Academy of Romanian Scientists
Annals Series on Biological Sciences

Volume 14, No. 1, 2025, pp. 185-192
Online ISSN 2285 – 4177
ORIGINAL PAPERS

APOPTOSIS IN CANCER THERAPY

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Abstract. *Apoptosis is a physiological cell suicide program that is critical for the development and maintenance of healthy tissues. Therefore, aberration of this process can be detrimental. Many types of cancer are characterized by a dysregulated apoptotic pathway, including down-regulated death receptor pathway function and abnormal Bcl-2 pathway function. The major problem in cancer treatment is the development of cells resistant to drugs and antiapoptotic machinery. Therefore, apoptosis induction can be the most potent defense against cancer. The relation between carcinogenesis and dysregulation of apoptosis is well known; therefore, any therapeutic strategy that specifically triggers apoptosis in cancer cells might have potential therapeutic value. One possibility is to use biological active compounds (naturally occurring antioxidant compounds) to eliminate premalignant/malignant cells by inducing them to undergo apoptosis. Death receptors belonging to the tumor necrosis factor (TNF) receptor gene superfamily (Fas/CD95/APO-1, TRAIL/APO-2L) play a central role in apoptosis because, by activating them, we can finally cause the cell's apoptotic demise. TRAIL (TNF-related apoptosis-inducing ligand) is a death receptor able to induce apoptosis in a wide range of transformed cell lines but not in normal cells. We use biologically active agents such as polyphenols (e.g., resveratrol, vineatrol) to increase tumor cells' sensitivity to apoptosis. Our study presents the results regarding B-cell chronic lymphocytic leukemia, a neoplastic disorder characterized by defective apoptosis. The tumoral cells do not frequently express Fas receptors, so they are resistant to its apoptotic action. They also present up-regulated expression of some antiapoptotic proteins (i.e., iNOS and Bcl-2). We try to modulate apoptosis with biologically active compounds.*

Keywords: apoptosis, biologic active compounds, CLL-B

DOI 10.56082/annalsarscibio.2025.1.185

Introduction

Programmed cell death has attracted much attention in during the years. After the discovery of the phenomenon by Carl Vogt in 1842 (1), when he described the death of notochordal and cartilage cells during development, and after the description of morphological changes by Flemming in 1885 (2), the research on apoptosis remained pending for many years. It was rediscovered in the second half of this century by Lockshin and Williams (3) who introduced the

term “programmed cell death” to describe the specific phenomenon of cell elimination during the evolutionary process of the transformation of the moth larva into the adult and then labeled, for the first time in an article, with the name apoptosis in 1972 by Kerr, Wyllie and Currie (4). In this way, an unparalleled page was opened in this field of research, considering that around 13,000 articles per year continue to appear.

The name apoptosis comes from Greek and means the falling of leaves from trees or the loss of petals from a flower, an analogy with the life cycle and the death of an organism.

Apoptosis is an active and well-defined process that plays an important role in the development, differentiation, proliferation/homeostasis, regulation and functioning of the immune system, the suppression of cells produced in excess, susceptible to evolving towards malignancy or cells that acquire irreparable genetic damage.

For this reason, any dysfunction, deregulation of the apoptotic process leads to a variety of pathologies.

Table 1. Disease As Consequence Of Dysregulated Apoptosis

Affected system	**Exemple**	**Apoptosis**	
		Hyperactivation	**Inhibation**
Neuro-degenerative diseases	Alzeimer	+	
	SLA	+	
	Parkinson	+	
Immune disorders	Autoimmune diseases		+
	AIDS	+	
	Diabetes	+	
	Thyroiditis	+	
Ischemia	Myocardial infarction	+	
Reperfusion	Stroke	+	
Neoplasis	Lymphoma		+
	Astrocytoma		+
	Hepatoma		+
	Melanoma		+
Various	Aging	+	
	Alopecia	+	

Apoptosis And Necrosis Morphological Characteristics

Unlike apoptosis, necrosis is an accidental cell death in which there is no mechanism for regulating cellular functions, no biological control and which is

distinguished by a rapid alteration of intracellular structures coupled with a loss of plasma membrane integrity. Necrosis leads to inflammatory reactions and numerous tissue lesions. Apoptosis and necrosis can occur simultaneously in tissues and cells in culture exposed to the same stimulus.

