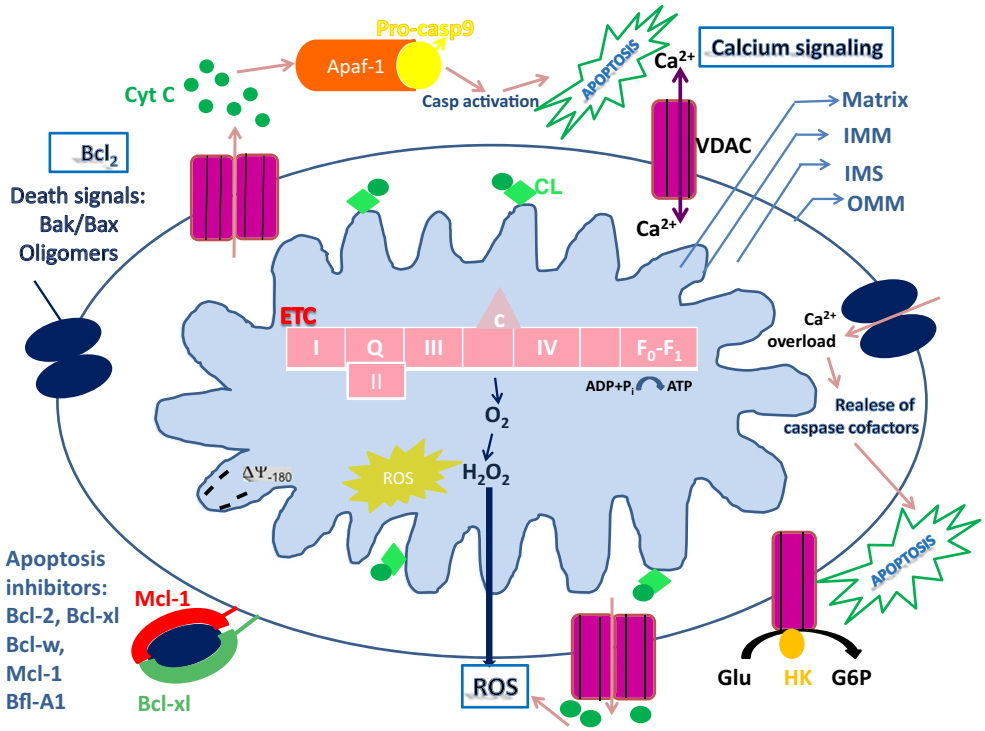

Fig. 1 Mitochondrial targeted pathways by peptides for cancer therapy. There are four possible pathways for induction of apoptosis in malignant cells by the therapeutic peptides. First, Bcl-2 family proteins consist of anti-apoptotic and pro-apoptotic members. They are the key regulatory proteins of apoptosis. Oligomerization of pro-apoptotic Bcl-2 family members (Bax and Bak) initiates MOMP by pore formation and disruption of membrane, and allows the release of Cyt c. Also, targeting the anti-apoptotic Bcl-2 proteins can promote cell death. Second, as mitochondria are the most prominent source of intracellular ROS, induction of apoptosis through elevated ROS is a central pathway in cancer. Third, overloaded calcium and finally activated VDAC. VDACs are a family of pore-forming proteins which serves as a key regulator of mitochondrial metabolite flux and apoptosis. Also, it should mention that all of these pathways are related to each other. For example, Bax activation leads to increased ROS production or transient expression of VDAC increases mitochondrial matrix Ca^{2+} concentration and mitochondrial Ca^{2+} is another powerful signal for ROS production. See text for abbreviation definitions

are anti-apoptotic (e.g., Bcl-2, Bcl-xL, Bcl-w, Mcl-1, and Bfl1) with the exceptions of Bak and Bax. In stress conditions, BH3-only proteins bind to the BH3 domain of anti-apoptotic Bcl-2 proteins, leading to displacing and releasing of pro-apoptotic BAK or BAX, followed by pore formation in the mitochondrial outer membrane, and release of pro-apoptotic factors such as Cyt c that leads to caspase activation and commits the cell to death [18, 23]. On the other hand, Bcl-2 and Bcl-xL exert their anti-apoptotic activity by diverting Bax and preventing it from forming MOM channels (Fig. 1). Cyt c is attached to IMM-specific phospholipid cardiolipin [6]. Cardiolipin is the only phospholipid in the mitochondria oxidized in the early stages of apoptosis that is triggered by the mitochondria-dependent generation of ROS. As it will be mentioned in the following section, increased ROS is a crucial factor in the release of Cyt c from the IMS. Therefore, a therapeutic approach is to inhibit Bcl-2 proteins using agents that mimic the BH3 domains of the pro-apoptotic Bcl-2 family members, which are called BH3 mimetic peptides. In cell-free assays, BH3 mimetic peptides bind to hydrophobic grooves of these anti-apoptotic proteins and disrupt complexes formed between pro-apoptotic and anti-apoptotic Bcl-2 family proteins [24]. A number of Bcl-2 family peptides have been described in Table 1, some of them are through in vitro and in vivo studies, and some have advanced into clinical trials.

ABT-737

ABT-737, as a novel anti-cancer drug, is a small molecule of BH3-mimetic peptides [24, 25]. Apoptosis induced by ABT-737 is related to dissociation of pro-apoptotic and anti-apoptotic Bcl-2 family members, disruption of the complex of Bax and Bcl-2 which triggers conformational alteration of Bax, Cyt c release from the mitochondria, and the activation of caspases. Recent studies revealed that Bcl-2 is a better ABT-737 target than Bcl-xL and Bcl-w. Furthermore, ABT-737 markedly increased the response to radiation as well as multiple chemotherapy agents in vitro, and showed good activity as a single agent in small cell lung cancer xenograft models. According to in vitro experiments, ABT-737 has monotherapy toxicity to leukemia and lymphoma, and also is able to induce apoptosis in multiple myeloma, glioma, and

