MODULATION OF APOPTOSIS BY HERBS

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Apoptosis or programmed cell death is a common process in multicellular organism. It enables the elimination of single cells or their assemblies when they are damaged or mutated to such an extent that their future existence might be dangerous to the whole organism. In particular, programmed cell death plays an important role during embryogenesis, metamorphosis, endocrine tissue atrophy, tumor regression and in the growth and maturation of individual organs. In recent years, there has been an increasing interest in the elucidation of mechanisms of apoptosis due to the recent discoveries that it plays a pivotal role not only in the normal development but also in a number of disease conditions.

Apoptosis is a highly regulated physiological process of cell death and is characterized by a unique pattern of morphological changes in both nucleus and cytoplasm. There is extensive chromatin clumping, condensation of cytoplasm, membrane blebbing and apoptotic body formation. Surrounding phagocytic cells without eliciting any inflammatory changes unlike necrosis ultimately engulf these membrane bound bodies. Apoptosis includes 3 stages namely (i) initiation by specific stimuli which may be intrinsic or extrinsic, (ii) execution phase wherein apoptosis morphology is appreciated and (iii) elimination of apoptotic cells via phagocytosis without inflammation.

Besides playing a vital role in fundamental biological processes including organ development, wound healing, immune responses, oncogenesis, atherogenesis, programmed cell death is crucial in regulating total cell number. Furthermore, its another important role is to remove the genetically damaged cells. These functions serve to protect animals and human beings from various diseases including cancer. Any disruption in apoptotic process may lead to unwanted cell survival and can cause development of abnormalities and facilitate tumor development. The disruption of apoptosis may lead to pathogenesis of many conditions like Alzheimer's disease, rheumatoid arthritis, AIDS, cancer and ischemic stroke etc. Apoptosis of the immune system has recently drawn interest since immune dysfunction can affect the survival of the host in various ways as there may be autoimmune diseases, cancer, immunopathological and immunotoxicological alterations, immunosuppression and vaccination failure etc. The programmed cell death also helps in adaptation of an organism to the environment and resolution of inflammation by safe elimination of unwanted cells.

It has been documented that programmed cell death process once started, is essentially irreversible. Under non-physiological conditions the same initial insult can lead to apoptosis and necrosis and the balance between cell death by these two processes seem to depend upon the intensity of the injury and level of available intracellular ATP. ATP levels are maintained until late in the apoptotic process and are required for caspases activation of Apaf-1 and subsequent translocation into the nucleus. Depletion of ATP levels following apoptogenic stimulus switches the mode of cell death from apoptosis to necrosis.

Apoptosis and necrosis

There are marked differences between apoptosis and necrosis and both can not be regarded as synonym. Unlike programmed cell death, necrosis is an accidental cell death, which is characterized by ATP depletion, cell swelling and lysis that results in the release of components showing tissue inflammation. It occurs due to failure to control cellular homeostasis and thus considered as non-homeostatic mode of cell death. Necrosis may be the consequence of cellular response to severe accidental or tissue insult and is always pathological. The main features of apoptosis and necrosis are listed below.

Apoptosis

- A functional energy producing system is required
- Chromatin condensation
- Disappearance of nucleolus
- Occurrence of blebs
- Margination of chromatin at the inner surface of the nuclear membrane
- Activation of nucleases and fragmentation of DNA
- Shrinkage of cells
- Breakage into apoptotic bodies
- Intracellular contents are not released
- Induction is by an active genetically regulated process which requires coordinated expression of many genes
- Inflammatory reaction is not elicited
- Prevented by increased expression of some genes or by external signals

Necrosis

- A functional energy producing system is not required
- Cell damage
- Major site of damage is membrane
- Ability to regulate osmotic pressure is lost
- Inflammatory reaction is elicited
- Intracellular contents are released