These morphological changes are the consequence of biochemical and molecular events that occur in apoptotic cells, after the activation of proteolytic enzymes that can mediate DNA hydrolysis into oligonucleosomal fragments or the hydrolysis of specific protein substrates that normally determine the integrity and shape of the cytoplasm and organelles.

Apoptosis pathways overview

There are two major pathways for the induction of apoptosis:

1) the receptor-mediated or extrinsec pathway (for type I cells which have the capacity to induce direct and mainly caspase-dependent apoptosis). It is mediated by the activation of "death receptors" (cell surface receptors) after the ligation with specific ligants. The death receptors they belong to the tumor necrosis factor receptor (TNFR) gene superfamily, including TNFR-1, Fas/CD95 (Fas Cell Surface Death Receptor) and TRAIL (Tumor Necrosis Factor- relatid apoptosis-inducing ligant)
2) the mitochondrial patway or the intrinsec pathway (for type II cells in which the signal from the activated receptor needs to be amplified via mitochocondria-dependent apoptosis. It is mediated by stress, radiations, drug, or apoptosis inducers (etoposide, biologic active compounds, etc. The link between caspase signalling cascade and mitochondria is provided by Bid (Bcl-2 interacting domain, a member of Bcl-2 family- B-cell lymphoma 2) (5).

Although the extrinsic and the intrinsic-induced apoptosis pathways are acting independently, accumulating evidence suggests that cross-talk between the two pathways exist.

TNF family

All the members of the family are type II transmembrane glyciproteins with a cystein rich extracellular subdomains which allow them to recognignize their ligants with specificity. In order to be active they form oligomers, most often trimers (6).

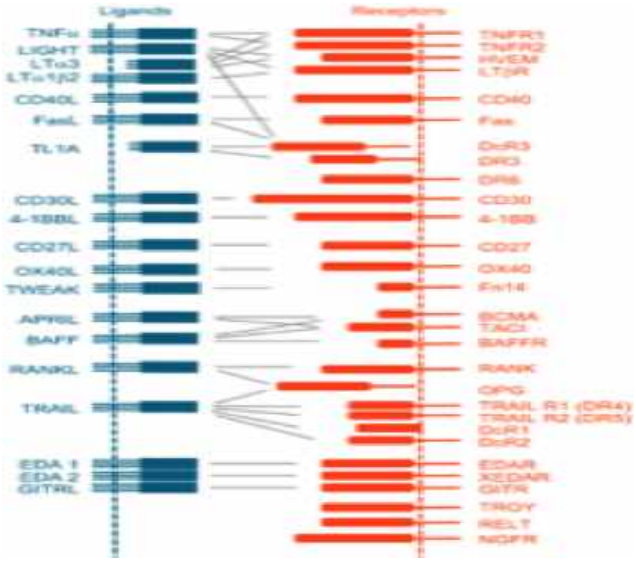

Fig 1. The TNF family. They belong to the tumor necrosis factor receptor (TNFR) gene superfamily, including TNFR-1, Fas/CD95 and TRAIL (C. Dostert, et all. Physiol Rev 99: 115–160, 2019)

The extrinsic pathway of apoptosis, that of death receptors

Following the signal received in response to the engagement of TNF family receptors on the cell surface. the recruitment of adaptor molecules (FADD, TRADD) via their DD domains (death domains) takes place. The DISC complex (death inducing signaling complex) is thus formed. In addition to the DD domains, the adaptor molecules also possess DED domains (death effector domains) through which they recruit procaspase 8 molecules, which in turn possess such domains. Following recruitment to the DISC, the procaspase 8 molecules in proximity begin to be activated by the phenomenon of autoproteolysis. In turn, the active caspase 8 induces hydrolysis and therefore the activation of the effector caspases (e.g. caspase 3) in the caspase cascade., is that of surface receptors, the so-called "death receptors" of which the receptors for TNF and those for Fas are part, which with the help of adaptor molecules recruit and activate caspases.