Table 1 Bcl-2 family anti-cancer peptides

Peptides	Study design	Results	Refs.
ABT-737	Preclinical (AML, multiple myeloma, lymphoma, CLL, ALL, and SCLC, cervical cancer, and breast cancer)	Programmed cell death of malignant cells effectively kills AML blast, progenitor, and stem cells without affecting normal hematopoietic cells	[31–34, 79]
ABT-263 (Navitoclax)	Phase I clinical (CLL, lymphomas)	Similar biological properties with ABT-737 with a longer oral half-life enhanced the activity of other chemotherapy agents in preclinical studies	[39, 40]
ABT-199	Preclinical (CLL)	Selective pharmacological inhibition of Bcl-2 shows promise for the treatment of Bcl-2-dependent hematological cancers	[26]
ATAP (amphipathic tail-anchoring peptide)	In vitro	Induce caspase-dependent apoptosis, induce MOMP without the participation of Bax and Bak	[80]
Ant-BH3		Targeted Bcl-x_L and lead to MOMP	[18]
p15tBID	In vitro and in vivo	Initiate BAK oligomerization and Cyt c release and finally apoptosis	[81]
Smac peptide	In vitro and in vivo (various types of malignant tumor cells)	Enhanced effects of chemo-therapeutic agents promote cell death by preventing the IAP inhibition of caspase	[82]
Obatoclax (GX15-070)	Phase I/II clinical trials (Leukemia, lymphoma, multiple myeloma, and non-SCLC)	Added to carboplatin/etoposide chemotherapy as initial treatment for extensive-stage small-cell lung cancer	[43, 44]
TLSGA-FELSRDK (TLS)	In vivo (SKOV3 ovarian cancer)	Induces early-stage apoptosis	[45]

See text for abbreviation definitions

small cell lung cancer (SCLC) cell lines at higher concentrations [26–29]. The anti-tumor activities of ABT-737 have been characterized in several animal models. Other studies have shown preclinical activity of ABT-737 as a single agent or in combination with various cytotoxic agents against acute myeloid leukemia (AML), multiple myeloma, lymphoma, chronic lymphocytic leukemia (CLL), and SCLC. Combining ABT-737 with the second agent such as cyclin-dependent kinase inhibitor (flavopiridol), arsenic trioxide, or fenretinide has achieved synergy via inactivation of Mcl-1, paving the way for future combination chemotherapy strategies targeting the Bcl-2 family [30–34].

ABT-263

Christin et al. (2008) modified a version of ABT-737 (ABT-263, Navitoclax), as a second generation to make the drug orally available (ABT-263 shares similar biological properties with ABT-737). ABT-263 is a small molecule inhibitor of Bcl-2 and Bad-like BH3 mimetic that binds to serum proteins resulting in a longer oral half-life [35, 36]. ABT-263 possesses high affinity for Bcl-xL, Bcl-2, and Bcl-w, but not for Mcl-1. The oral bioavailability of ABT-263 in preclinical animal models is 20 to 50 %, depending on formulation. ABT-263 disrupts Bcl-2/Bcl-xL interactions with pro-death proteins (e.g., Bim), leading to the initiation of apoptosis within 2 h post-treatment. Disruption of this complex and release of Bim either activate Bax and/or Bak directly or antagonize anti-apoptotic proteins such as Mcl-1 to release activated Bax and Bak, and induce cell death [36]. ABT-263 can cause complete regression of the tumors in xenograft models of SCLC and acute lymphoblastic leukemia (ALL) cancer cell [37, 38]. It enhanced the activity of other chemotherapy agents in preclinical studies of B cell lymphoma and multiple myeloma. ABT-263 is a non-myelo-suppressive factor and has shown activity as a single agent against refractory or relapsed lymphoid malignancies with minimal systemic toxicities. The fact that these BH3 mimetics cannot bind to MCL-1 is one of the main causes of resistance particularly when cancer cells overexpress MCL-1 [39]. ABT-263 is now in phase I/II clinical trial of various cancer types [40].

ABT-199

Treatment with ABT-263 (Navitoclax) causes on-target, dose-limiting thrombocytopenia because platelets are dependent on the anti-apoptotic protein BCL-XL for their survival. In this regard, Souers et al. (2013) developed ABT-199, a modified BH3-mimetic derivative of ABT-263 which maintains the specificity for Bcl-2, but lacks affinity for Bcl-xl [41]. This compound inhibits the growth of Bcl-2-dependent tumors in vivo and spares human platelets. A single dose of ABT-199 resulted in tumor lysis in refractory CLL which shows promise for treatment of Bcl-2-dependent hematological cancers. Pan et al. (2013) evaluated the anti-cancer effects of ABT-199 on AML, and compared its efficacy with ABT-737/navitoclax, drugs that have both shown activity in

ex vivo treatment of AML cell lines and AML primary patient samples in clinical trials [26, 42].

Obatoclax

Obatoclax, also known as GX15-070, is a cell permeable hydrophobic small molecule BH3 mimetic and an inhibitor of pro-survival Bcl-2 members, including MCL-1 and Bcl-xL. In fact, obatoclax occupies a hydrophobic pocket within the BH3 binding groove of a broad spectrum of Bcl-2 family members and directly induces apoptosis by oligomerization of Bak and release of Cyt c. It potently interferes with the direct interaction between Mcl-1 and Bak. Obatoclax is currently undergoing evaluation in phase I/II clinical trials for patients with hematologic malignancies and can overcome the resistance to apoptosis conferred by MCL-1. It can be used alone or in combination with other anti-cancer drugs, for treating a variety of hematological malignancies such as leukemia, lymphoma, and solid tumor malignancies [43, 44].

TLSGA-FELSRDK

TLSGA-FELSRDK (TLS) was identified by Chuying Ma's group to enhance the efficiency of ovarian cancer treatment [45]. This peptide is a targeting ligand that can specifically trigger cellular uptake of chemotherapeutics. Cell surface proteins and two C-terminal basic residues (R/K residue) of TLS are required for effective cell penetration. This peptide improves chemosensitivity in ovarian carcinoma cell line (SKOV3) by the synergistic anti-proliferative effects with doxorubicin. TLS can modulate abnormal pathways in cancer and target molecules playing a direct role in apoptotic pathways such as Bcl-2 family [46]. So, it induces early-stage apoptosis, which might be related to the down-regulation of Bcl-2. Also, TLS can selectively penetrate into ovarian cancer cells, which makes it an ideal candidate for the development of peptide-based therapy in ovarian cancer. Taken together, TLS, acting as a combination of a targeting ligand and a therapeutic agent, is a candidate for the development of peptide-based therapies in ovarian cancer [47].