Apoptosis pathways

Apoptosis is a distinct mechanism of cell death and involves the activation of cascades of molecular events leading to the cell death. This systematic self-destruction of cells may be due to a wide variety of intrinsic and extrinsic stimuli including pathogens and environmental contaminants. There are two major pathways of apoptosis: the extrinsically mediated pathway and intrinsically mediated pathway. In both pathways, a series of molecular and biochemical steps lead to the activation of common effectors or executioner cysteine proteases, the caspases, resulting in cleavage of a number of nuclear and cytoplasmic substances. Recent evidence suggests that these two pathways are linked in certain cell types. The intrinsic and extrinsic pathways are linked by Bid, a pro-apoptotic Bcl-2 family member. Caspase-8 dependent cleavage of Bid allows translocation of this

protein to mitochondrion, where it directly or indirectly facilitates cytochrome C release and thus, leading to apoptosis through intrinsic pathway.

Oxidative stress and apoptosis have both been associated with chemical exposures and toxicity. It has been documented that certain chemical exposures can result in the alteration of secondary messengers, such as free radicals or reactive oxygen species (ROS), and these alterations have been linked to the induction of apoptosis in immune cells. Free radicals influence gene expression, regulate cellular responses to cytokines, as well as proliferative events of a cell. All these events have also been implicated as possible triggering mechanisms of apoptosis.

Herbs modulating apoptosis

In the recent years, there had been concerted efforts to study pharmacological modulation of apoptosis. The attempt to influence the natural phenomenon of programmed cell death is of considerable applied importance as it is affected in several clinical situations. Apoptosis is reduced in cancer while it is increased in neurodegenerative diseases and related disorders. Therefore, chemicals that can modify programmed cell death are likely to be potentially useful drugs. A few plants have been shown to possess suppressive action on apoptosis and these are summarized in Table 1.

Table 1. Herbs associated with inhibition of apoptosis

Plant	Compound/ Extract	Animal/Cell system (disease/condition)	Mechanism of action	Ref
Pueraria lobata	Puerarin	Rat pancreatic islets (pancreatic islets damage)	Prevents ROS generation	1
Sophora japonica	Quercetin, rutin	Rat (cerebral infarct induced by ischemia-reperfusion)	-----	2
Angelica spp.	-----	Rat (Focal cerebral ischemia injury)	Deceases expression of bax protein	3
Captidis rhizome	Extract	Pancreatic beta- cells (diabetes mellitus)	Inhibition of deltapsim disruption	4
Tripterygium wilfordi	Monomer	PC 12 cells (Alzheimer's disease)	Inhibition of intracellular Ca^{2+} increment	5
Uncaria rhynchophylla	Alkaloid fraction	Cultured hippocampal slices (Epilepsy)	Inhibited c-jun, p53 and bax genes	6
Chinese Herbal Drug MAGNOLOL		Rat (warm ischemia-reperfusion injury of liver)	Upregulation of Bcl-XL and suppression of Bcl-xS genes	7

From Pacific Yew (*Taxus brevifolia*), which gave taxol, plants have been a source of clinically useful drugs. Recently, a variety of herbs have been investigated for their potential to influence the process of programmed cell death. Attempts have been made to investigate the plants for their ability to induce apoptosis in cancer cells in order to arrest their proliferation. The relevant information on some plants known to induce apoptosis in a variety of cancer cells is presented in Table 2. Various cell lines such as HL60, human hepatocellular carcinoma cell line (KIM-1), cholangiocarcinoma cell line (KMC-1), B-cell hybridomas, U937 a monocytic cell line, HeLa cells, human lymphoid leukemia (MOLT-4B), K562 , A549, J5DBTRG-05MG, HT1299, DMS114, H 358, CalU1, SKLu1, H23, HT1080, 95-D, PLA-801 and others have been studied. Several extracts and compounds isolated from plants

have been found to induce programmed cell death and these include extracts of plants like mistletoe and *Semicarpus anacardium*, compounds like bryonolic acid (*Trichosanthes kirilowii var. Japonica)*, crocin (saffron) and allicin (*Allium sativum*) have also been found to induce programmed cell death and therefore, exert antiproliferative effect. The Chinese herbal medicine "Sho-saiko-to" induces apoptosis in selected cancerous cell lines. Panax ginseng has been documented to prevent irradiation-induced apoptosis in hair follicles, which is suggestive of important therapeutic implications.