The extrinsic pathway of apoptosis, that of the mitochondria

The second pathway occurs when the externally induced apoptotic stimulus (stress, radiation, apoptotic agents, drugs, etc.) is not strong enough to induce the activation of the caspase cascade and to determine apoptosis. The pathway is modulated by the Bcl-2 protein family (5); it leads to the release of cytochrome c from the mitochondria, the activation of Apaf-1(apoptosis factor-1) and the activation of the caspase cascade. The link between the caspase cascade

and the mitochondria is made by Bid, a member of the Bcl-2 family. Bid is hydrolyzed by caspase 8 and its truncated form, tBID, is translocated to the mitochondria where it acts simultaneously with Bax and Bak, the 2 proapoptotic members of the Bcl-2 family, in order to release into the cytosol proapoptotic factors such as cytochrome c, located on the inner mitochondrial membrane. Once released into the cytosol, cytochrome c, in the presence of Apaf-1 induces the formation of the apoptosome and following the binding of procaspase 9 to this heptameric structure, its activation occurs. In turn, active caspase 9 hydrolyzes procaspase 3, which in turn will cause the hydrolysis of specific protein substrates, ultimately amplifying the apoptotic signal and eventually executing it with all the known morphological and biochemical characteristics.

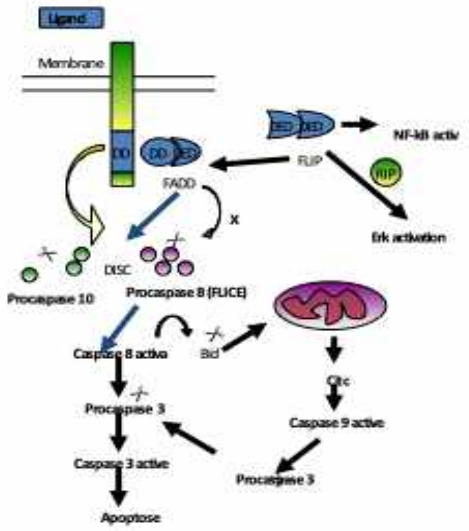

Fig. 2 The intrinsec and the extrinsec pathways of apoptosis. DD (death effector domain); FADD-Fas-associated death domain; Bid- Bcl-2; interacting domain; Flip - FLICE inhibitory protein (personal data)

Chronic limphcytic leukemia type B

B-cell chronic lymphocytic leukemia (B-CLL) is a neoplasic disorder characterized by defective apoptosis. The cells are resistant to apoptosis cell death *in vivo,* but display rapid death by apoptosis *in vitro*, suggesting the lack of essential growth factors in the culture medium, or the absence of a survival signal that could be triggered by interaction with other cells. The cells are frozen in the G_0 phase of the cell cycle, cannot develop an appropriate mitogenic response and escape apoptosis, so there is a progressive accumulation in G_0 of malignant CD5+, $CD23^+$ B cells. The major problem in the treatment of leukemia is the development of resistant leukemic cells to drugs, and the development of

antiapoptotic machinery because they have high bcl2/bax ratio, low expression, and a lack of susceptibility to Fas-mediated apoptosis (intrinsec apoptosis).

During the years, studies shown that apoptosis can be reverted by some factors: IL-4, IL-13, IFNs, growth factors, BCR stimulation or apoptosis inductors; IL-4, does not significantly affect tumor cell replication, exerts an antiapoptotic effect. In a similar way, interleukin 13 (IL-13), which mimics some of the activities of IL-4 in these cells, inhibits interleukin 2 (IL-2)-dependent proliferation and thus protects cells from apoptosis. Granulocyte-colony stimulating factor (G-CSF) wich helps the bone marrow make more white blood cells (7), soluble CD23 (sCD23), (8) or CD40 engagement (9) also protect B-cell CLL from programmed cell death, either spontaneous or induced by pro-apoptotic molecules such as drugs used in the clinic (chlorambucil, fludarabine, adenosine deaminase inhibitors, etc).