ROS pathway in MTPs for cancer therapy

The mitochondrial electron transport chain (ETC) is the primary producer of ROS in most of the eukaryotic cells located in IMM (Fig. 1). Mitochondrial ETC generates primary ROS at two complexes I and III. HO^{2-} and O^{2-} radicals are produced from ROS in a number of cellular reactions and by different enzymes such as lipo-oxygenase, xanthineoxidase, superoxide dismutase, and peroxidase [48, 49]. The rate of mitochondrial ROS production can be altered by the several physiological and pathological conditions. Elevated ROS associated with mitochondrial dysfunction causes both necrotic and apoptotic cell death. It has been suggested that ROS play a key role in promoting Cyt c release from the mitochondria [50–52], which triggers activation of the caspase cascade in the cytoplasm that ultimately leads to apoptosis [53, 54]. As mentioned above, Cyt c is normally bound to the IMM via cardiolipin [55], and its release from the mitochondria is a critical step in apoptotic pathway initiation that can be achieved through mPTP (Ca^{2+} dependent) followed by mitochondrial swelling and rupture of the outer membrane or by the BCL-2 family pore formation (Ca^{2+} independent). Inhibitors of ETC tend to increase ROS production leading to the activation of some transcription factors including HIF-1 and NF-Kb, and expression of some proteins that induce cell death. Increased ROS also makes damage to mitochondrial DNA and proteins, lipids peroxidation, and ultimately leads to apoptosis. Also, increased production of ROS followed by a decreased membrane potential can be the starting point of apoptosis. On the other hand, ROS causes cell signaling and cyto-protection, so it can play a significant role in cell survival. ROS modulating peptides have been described in Table 2. Among these peptides, GO-203 has advanced into clinical trials [56].

GO-203

GO-203 is a cell-penetrating peptide in phase I clinical trials and contains a poly-Arg cell transduction domain linked to the CQCRRKN sequence ([R]-CQCRRKN; all D-amino acids) that binds directly to the MUC1 (a polymorphic epithelial mucin protein) C-terminal domain (MUC1-C). GO-203 inhibited abnormal expression of MUC1-C in multiple types of cancers such as chronic myeloid leukemia (CML) and is associated with an increase in ROS. CML is caused by the reciprocal translocation and expression of the Bcr-Abl fusion protein. MUC1-C functions as a cell surface receptor that accumulates in the cytoplasm and is targeted to the nucleus. MUC1 is expressed in CML blasts, stabilizes the oncogenic Bcr-Abl protein, and blocks CML cell differentiation. Blocking MUC1-C with GO-203 peptide leads to disrupt redox balance in CML cells and an increase in ROS, thereby decreases Bcr-Abl and β-catenin expression, and can also induce terminal myeloid cell differentiation [57].

TAT-FHIT

FHIT is a protein that can generate ROS. Transfection or transduction of FHIT gene leads to apoptosis induction in different cancers. FHIT makes complex with ferrodoxin

Table 2 Anti-cancer peptides targeting ROS, Ca^{2+}, and VDAC

Pathways	Peptides	Study design	Results	Refs.
ROS	GO-203	Phase I clinical GO-203-2c given intravenously daily×21 Repeated every 28 days in patients with advanced solid tumors: lymphomas	Disruption of redox balance and an increase in ROS targeted C-terminal protein of MUC1 and disrupts MUC1-C ROS suppression	[57, 83, 84]
	TAT-FHIT	In vitro HCC cells	Leads to apoptosis by generating ROS and stimulating calcium uptake at the mitochondria	[58, 59]
VDAC1-based peptide	Antp-LP4	In vitro (several cancer cell lines)	Decreases mitochondrial membrane potential and cellular ATP levels, decrease the anti-apoptotic effects of HK-I, Bcl-2 or Bcl-xL, Cyt c release	[85, 86]
	Tf-LP4	In vitro (CLL patient-derived PBMCs and in MEC-1 cells)	Induced cell death	[85, 86]
	TAT-LP4	In vitro (CLL PBMCs and MEC-1 cells)	Less effective in inducing cell death	[85, 86]
	TAT-HK	Ex vivo	Mitochondrial membrane depolarization, increased cell death	[87, 88]
Calcium	MTD-peptides	Treated cells, showed cellular swelling, and induce necrosis rather than apoptosis	Penetrate any type of cytoplasmic membrane, cause Ca^{2+} leak from mitochondria by opening of mPTP	[66]

See text for abbreviation definitions

reductase and applies its pro-apoptotic effect by generating ROS and stimulating calcium uptake at the mitochondria. Yu et al. (2012) used a HIV-Tat-derived protein transduction domain fusion with FHIT (TAT–FHIT) for induction of apoptosis in hepatocellular carcinoma (HCC) cells. They demonstrated that TAT-FHIT strongly inhibits growth and induce apoptosis in HCC cells in vitro [58, 59].

Ca^{2+} pathway in MTPs for cancer therapy

Ca^{2+} influences both cell survival and death. It has been known that Ca^{2+} signaling has contributed in both the initiation and continuation of cell death. While severe Ca^{2+} dysregulation can promote cell death through necrosis, controlled increase of intracellular Ca^{2+} promotes cell death through apoptosis (Fig. 1). In addition, other types of cell death, outstandingly anoikis and autophagy, are regulated by Ca^{2+} transients [60, 61]. It has been proved that Ca^{2+} is involved in regulating release of pro-apoptotic proteins. Mitochondrial Ca^{2+} overload triggers the loss of mitochondrial membrane potential and release of caspase cofactors, and also activate a mechanism of MOMP that involves the opening of mPTP. The mPTP may act as a mitochondrial Ca^{2+} release channel and plays an important role in apoptosis. So, these processes can lead to releasing of Cyt c and finally cell death. Ca^{2+}-dependent processes can be responsible for the processing of AIF, resulting in its translocation from the mitochondria into cytosol [62, 63]. Ca^{2+} is taken up into the mitochondria via a uniporter in IMM. Despite the low affinity of mitochondrial Ca^{2+} transporters under the normal physiological conditions, large Ca^{2+} fluxes occur across the mitochondrial membranes under the pathological conditions, and much larger amounts of Ca^{2+} can accumulate in the mitochondria by adjoining of the mitochondria to endoplasmic reticulum (ER) and formation of Ca^{2+} "hotspots" at the entrance of ER channels [64, 65]. Indeed, transient expression of VDAC increases mitochondrial matrix Ca^{2+} concentration by the fast diffusion of Ca^{2+} from ER through IMM. Also, the mitochondrial Ca^{2+} is another powerful signal for ROS production [60].

