

Apoptosis: Modulators for Anticancer Therapy

Khushbu Verma¹, Ajay Pratap Singh²

¹Assistant Professor, Department of Pharmacology, Sai Meer College of Pharmacy, Farrukhabad, India

²Senior Content Developer, Department of Science, Entrance Systems and Solutions Pvt. Ltd., Delhi, India

Abstract: Apoptosis, the cell's natural mechanism for death, may be a promising target for antitumor therapy. Each of the intrinsic and extrinsic pathways use caspases to hold out programmed cell death through the cleavage of many proteins. In cancer, the apoptotic pathway is usually pent-up through a wide sort of means that together with overexpression of anti-apoptotic proteins and under-expression of pro-apoptotic proteins. Several of those changes cause intrinsic resistance to the foremost common anticancer medical aid, therapy. Promising new antitumor therapies square measure plant-derived compounds that exhibit antitumor activity through activating the apoptotic pathway.

Keywords: Apoptosis

1. Introduction

Apoptosis is a very necessary process of our life cells for the healthy condition of body. Every body cell requires maintained homeostasis balance between cell proliferation and cell death rate for the normal physiological process. Naturally occurrence of functional cell death cycle on the basis of cell ageing (maturity) are called apoptosis. The apoptosis plays a vital role to eliminate or destroy unwanted, unnecessary cells and its highly regulated process. It is characterized due to indigenous protease activated and which induced genetic of controlled auto digestion of the cells.

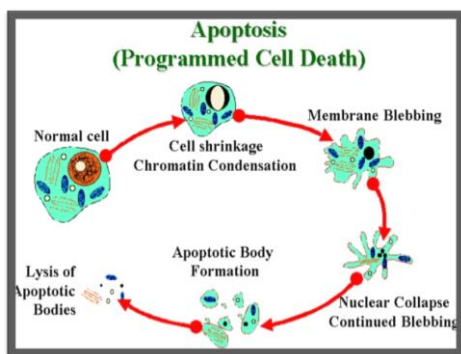


Fig. 1. Apoptosis

The occurrence of apoptotic cell death in continuous cycle for whole life helps to avoiding the formation of cancerous cells. Every second adult human losses approximately 2.5 million red blood cells and these are replaced at a rate of about 2 millions per second. No any biologist identified the exact number of cells, but we can approximate to about 10-50 trillion and about 300 million cells die every minute in our bodies. Due

to lot of study on the cell. Researchers know that the body replaces itself with a largely new set of cells. Some of our most important parts of the body are revamped in every seven years to ten years. Scientists calculate the speed of death in cells. Death travels in unremitting waves through a cell moving at a rate of 30 micrometer (1000 of an inch) every minute, that means for instance, that a nerve cell whose body can reach a size of 100 micrometer, could take as long as 3 minutes and 20 seconds to die.

2. Stages for developing apoptosis

There are four stages involved for the developing of apoptosis. First, the cell receives the signal that evokes the pathway for apoptosis. Second, the cell can still be rescued if it is exposed to survival factors. Third stage, in which the rescue is not possible, and fourth, finally occurs disassembly of the cell into membrane enclosed vesicles.

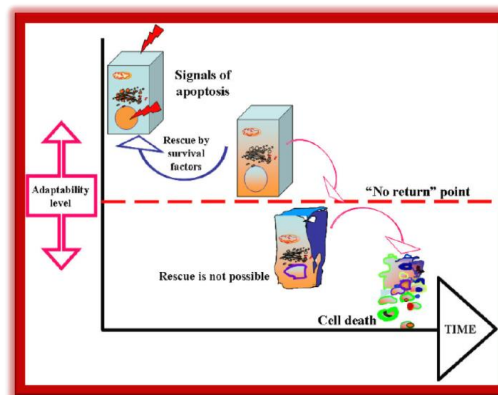


Fig. 2. Stages for developing apoptosis

3. Mechanism/pathway of apoptosis

Apoptosis can be induced through three different pathways:

1. Targeting mitochondria functionality (mitochondrial, cellular or apoptosis intrinsic pathway)
2. Direct transduction of the signal via adaptor proteins (death receptor or apoptosis extrinsic pathway)
3. Perforin/Enzymes pathway