Studies on the expression of various members of the bcl-2 family (bcl-2, bcl-xL, bcl-xS, bax) in CD5+ B-CLL have shown an imbalance in the ratio between members of this family, the ratio being in favor of anti-apoptotic members, which leads to the accumulation of leukemic cells (10).

For many years studies on B-CLL are hampered by the paucity of representative established cell lines or by the absence of clinical models. Therefore, the vast majority of the investigations have been conducted on *ex vivo* cultures from B-CLL patients.

Biologic activ compounds

Natural cure can offer a great benefit for public health with a reasonable cost and a poor risk. In present more than 400 natural compounds are considered biologic active agents and studied in research laboratory. More than 40 of these compounds are studied in clinical trials. Some of these agents are investigated individually and other are tested in combination to avoid in that manner the chemoresistance process. All these agents have been tested in laboratory and also *in vivo* (animals studis) and considering their antitumoral effects they have been proposed as potential adjuvants in cancer therapy.

One of these biological active compound is resveratrol (3,5,4′-trihidroxi-trans-stilben) is a polyphenol with strong antioxidant action. Resveratrol is a polyphenol with a strong antioxidant action, which has been and is being intensively studied due to its properties in many studies regarding its anti-cancer activity. (11)

B-CLL being a neoplasic disorder characterized by defective apoptosis, we tried to revert this process by using the resveratrol as apoptosis inductor. In order to achieeve this goal we use a commercial cell line (MEC-1- ACC 497- DMSZ-

German Collection of Microorganisms and Cell Culture, Gmbh) along with different resveratrol concentrations and times as we see in Figure 3.

As shown in Figure 4, resveratrol decreases mitochondrial transmembrane potential, triggering the apoptotic process. At the same time, by decreasing the expression of the antiapoptotic protein bcl-2, it modifies the bcl-2/bax ratio, inducing the apoptotic process in the leukemia cells of the cell line used in the study.

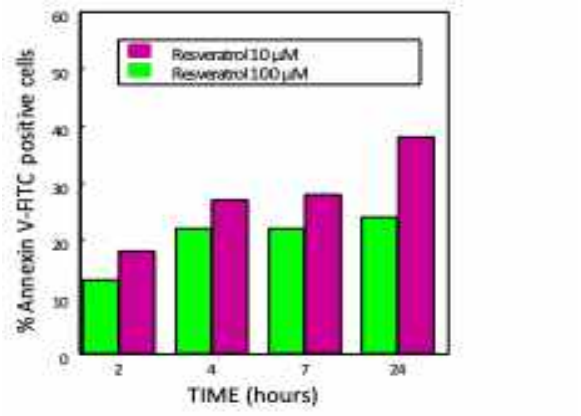

Fig 3. Resveratrol induces apoptosis in leukemic cell line (personal data)

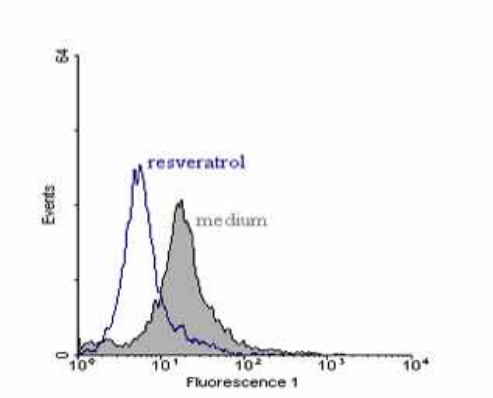

Fig. 4. Resveratrol induces a decrease in the expression of the antiapoptotic protein bcl-2, in leukemic cell line (personal data) (personal data)

Conclusions

There are many biological active compounds with active phytochemicals with anticancer potential and pharmacological properties, and many studies support their use in cancer therapy. Resveratrol is one of them, and the results presented show its potential, not as a drug but as an adjuvant.

Conflit of Interes: *The author declares no conflict of interest regarding this article*

Founding: *No funding for this study*

Acknowledgment: *None*

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