MTD peptides

Evidences indicate that mitochondrial-targeting domain (MTD) peptide increase the calcium concentration in the cytosol. MTD cause Ca^{2+} leak from mitochondria by opening of mPTP. MTD peptides have derived from Noxa (a pro apoptotic member of BCL-2 family) which has two functional domains including BH3 and MTD. BH3 domain inhibits anti-apoptotic proteins such as Mcl-1 and A1/Bfl-1 that leads to cell death. Furthermore, cells treated with MTD peptide showed cellular swelling on membrane and cytoplasmic distribution that indicates these peptides induce necrosis rather than apoptosis. This peptide can penetrate any type of cytoplasmic membrane, so it was fused to synthetic tumor vasculature targeting motifs and tumor-homing MTD peptides to enhance its selectivity [66].

TAT-IDPS

Disrupting inositol 1,4,5-trisphosphate receptor (IP3R) complexes with Bcl-2 by use of a cell-permeable peptide (stabilized TAT-fused IP3R-derived peptide (TAT-IDPS)) that selectively targets the BH4 domain of Bcl-2 but not that of Bcl-Xl potentiated pro-apoptotic Ca^{2+} signaling in CLL cells. However, the exact molecular reason for sensitivity of cancer cells to disrupting IP3R/Bcl-2 complexes are not yet been elucidated. In addition, certain chronically activated B cell lymphoma cells showed addiction to high Bcl-2 levels for their survival not only to balance out pro-apoptotic Bcl-2-family members but also to suppress IP3R hyperactivity. Above all, cancer cells expressing high levels of IP3R2 are dependent to formation of IP3R/Bcl-2 complex, and disruption of these complexes by peptide tools results in pro-apoptotic Ca^{2+} signaling and leads cell to death [67].

VDAC pathway MTPs for cancer therapy

At the outer mitochondrial membrane, the multifunctional VDAC is a family of pore-forming proteins which serves as a key regulator of mitochondrial metabolite flux and apoptosis. VDAC mediates the transport of anions, cations, ATP, Ca^{2+}, and metabolites (Fig. 1). Thus, VDAC plays an important role in regulating the relationship between mitochondrion and cytosol [18]. Evidences indicate that VDAC activity can be modulated by a variety of proteins, notably members of the Bcl-2 family. Both anti- and pro-apoptotic proteins interact with VDAC to regulate mitochondria-mediated apoptosis. VDAC interacts with the Bcl-2 family proteins which can modulate its activities. Bcl-2 or Bcl-XL overexpression was reported to promote an open conformation of VDAC. In contrast, it was also found that Bcl-2 induced VDAC closure, whereas Bax enhanced VDAC opening [68, 69]. Another study reported that tBid induced the closure of VDAC, whereas Bax had no effect [70]. Obviously, the effect of the Bcl-2 proteins on changing the VDAC pore size is controversial, and more investigations are required to specify the role of the Bcl-2 family proteins in this case [71]. In contrast, in cancer, VDAC is usually inhibited by the anti-apoptotic Bcl-2 proteins (e.g., Bcl-2 and Bcl-xL) and HK I and HK II. They have three isoform in mammals including VDAC1, VDAC2, and VDAC3. It has been proposed that VDAC serves as a component of the apoptosis machinery participating in the release of Cyt c. VDAC1 also has influence in the control of ROS production [18, 71].

Cell-penetrating VDAC1-based peptides activate the mitochondria-mediated apoptotic pathway, followed by loss of potential of mitochondrial membrane, release of mitochondrial Cyt c, membrane blebbing, and condensation of nuclei, DNA fragmentation, decreased cellular ATP levels, and detachment of HK. Numbers of cell-penetrating VDAC1-based peptides were designed toward some cancer models that are listed in Table 2.

Other peptides with targeted mitochondria for cancer therapy

KillerFLIP

Pennarun et al. (2013) have identified a 15-mer peptide READ FFWSLCTADMS that was derived from the C-terminal domain of the long isoform of c-FLICE-like inhibitory protein (c-FLIPL), an anti-apoptotic protein and named killerFLIP. There are three spliced variants of c-FLIP (Table 3). C-$FLIP_L$ is one of them and found at the protein level with its short isoform (c-$FLIP_S$) and c-$FLIP_R$. Structurally, c-$FLIP_L$ is similar to procaspase 8 and has two death effector and a caspase-like domain. The biochemical properties of killerFLIP are similar to those of cationic lytic peptides, which participate in defense against pathogens, and have also established anticancer effects. The major mechanism of these peptides is plasma membrane permeabilization of cancer cells independent of apoptosis, but great changes were also made in mitochondrial membrane and subsequent activation of the intrinsic pathway of apoptosis. When killerFLIP are introduced into cells via a TAT cell delivery system, they showed a significant cytotoxicity in different cancer cell lines and also inhibition of tumor growth in animal models. So, results demonstrate that KillerFLIP-induced cell death is mostly due to direct plasma membrane permeabilization, not activation of apoptosis or necroptosis [72].

Host defense peptides

The role of host defence peptides, also termed antimicrobial peptides, has been determined in the killing of prokaryotic and eukaryotic cells. These peptides are mostly cationic and have an amphipathic structure. The host defense membrane-active peptides can be divided into two major groups. Peptides which act against normal mammalian cells are toxic to bacteria and both mammalian cancer and non-cancer cells. In fact, they can trigger necrosis and apoptosis via the cell membrane lytic effect and mitochondrial membrane disruption and then release of Cyt c [73].