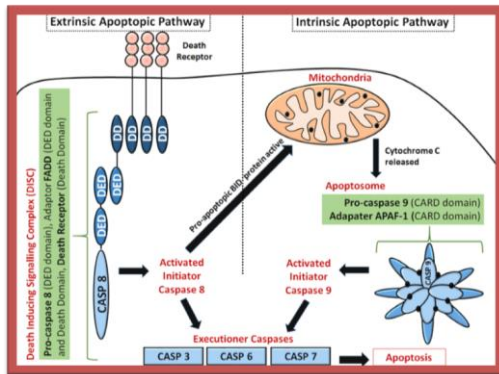


Fig. 3. Extrinsic and Intrinsic pathway

A. Intrinsic pathway

When the strain and harm to the cell occurred then intrinsic pathway to caspase-mediated cell death is started. These forms of stress and harm will cause harm the deoxyribonucleic acid, O deprivation, and different stresses that impair a cell’s ability to operate. In response to those damages or stresses, the cell set to activated a group of proteins known as “BH3-only proteins. The encouragement and discouragement of caspase-mediated cell death depends on which kind BH3-only proteins square measure activated. Pro-apoptotic BH3-only proteins activate BAX and BAK – the similar form of proteins is activated by tBID when it’s created through the adventitious pathway to caspase-mediated cell death. Activated BAX and BAK cause a condition called “MOMP.” MOMP stands for “mitochondrial outer membrane porosity.”

Due to substance molecules the steps leading up to MOMP is stopped in their tracks. however once MOMP has been achieved, the cell can complete the death method. MOMP plays a significant role in caspase-mediated cell death by permitting the discharge of cytochrome into the protoplasm. Under traditional circumstances, cytochrome plays a crucial role within the mitochondrial negatron transport chain. throughout MOMP, however, cytochrome will depart from the mitochondria and act as a sign molecule within the cell protoplasm. Cytochrome-C within the cell protoplasm prompts the formation of the ominous-sounding “apoptosome”. Once apoptosome is created turns pro-caspase-9 into caspase-9. Caspase-9 plays various functions to uphold caspase-mediated cell death. Among the foremost vital is that the activation of caspases-3 and -7. Once activated, caspases-3 and -7 begin the breakdown of cellular materials. Caspase-3 condenses and breaks down the cell’s deoxyribonucleic acid.

B. Extrinsic pathway

In the “extrinsic” pathway to necrobiosis, a proof is received from outside the cell instructing it to commit programmed death. this could occur if the cell is not any longer required, or if it’s pathologic. Like several pathways for transportation concerning complicated changes during a cell, the unessential pathway to necrobiosis involves several steps, every of which might be “up regulated” or “down regulated” by organic

phenomenon or by different molecules: Like most communication between cells, the unessential pathway of necrobiosis starts with a proof molecule binding to a receptor on the surface of the cytomembrane. 2 common kinds of chemical messengers that trigger the unessential pathway to necrobiosis ar FAS and path. These molecules are also excreted by neighboring cells if a cell is broken or not required. The receptors that bind to FAS and path are known as “FASR” for “FAS Receptor” or “TRAILR” for “TRAIL Receptor.” As with most receptor proteins, once FASR and TRAILR encounter to their signal molecule – generally known as a “ligand” – they bind thereto. The binding method causes changes to the receptor’s intracellular domain. In response to the changes within the intracellular domain of TRAILR or FASR, a super molecule within the cell known as FADD conjointly changes. FADD’s name is either amusing or terrifying: it stands for “FAS-Associated Death Domain” super molecule. Once FADD has been activated by changes to the receptor, it interacts with 2 extra proteins, that endure to begin the method of death. Pro-caspase-8 Associate in Nursing d pro-caspase-10 ar inactive proteins till they move with an activated FADD. however, if 2 of those molecules encounter Associate in Nursing activated FADD, the elements of the proteins that keep them inactive ar “cleaved” or “cut” away. The pro-caspases then become caspase-8 and caspase-10 – that are romantically remarked by scientists as “the starting of the end” because of their role in beginning necrobiosis. Caspases-8 and -10 disperse through the protoplasm and trigger changes to many different molecules throughout the cell, together with messengers that begin the breakdown of DNA when being activated by the caspases. Another inactive molecule known as BID is reworked into tBID once the activated caspases cleave off a part of BID that keeps the molecule inactive. when BID is reworked into tBID, tBID moves to the mitochondria. tBID activates the molecules BAX and BAK. The activation of BAX and BAK are the primary steps shared by each the unessential and intrinsic pathways to necrobiosis. Steps 1-4 listed here are distinctive to the unessential pathway. however, when BAX and BAK are activated, the following steps are an equivalent between each pathway. As such, steps 3-7 of the intrinsic pathway, listed below, are steps 5-9 of the unessential pathway!