LL-37/hCAP-18

Cathelicidin, found in most mammalian species, are a family of bacteriocidal polypeptides secreted by macrophages and polymorphonuclear leukocytes. They include a highly conserved N-terminal domain, cathelin, and a variable C-terminal peptide, which is released proteolytically upon

Table 3 Other peptides which can target mitochondria for cancer therapy

Peptides	Study design	Results	Refs.
R7-kla	In vitro and in vivo (HT1080 human fibrosarcoma cell line)	Target mitochondrial membrane and showed better cytotoxicity compared with kla	[78]
RGD-4C-GG-D (KLAKLAK)2	In vivo	Targeted mitochondrial membrane and lead to MOMP, reduced the growth of breast carcinoma in nude mice, induced apoptosis of endothelial cells	[89]
BHAP (bifunctional: contains an AHNP anti-HER2 motif and [KLAKLAK])	In vitro and in vivo (selective internalization into HER-2-overexpressing human breast cancer cells)	Apoptosis-inducing peptide simultaneously perturb the growth factor receptor signaling and the mitochondrial activity	[18]
killerFLIP	In vitro and in vivo (cancer cell lines including leukemia, prostate and colon cancer but not in normal epithelial and endothelial cells well tolerated in mice)	First plasma membrane permeability and then mitochondrial membrane, and activation of apoptotic intrinsic pathway triggers cell death	[72]
LL-37/hCAP-18	In vivo (human oral squamous cell carcinoma SAS-H1 cells colon cancer)	Induce apoptosis	[74–77]
Buforin II and Buforin IIb	In vivo (broad spectrum of cancer cells)	Oncolytic activity with caspase-9 activation and cytochrome c release	[90]
Hunter-killer peptides (HKPs)	In vivo (lung, prostate, and breast carcinoma human xenografts)	Kill malignant blood vessels without harming the normal vasculature	[76]

See text for abbreviation definitions

demand. The single human member of this family is an 18-kDa cationic antimicrobial protein (hCAP18) which is mainly produced by leucocytes, epithelial, and mucosal cells. HCAP18 is the pro-protein, and LL-37 is its mature processed form which has oncolytic and tumorigenesis activity. A 27-mer peptide of the C-terminal domain of hCAP-18 which corresponds to amino acid residues 6–32 of LL-37 was found to induce apoptosis in both drug-sensitive and drug-resistant variants of human oral squamous cell carcinoma. LL-37 activates a p53-mediated pathway, caspase-independent apoptotic cascade that contributes to suppression of colon cancer. Exposure of colon cancer cells to LL-37 induces phosphatidylserine externalization and DNA fragmentation in a manner independent of caspase activation. Apoptogenic function was interceded by nuclear translocation of the pro-apoptotic factors, AIF and endonuclease G, through p53-dependent up-regulation of Bax and Bak and down-regulation of Bcl-2 (Table 3). LL-37 activates a GPCR-p53-Bax/Bak/Bcl-2 signaling cascade [74, 75]. The combination of CpG oligodeoxynucleotides with LL-37 peptide generated significantly better anti-tumor effects and enhanced survival in murine ovarian tumor-bearing mice. Unfortunately, LL-37 and its peptide fragments appear to be cytotoxic to both human peripheral blood leucocytes and untransformed endothelial cells with results in hemolysis [76, 77].

R7-kla

R7-kla is a common cytotoxic peptide (r7-kla) which derived from a natural antimicrobial peptide. R7-kla was synthesized by incorporating a mitochondrial membrane-disrupting peptide, kla (klaklakklaklak), with a cell-penetrating domain, r7 (rrrrrrr), to increase cellular uptake. The mechanism of peptide entry into cells involves peptide binding to the heparan sulfate on cell surface and subsequently endocytosis. R7-kla is an apoptosis inducer which causes mitochondrial membrane damage, triggering apoptosis and subsequently induced cell death in both in vitro and in vivo. Killing effects of r7-kla to different tumors were almost the same (Table 3). So, it can be used as an anti-tumor agent particularly when joint with the proper delivery systems. These cationic peptide sequences do not efficiently cross the eukaryotic plasma membranes, and so it usually has low toxicity, unless it is coupled to selective targeting domains allowing cell internalization. But they are able to interact with negatively charged prokaryotic plasma membrane and disrupt it. Therefore inside the cell, KLA can disrupt the mitochondrial membrane, and following the release of Cyt c, apoptosis is triggered. The anti-tumoral effect of KLA is based on physical disruption of the membrane bilayer and is therefore expected to be less susceptible to multiple drug resistance. With the D-configuration of KLA (kla) attached to a hepta-arginine cell-penetrating domain (r7), r7-kla showed better cytotoxicity against the HT1080 human fibro-sarcoma cell line compared with the clinically used anti-tumor agents evaluated in the current study [78].

Conclusion and future studies

One of the prominent characteristics of cancer cells is improved resistance to mitochondrial apoptosis. Targeting mitochondrial proteins and membranes is an approach aiming at inducing apoptosis or cell death. Regarding this, many (pre)

clinical studies have been carried out and encouraging results have been obtained. Although there is a lot of information about the function and structure of mitochondria, but the knowledge in the field of drugs targeting to this organelle is still not enough, and there is a need for improvement in efficiency of drug delivery systems to the mitochondria. This review focused on the most important mitochondrial pathways which have key roles in regulation of cell death in response to various stimuli. We collected and categorized different therapeutic peptides which can target these pathways as a novel tool in cancer therapy. Synthesis of new therapeutic peptides and drug discovery in the area of mitochondrial targeting can provide novel agents for treatment of cancers. Several challenges such as low bioavailability or difference of mitochondrial function among tissues needs to be recognized and overcame to achieve full therapeutic potential of these peptides. It is clear that new therapeutic approaches such as drug delivery systems, by considering all aspects of mitochondrial function in diseases, are essential for the future development of mitochondrial targeting peptides.

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Conflicts of interest None

References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin. 2013;63:11–30.
2. Alizadeh AM, Shiri S, Farsinejad S. Metastasis review: from bench to bedside. Tumour Biol. 2014;35(9):8483–523.
3. Imanieh MH, Bagheri F, Alizadeh AM, Ashkani-Esfahani S. Oxytocin has therapeutic effects on cancer, a hypothesis. Eur J Pharmacol. 2014;741:112–23.
4. Urruticoechea A, Alemany R, Balart J, Villanueva A, Vinals F, Capella G. Recent advances in cancer therapy: an overview. Curr Pharm Des. 2010;16:3–10.
5. Wu H-C, Chang D-K, Huang C-T. Targeted therapy for cancer. J Cancer Mol. 2006;2:57–66.
6. Neuzil J, Dyason JC, Freeman R, Dong L-F, Prochazka L, Wang X-F, et al. Mitocans as anti-cancer agents targeting mitochondria: lessons from studies with vitamin E analogues, inhibitors of complex II. J Bioenerg Biomembr. 2007;39:65–72.
7. J. Thundimadathil. Cancer treatment using peptides: current therapies and future prospects. J amino acids. 2012;2012.
8. Sun L. Peptide-based drug development. Mod Chem Appl. 2013;1:e103.
9. Aina OH, Sroka TC, Chen ML, Lam KS. Therapeutic cancer targeting peptides. Pept Sci. 2002;66:184–99.
10. Li ZJ, Cho CH. Peptides as targeting probes against tumor vasculature for diagnosis and drug delivery. J Transl Med. 2012;10:S1.
11. Armstrong JS. Mitochondria: a target for cancer therapy. Br J Pharmacol. 2006;147:239–48.
12. Whelan RS, Konstantinidis K, Wei A-C, Chen Y, Reyna DE, Jha S, et al. Bax regulates primary necrosis through mitochondrial dynamics. Proc Natl Acad Sci. 2012;109:6566–71.
13. Szewczyk A, Wojtczak L. Mitochondria as a pharmacological target. Pharmacol Rev. 2002;54:101–27.
14. Smith RA, Hartley RC, Cocheme HM, Murphy MP. Mitochondrial pharmacology. Trends Pharmacol Sci. 2012;33:341–52.
15. Frezza C, Gottlieb E. Mitochondria in cancer: not just innocent bystanders. in Seminars in Cancer Biol. 2009;4-11.
16. Lowe SW, Lin AW. Apoptosis in cancer. Carcinogenesis. 2000;21:485–95.
17. Martel C, Huynh LH, Garnier A, Ventura-Clapier R, Brenner C. Inhibition of the mitochondrial permeability transition for cytoprotection: direct versus indirect mechanisms. Biochem Res Int. 2012;2012:213403.
18. Constance JE, Lim CS. Targeting malignant mitochondria with therapeutic peptides. Ther Deliv. 2012;3:961–79.
19. Martinou J-C, Youle RJ. Mitochondria in apoptosis: Bcl-2 family members and mitochondrial dynamics. Dev Cell. 2011;21:92–101.
20. Jacotot E, Costantini P, Laboureau E, Zamzami N, Susin SA, Kroemer G. Mitochondrial membrane permeabilization during the apoptotic process. Ann N Y Acad Sci. 1999;887:18–30.
21. Alizadeh AM, Faghihi M, Khori V, Sohanaki H, Pourkhalili K, Mohammadghasemi F, et al. Oxytocin protects cardiomyocytes from apoptosis induced by ischemia–reperfusion in rat heart: role of mitochondrial ATP-dependent potassium channel and permeability transition pore. Peptides. 2012;36:71–7.
22. Adams J, Cory S. The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene. 2007;26:1324–37.
23. Hosseini J, Mahmoodi M, Jalili A, Fakhari S, Hosseini-zijoud S-M, Tahamtan M, et al. Aloe-emodin Induces apoptosis through the up-regulation of Fas in the human breast cancer cell line MCF-7. Life Sci J. 2014;11(2s):47–53.
24. Jabbour AM, Heraud J, Daunt C, Kaufmann T, Sandow J, O'Reilly L, et al. Puma indirectly activates Bax to cause apoptosis in the absence of Bid or Bim. Cell Death Differ. 2008;16:555–63.
25. Ren D, Tu H-C, Kim H, Wang GX, Bean GR, Takeuchi O, et al. BID, BIM, and PUMA are essential for activation of the BAX-and BAK-dependent cell death program. Science. 2010;330:1390–3.
26. Pan R, Hogdal LJ, Benito JM, Bucci D, Han L, Borthakur G, et al. Selective BCL-2 inhibition by ABT-199 causes on-target cell death in acute myeloid leukemia. Cancer Discov. 2014;4:362–75.
27. Chonghaile TN, Letai A. Mimicking the BH3 domain to kill cancer cells. Oncogene. 2008;27:S149–57.
28. Rooswinkel R, Van De Kooij B, Verheij M, Borst J. Bcl-2 is a better ABT-737 target than Bcl-xL or Bcl-w and only Noxa overcomes resistance mediated by Mcl-1, Bfl-1, or Bcl-B. Cell Death Dis. 2012;3:e366.
29. Kang MH, Reynolds CP. Bcl-2 inhibitors: targeting mitochondrial apoptotic pathways in cancer therapy. Clin Cancer Res. 2009;15:1126–32.
30. Kang MH, Wan Z, Kang YH, Sposto R, Reynolds CP. Mechanism of synergy of N-(4-hydroxyphenyl)retinamide and ABT-737 in acute lymphoblastic leukemia cell lines: Mcl-1 inactivation. J Natl Cancer Inst. 2008;100:580–95.
31. Chauhan D, Velankar M, Brahmandam M, Hideshima T, Podar K, Richardson P, et al. A novel Bcl-2/Bcl-XL/Bcl-w inhibitor ABT-737 as therapy in multiple myeloma. Oncogene. 2006;26:2374–80.
32. Oltersdorf T, Elmore SW, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA, et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature. 2005;435:677–81.
33. Tagscherer KE, Fassl A, Campos B, Farhadi M, Kraemer A, Böck B, et al. Apoptosis-based treatment of glioblastomas with ABT-737, a novel small molecule inhibitor of Bcl-2 family proteins. Oncogene. 2008;27:6646–56.
34. Trudel S, Stewart AK, Li Z, Shu Y, Liang S-B, Trieu Y, et al. The Bcl-2 family protein inhibitor, ABT-737, has substantial antimyeloma activity and shows synergistic effect with dexamethasone and melphalan. Clin Cancer Res. 2007;13:621–9.