4. Perforin and Granzyme

Perforin may be a pore-forming supermolecule and conjointly referred to as living substance grain toxins. Granzyme may be a family of structurally connected aminoalkanoic acid proteases hold on inside the cytotoxic granules of cytotoxic lymphocytes (CLs). Perforin and granzyme induce target-cell cell death hand in glove (Figure 4). Granzyme is critical for triggering cell death of target cells, however they rely upon being fitly delivered by perforin. each perforin and granzyme bind to the target-cell surface as a part of one molecule advanced related to serglycin, that more diminishes the likelihood of passive diffusion of granzymes.

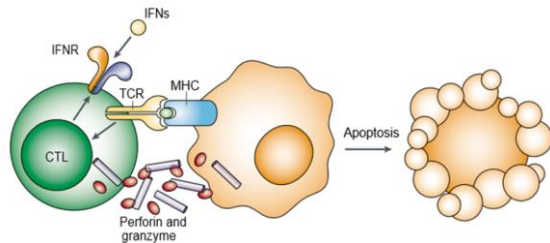


Fig. 4. Perforin and granzyme induce target-cell apoptosis cooperatively

In humans, there are granzyme A, B, H, K, and M, while in mice there are granzyme A, B, C, D, E, F, G, K, L, M, and N. Granzyme A (GrA) and granzyme B (GrB) are the most abundant granzymes and have been the most studied. The functions of granzymes A and B in inducing target-cell apoptosis have been investigated extensively *in vitro*, and they are better understood than the role of perforin at the molecular level.

A. Process and Regulation of Perforin/Granzyme Apoptosis Pathway

Once secreted by cytotoxic lymphocytes, granzymes enter into target cells, that could be a very important step in necrobiosis. the foremost lytic macromolecules packaged inside the granules are completely different granzymes and therefore the pore-forming protein perforin, that facilitates the incorporation of granzymes by cells.

Granzyme B primarily triggers proteinase activation indirectly, instead of by direct proteinase process. It achieves this by directly activating pro-apoptotic ‘BH3-only’ members of the BCL-2 family, like BH3-interacting domain death agonist (Bid). Bid at the side of pro-apoptotic BCL-2 family Bax and/or Bak proteins lead to the escape of pro-apoptotic mitochondrial mediators, like cytochrome, into the cytoplasm. cytochrome unleash activates professional caspase-9, and by binding to apoptotic enzyme activating issue 1 (Apaf-1), professional caspase-9 becomes mature caspase-9, that continues to create the apoptosome and activates downstream caspase-3. Activated caspase-3 is in a position to cleave specific substrates like ICAD (inhibitor of the caspase-activated DNase, CAD), permitting the CAD to translocate to the nucleus to

fragment desoxyribonucleic acid. Besides Bid, granzyme B will inactivate Mcl-1 that could be a member of the anti-apoptotic Bcl-2 family to unleash the pro-anti-apoptotic Bcl-2 family macromolecule Bim on the outer mitochondrial membrane. And granzyme B may also mediate the effector caspase-3 and instigator caspase-8 to control the caspase-mediated cell death pathway.

Contrast to granzyme B, granzyme M doesn’t captivated with mitochondrial to control, however granzyme M could activate granzyme B by the cleavage of proteolytic enzyme substance nine (PI-9) that is that the granzyme B substance. Granzyme M can also directly cleave the ICAD to unleash CAD like granzyme B.

Granzyme A induces loss of mitochondrial inner membrane potential and therefore the unleash of reactive element species (ROS). It generates fibre desoxyribonucleic acid nicks, instead of oligonucleosomal desoxyribonucleic acid fragments. In response to ROS, the ER-associated SET advanced, as well as SET, Ape1, pp32, HMG2, NM23-H1 and TREX1, translocates to the nucleus, wherever granzyme A cleaves 3 members of the SET advanced that ar concerned in desoxyribonucleic acid repair: HMG2, Ape1, and SET.

B. Modulators that induced apoptosis in cancer treatment

When naturally occurring apoptotic process not done work proper and then number of unnecessary cells collected which formed the tumor called cancer. So, for the treatment of cancer oncologist used some agents that are stimulate the apoptotic process for removing of unwanted cells from body.