35. Ackler S, Mitten MJ, Foster K, Oleksijew A, Refici M, Tahir SK, et al. The Bcl-2 inhibitor ABT-263 enhances the response of multiple chemotherapeutic regimens in hematologic tumors in vivo. Cancer Chemother Pharmacol. 2010;66:869–80.
36. Rudin CM, Hann CL, Garon EB, De Oliveira MR, Bonomi PD, Camidge DR, et al. Phase II study of single-agent navitoclax (ABT-263) and biomarker correlates in patients with relapsed small cell lung cancer. Clin Cancer Res. 2012;18:3163–9.
37. Wilson WH, O'Connor OA, Czuczman MS, LaCasce AS, Gerecitano JF, Leonard JP, et al. Navitoclax, a targeted high-affinity inhibitor of BCL-2, in lymphoid malignancies: a phase 1 dose-escalation study of safety, pharmacokinetics, pharmacodynamics, and antitumour activity. Lancet Oncol. 2010;11:1149–59.
38. Vogler M, Dinsdale D, Dyer MJ, Cohen GM. Bcl-2 inhibitors: small molecules with a big impact on cancer therapy. Cell Death Differ. 2008;16:360–7.
39. Tse C, Shoemaker AR, Adickes J, Anderson MG, Chen J, Jin S, et al. ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. Cancer Res. 2008;68:3421–8.
40. Shoemaker AR, Mitten MJ, Adickes J, Ackler S, Refici M, Ferguson D, et al. Activity of the Bcl-2 family inhibitor ABT-263 in a panel of small cell lung cancer xenograft models. Clin Cancer Res. 2008;14:3268–77.
41. Souers AJ, Leverson JD, Boghaert ER, Ackler SL, Catron ND, Chen J, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nat Med. 2013;19:202–8.
42. Vaillant F, Merino D, Lee L, Breslin K, Pal B, Ritchie ME, et al. Targeting BCL-2 with the BH3 mimetic ABT-199 in estrogen receptor-positive breast cancer. Cancer Cell. 2013;24:120–9.
43. Paik PK, Rudin CM, Brown A, Rizvi NA, Takebe N, Travis W, et al. A phase I study of obatoclax mesylate, a Bcl-2 antagonist, plus topotecan in solid tumor malignancies. Cancer Chemother Pharmacol. 2010;66:1079–85.
44. Nguyen M, Marcellus RC, Roulston A, Watson M, Serfass L, Madiraju SM, et al. Small molecule obatoclax (GX15-070) antagonizes MCL-1 and overcomes MCL-1-mediated resistance to apoptosis. Proc Natl Acad Sci. 2007;104:19512–7.
45. Ma C, Yin G, You F, Wei Y, Huang Z, Chen X, et al. A specific cell-penetrating peptide induces apoptosis in SKOV3 cells by down-regulation of Bcl-2. Biotechnol Lett. 2013;35:1791–7.
46. Manion MK, Hockenbery DM. Targeting BCL-2-related proteins in cancer therapy. Cancer Biol Ther. 2003;2:S105–14.
47. Laakkonen P, Akerman ME, Biliran H, Yang M, Ferrer F, Karpanen T, et al. Antitumor activity of a homing peptide that targets tumor lymphatics and tumor cells. Proc Natl Acad Sci U S A. 2004;101:9381–6.
48. Kardeh S, Ashkani-Esfahani S, Alizadeh AM. Paradoxical action of reactive oxygen species in creation and therapy of cancer. Eur J Pharmacol. 2014;735:150–68.
49. Blokhina O, Virolainen E, Fagerstedt KV. Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot. 2003;91:179–94.
50. Galindo MF, Jordan J, Gonzalez-Garcia C, Cena V. Reactive oxygen species induce swelling and cytochrome c release but not transmembrane depolarization in isolated rat brain mitochondria. Br J Pharmacol. 2003;139:797–804.
51. Faghihi M, Alizadeh AM, Khori V, Latifpour M, Khodayari S. The role of nitric oxide, reactive oxygen species, and protein kinase C in oxytocin-induced cardioprotection in ischemic rat heart. Peptides. 2012;37:314–9.
52. Petrosillo G, Ruggiero FM, Paradies G. Role of reactive oxygen species and cardiolipin in the release of cytochrome c from mitochondria. FASEB J. 2003;17:2202–8.
53. Liu X, Kim CN, Yang J, Jemmerson R, Wang X. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. Cell. 1996;86:147–57.
54. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, et al. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell. 1997;91:479–89.
55. Nicholls P. Cytochrome c binding to enzymes and membranes. Biochim Biophys Acta. 1974;346:261–310.
56. Zhao K, Zhao G-M, Wu D, Soong Y, Birk AV, Schiller PW, et al. Cell-permeable peptide antioxidants targeted to inner mitochondrial membrane inhibit mitochondrial swelling, oxidative cell death, and reperfusion injury. J Biol Chem. 2004;279:34682–90.
57. Yin L, Kufe D. MUC1-C oncoprotein blocks terminal differentiation of chronic myelogenous leukemia cells by a ROS-mediated mechanism. Genes Cancer. 2011;2:56–64.
58. Wierzbicki PM, Adrych K, Kartanowicz D, Dobrowolski S, Stanislawowski M, Chybicki J, et al. Fragile histidine triad (FHIT) gene is overexpressed in colorectal cancer. J Physiol Pharmacol. 2009;4:63–70.
59. Yu GR, Qin WW, Li JP, Hua W, Meng YL, Chen R, et al. HIV-TAT-fused FHIT protein functions as a potential pro-apoptotic molecule in hepatocellular carcinoma cells. Biosci Rep. 2012;32:271–9.
60. Zhivotovsky B, Orrenius S. Calcium and cell death mechanisms: a perspective from the cell death community. Cell Calcium. 2011;50:211–21.
61. Orrenius S, Nicotera P, Zhivotovsky B. Cell death mechanisms and their implications in toxicology. Toxicol Sci. 2011;119:3–19.
62. Norberg E, Gogvadze V, Ott M, Horn M, Uhlen P, Orrenius S, et al. An increase in intracellular Ca2+ is required for the activation of mitochondrial calpain to release AIF during cell death. Cell Death Differ. 2008;15:1857–64.
63. Carafoli E. Calcium signaling: a tale for all seasons. Proc Natl Acad Sci. 2002;99:1115–22.
64. Thor H, Hartzell P, Orrenius S. Potentiation of oxidative cell injury in hepatocytes which have accumulated Ca^{2+}. J Biol Chem. 1984;259:6612–5.
65. Hajnoczky G, Robb-Gaspers LD, Seitz MB, Thomas AP. Decoding of cytosolic calcium oscillations in the mitochondria. Cell. 1995;82:415–24.
66. Seo YW, Woo HN, Piya S, Moon AR, Oh JW, Yun CW, et al. The cell death-inducing activity of the peptide containing Noxa mitochondrial-targeting domain is associated with calcium release. Cancer Res. 2009;69:8356–65.
67. Zhong F, Harr MW, Bultynck G, Monaco G, Parys JB, De Smedt H, et al. Induction of Ca(2)+-driven apoptosis in chronic lymphocytic leukemia cells by peptide-mediated disruption of Bcl-2-IP3 receptor interaction. Blood. 2011;117:2924–34.
68. Brenner C, Cadiou H, Vieira HL, Zamzami N, Marzo I, Xie Z, et al. Bcl-2 and Bax regulate the channel activity of the mitochondrial adenine nucleotide translocator. Oncogene. 2000;19:329–36.
69. Shimizu S, Ide T, Yanagida T, Tsujimoto Y. Electrophysiological study of a novel large pore formed by Bax and the voltage-dependent anion channel that is permeable to cytochrome c. J Biol Chem. 2000;275:12321–5.
70. Rostovtseva TK, Antonsson B, Suzuki M, Youle RJ, Colombini M, Bezrukov SM. Bid, but not Bax, regulates VDAC channels. J Biol Chem. 2004;279:13575–83.
71. Gustafsson AB, Gottlieb RA. Bcl-2 family members and apoptosis, taken to heart. Am J Physiol Cell Physiol. 2007;292:C45–51.
72. Pennarun B, Gaidos G, Bucur O, Tinari A, Rupasinghe C, Jin T, et al. killerFLIP: a novel lytic peptide specifically inducing cancer cell death. Cell Death Dis. 2013;4:e894.
73. Steinstraesser L, Kraneburg U, Jacobsen F, Al-Benna S. Host defense peptides and their antimicrobial-immunomodulatory duality. Immunobiology. 2011;216:322–33.