C. Apoptotic modulators

Bcl₂ family proteins are classified into two types

1. Antiapoptotic proteins. E.g. BCL-2, BCL-X, BCL-XL, BCL-W
2. Proapoptotic protein. E.g. BAX, BAK, BID, BUD, BIM, BIK

Apoptosis triggered by different stress signals

- a) ROS
- b) RNS
- c) DNA

Table 1
Summary of plant derived modulators and their mechanism for inducing apoptosis

Modulators	Originated from	Experimental model	Mechanism of action
Aloe-emodin	Rheum palmatum	Neuroectodermal tumor in mice	Kill the cancerous cells by inducing cytochrome C- release
Cinnamaldehyde	Cinnamomum cassia	Human survival carcinoma cell line	Exhibit reduced migration potential that could be explained due to
Black cohosh, mucrotys, bug bane, bugwort	Cimicifuga racemosa	Female breast cancer	By activating of caspases
Methnolic extract Rhein	From ethyl acetate extract of cassia fistula	Human colon cancer cell line COLO 320 Dm	Rhein induced the production of ROS and Ca ²⁺ and decrease d levels leading to cytochrome C-release promote the activation of Caspase-9 and -3 causing apoptosis
Curcumin (Curcuminoids)	Turmeric (Curcuma longa)	Animal and human breast, lungs colon kidney, ovaries and liver cell line	Curcumin exhibit anticancer activity by inducing apoptosis and inhibiting proliferation and invation of tumors by inhibiting the variety of signaling pathway like- BCL-2 and XIAP
Hydroalcoholic leaf extract alpha terithenylmethanol	Eclipta alpha or eclipta prospata	HepG ₂ O6 glioma and A498 cell line	It is induces apoptosis by generating reactive oxygen species via NADPH oxidize in human cancer cells
Betulinic acid	Dillenia indica	Human leukemic cell lines U937, HL60 and K562	Induced cell death in U937, HL60 and K562 cell lines by inducing apoptosis

D. Plant-derivative modulators that exhibit apoptotic activity in cancer treatment

Plant obtaining apoptotic (cell death) causing modulators are less toxic/harmful to normal cells. The plants could be utilized as medicine and therapies over the 5000 years ago.

Special for the anticancer properties graviola fruit tree plant are used in the mechanism of promoting apoptosis by increasing BAX and inhibiting BCL-2 protein.

5. Conclusion

The apoptotic pathway is an efficient approach for finding new antineoplastic therapies. There are various mutations found in intrinsic, extrinsic and perforine pathways in cancer, allowing the cells to avoid apoptosis which is a characteristic of cancer. The capability to target and activate an apoptotic pathway would provide a more common cancer therapy. Particularly promising compounds to trigger apoptosis are many plant-derived compounds that are additionally nontoxic to healthy cells.

Acknowledgement

It has given me the research experience that will be definitely helpful in my future career. On the successful completion of my paper, I would like to express my gratitude to those without whom this project would not have been materialized.

With an overwhelming sense pride and genuine and personal regards to Dr. Arpit katiyar (Head of the Department of Pharmacy) Sai Meer college of Pharmacy & Sciences) for this valuable advice and encouragement.

References

- [1] Danial, N.N.; Korsmeyer, S.J. Cell death: Critical control points. *Cell* 2004, 116, 205–219.
- [2] Hassan, M.; Watari, H.; AbuAlmaaty, A.; Ohba, Y.; Sakuragi, N. Apoptosis and molecular targeting therapy in cancer. *BioMed Res. Int.* 2014, 2014.
- [3] Lopez, J.; Tait, S.W.G. Mitochondrial apoptosis: Killing cancer using the enemy within. *Br. J. Cancer* 2015, 112, 957–962.
- [4] Zaman, S.; Wang, R.; Gandhi, V. Targeting the apoptosis pathway in hematologic malignancies. *Leuk. Lymphoma* 2014, 55, 1980–1992.
- [5] Arbiser, J.L.; Bonner, M.Y.; Gilbert, L.C. Targeting the duality of cancer. *NPJ Precis. Oncol.* 2017, 1.
- [6] Xu, W.; Jing, L.; Wang, Q.; Lin, C.-C.; Chen, X.; Diao, J.; Liu, Y.; Sun, X. Bas-PGAM5L-Drp1 complex is required for intrinsic apoptosis execution. *Oncotarget* 2015, 6, 30017–30034.
- [7] Liu, Y.; Zhu, X. Endoplasmic reticulum-mitochondria tethering in neurodegenerative diseases. *Transl. Neurodegener.* 2017, 6, 21.
- [8] Villa-Pulgarin, J.A.; Gajate, C.; Botet, J.; Jimenez, A.; Justies, N.; Varela-M, R.E.; Cuesta-Marbán, A.; Müller, I.; Modolell, M.; Revuelta, J.L.; et al. Mitochondria and lipid raft-located FoF1-ATP synthase as major therapeutic targets in the antileishmanial and anticancer activities of ether lipid edelfosine. *PLoS Negl. Trop. Dis.* 2017, 11, e0005805.
- [9] Bao, H.; Zhang, Q.; Zhu, Z.; Xu, H.; Ding, F.; Wang, M.; Du, S.; Du, Y.; Yan, Z. BHX, a novel pyrazoline derivative, inhibits breast cancer cell invasion by reversing the epithelial-mesenchymal transition and down-regulating Wnt/ β -catenin signaling. *Sci. Rep.* 2017, 7, 9153.
- [10] Lomonosova, E.; Chinnadurai, G. BH3-only proteins in apoptosis and beyond: An overview. *Oncogene* 2008, 27, S2–S19.
- [11] Elmore, S. Apoptosis: A review of programmed cell death. *Toxicol. Pathol.* 2007, 35, 495–516.
- [12] Green, D.R.; Llambi, F. Cell death signaling. *Cold Spring Harb. Perspect. Biol.* 2015, 7.
- [13] Goldar, S.; Khaniani, M.S.; Derakhshan, S.M.; Baradam, B. Molecular mechanisms of apoptosis and roles in cancer development and treatment. *Asian Pac. J. Cancer Prev.* 2015, 16, 2129–2144.
- [14] Liu, H.; Su, D.; Zhang, J.; Ge, S.; Li, Y.; Wang, F.; Gravel, M.; Roulston, A.; Song, Q.; Xu, W.; et al. Improvement of pharmacokinetic profile of TRAIL via trimer-tage enhances its antitumor activity in vivo. *Sci. Rep.* 2017, 7, 8953.
- [15] Adams, J.M.; Cory, S. The BCL-2 protein family: Arbiters of cell survival. *Science* 1998, 281, 1322–1326.
- [16] Yip, K.W.; Reed, J.C. BCL-2 family proteins and cancer. *Oncogene* 2008, 27, 6398–6406.
- [17] Elkholi, R.; Renault, T.T.; Serasinghe, M.N.; Chipuk, J.E. Putting the pieces together: How is the mitochondrial pathway of apoptosis regulated in cancer and chemotherapy? *Cancer Metab.* 2014, 2, 16.
- [18] Burmester, T.; Hankeln, T. What is the function of neuroglobin? *J. Exp. Biol.* 2009, 212, 1423–1428.
- [19] Fiocchetti, M.; Nuzzo, M.T.; Totta, P.; Acconcia, F.; Ascenzi, P.; Marino, M. Neuroglobin, a pro-survival player in estrogen receptor α -positive cancer cells. *Cell Death Dis.* 2014, 5, e1449.
- [20] Jinesh, G.G.; Kamat, A.M. Endocytosis and serpentine filopodia drive blebbishield-mediated resurrection of apoptotic cancer stem cells. *Cell Death Discov.* 2016, 2, 15069.
- [21] Jinesh, G.G.; Choi, W.; Shah, J.B.; Lee, E.K.; Willis, D.L.; Kamat, A.M. Blebbishields, the emergency program for cancer stem cells: Sphere formation and tumorigenesis after apoptosis. *Cell Death Differ.* 2013, 20, 382–395.
- [22] Jinesh, G.G. Exposing the deadly dark side of apoptotic cancer stem cells. *Oncoscience* 2017, 4, 124–125.
- [23] Jinesh, G.G.; Molina, J.R.; Huang, L.; Laing, N.M.; Mills, G.B.; Var-Eli, M.; Kamat, A.M. Mitochondrial oligomers boost glycolysis in cancer stem cells to facilitate blebbishield-mediated transformation after apoptosis. *Cell Death Discov.* 2016, 2, 16003.
- [24] Jinesh, G.G.; Kamat, A.M. Blebbishield emergency program: An apoptotic route to cellular transformation. *Cell Death Differ.* 2016, 23, 757–758.
- [25] Fridlender, M.; Kapulnik, Y.; Koltai, H. Plant derived substances with anti-cancer activity: From folklore to practice. *Front. Plant Sci.* 2015, 6, 799.