74. Wu WKK, Sung JJY, To KF, Yu L, Li HT, Li ZJ, et al. The host defense peptide LL-37 activates the tumor-suppressing bone morphogenetic protein signaling via inhibition of proteasome in gastric cancer cells. J Cell Physiol. 2010;223:178–86.
75. Ren SX, Cheng AS, To KF, Tong JH, Li MS, Shen J, et al. Host immune defense peptide LL-37 activates caspase-independent apoptosis and suppresses colon cancer. Cancer Res. 2012;72:6512–23.
76. Chuang C-M, Monie A, Wu A, Mao C-P, Hung C-F. Treatment with LL-37 peptide enhances antitumor effects induced by CpG oligodeoxynucleotides against ovarian cancer. Hum Gene Ther. 2009;20:303–13.
77. Al-Benna S, Shai Y, Jacobsen F, Steinstraesser L. Oncolytic activities of host defense peptides. Int J Mol Sci. 2011;12:8027–51.
78. Law B, Quinti L, Choi Y, Weissleder R, Tung C-H. A mitochondrial targeted fusion peptide exhibits remarkable cytotoxicity. Mol Cancer Ther. 2006;5:1944–9.
79. Zhang L, Ming L, Yu J. BH3 mimetics to improve cancer therapy; mechanisms and examples. Drug Resist Updat. 2007;10:207–17.
80. Ko J-K, Choi K-H, Pan Z, Lin P, Weisleder N, Kim C-W, et al. The tail-anchoring domain of Bfl1 and HCCS1 targets mitochondrial membrane permeability to induce apoptosis. J Cell Sci. 2007;120:2912–23.
81. Letai A, Bassik MC, Walensky LD, Sorcinelli MD, Weiler S, Korsmeyer SJ. Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics. Cancer Cell. 2002;2:183–92.
82. Mizukawa K, Kawamura A, Sasayama T, Tanaka K, Kamei M, Sasaki M, et al. Synthetic Smac peptide enhances the effect of etoposide-induced apoptosis in human glioblastoma cell lines. J Neuro-Oncol. 2006;77:247–55.
83. Yin L, Kosugi M, Kufe D. Inhibition of the MUC1-C oncoprotein induces multiple myeloma cell death by down-regulating TIGAR expression and depleting NADPH. Blood. 2012;119:810–6.
84. Raina D, Kosugi M, Ahmad R, Panchamoorthy G, Rajabi H, Alam M, et al. Dependence on the MUC1-C oncoprotein in non-small cell lung cancer cells. Mol Cancer Ther. 2011;10:806–16.
85. Shoshan-Barmatz V, Abu-Hamad S, Arzoine L, Zaid H. VDAC1 compositions and methods of use thereof for regulating apoptosis. ed: Google Patents, 2014.
86. Prezma T, Shteinfer A, Admoni L, Raviv Z, Sela I, Levi I, et al. VDAC1-based peptides: novel pro-apoptotic agents and potential therapeutics for B-cell chronic lymphocytic leukemia. Cell Death Dis. 2013;4:e809.
87. Chiara F, Castellaro D, Marin O, Petronilli V, Brusilow WS, Juhaszova M, et al. Hexokinase II detachment from mitochondria triggers apoptosis through the permeability transition pore independent of voltage-dependent anion channels. PLoS One. 2008;3:e1852.
88. Smeele KM, Southworth R, Eeerbeek O, Wu R, Koeman A, Ardehali H, et al. Acute dissociation of hexokinase from mitochondria abrogates normal functioning, ischemic tolerance and ischemic preconditioning of the intact beating heart. Circulation. 2010;122:A15296.
89. Smolarczyk R, Cichoń T, Graja K, Hucz J, Sochanik A, Szala S. Antitumor effect of RGD-4C-GG-D (KLAKLAK) 2 peptide in mouse B16 (F10) melanoma model. Acta Biochim Pol. 2005;53:801–5.
90. Lee HS, Park CB, Kim JM, Jang SA, Park IY, Kim MS, et al. Mechanism of anticancer activity of buforin IIb, a histone H2A-derived peptide. Cancer Lett. 2008;271:47–55.