Table 2. Induction of apoptosis by herbs

Plant	Compound/ Extract	Cell line	Mechanism of action	Ref
Angelica sinensis	Acetone extract	A549, HT29, J5DBTR G-05MG	Activation of caspase 9 and 3 mediated via the suppression of Bcl-2 and cdk 4 expression. No activation of caspase-8.	8
Bupleurum falcatum	Saikosaponin D	A549	Increasing the expression of p53 and p21/WAF1 proteins and induction of Fas/APO1	9
Bupleurum scorzonerifolium	Acetone extract	A549	Inhibited telomerase activity	10, 11
Coxi lachryma	Methanolic	A549	Inhibition of cyclin A expression and extract activation via caspase cascade	12
Curcuma longa	Curcumin	A549, HT1299	Decreased expression of p53, bcl-2 bcl-XL	13
Lithospermum radix	HIVS	DMS114	Decreased and suppressed the expression of TRAP1	14
Tripterygium wilfordii Hook F	Triptolide and its derivatives	A549, H 358, CalU1, SKLu1, H23, HT1080	Induced apoptosis via p53 and p21 pathway	15 – 18
Thalictrum acutifolium	Acutiaporberine	95-D, PLA-801	Inhibited expression of bcl-2 gene and activated expression of bax gene. p53 independent	19, 20
Scutellaria barbata	Ethanol extract	A549	Activation through caspase cascade. Increase caspase 3/7. Activation of cell cycle control genes such as STK6, MCM5, etc	21
Solanum incanum	Solamargine	A549	Increased the release of cytochrome c. Decreased the bcl-2 and bcl-XL. Increased Bax and caspase-3 activity	22
Plant pigments	Acacetin	A549	Via p53 pathway	23
Liliaceae plants	Isoliquiritigenin	A549	Via p53 pathway	24
Grapes, peanuts, pines and other Leguminosae family	Stilbenoids	A549	Via p53 pathway	25

The dietary plants such as soya bean, garlic, ginger and green tea etc. which have been suggested to decrease the incidence of cancer may act by inducing apoptosis. The toxic effects produced by a number of food items and herbal medicines have been attributed to their ability to induce programmed cell death. It has been suggested that rapid progression of the betel- and tobacco-related oral squamous cell carcinomas may be associated with a simultaneous involvement

of p53 and c-myc leading to inhibition of apoptosis. The molecular mechanisms by which herbs modulate apoptosis have been investigated. Some of these suggested mechanisms include endonuclease activation, induction of p53, activation of caspase 3 protease via a Bcl-2-insensitive pathway, potentiation of free-radical formation and accumulation of sphinganine etc.

It is known that normal p53 function plays an important role in inducing programmed cell death and cell cycle checkpoints in human cells following DNA damage. The p53 is the most commonly mutated tumor suppressor gene. Moreover, the sensitivity of cancer cells to chemotherapeutic agents is greatly influenced when the function of p53 is repealed. However, recent studies have shown that the antiproliferative activities of herbs in human cancer cells are induced via an apoptotic system with or without p53 pathway. Some herbs may interact with the Fas/FasL system which is a key signaling transduction pathway of programmed cell death in cells and tissues. Most herbs alter the cell cycle by increasing the proportion of cells in G1 phase while some herbs produce inhibition of cancer cells growth via the G2/M phase cell cycle arrest.

In conclusion, apoptosis is a highly conserved mechanism of self-defense. Malfunction of apoptotic signaling can play an important role in various diseases and insufficient apoptosis may lead to cancer, autoimmunity, persistent infections while excessive apoptosis contributes to neurodegeneration, autoimmunity, AIDS and ischaemia. Of current interest is the involvement of disrupted apoptosis function in tumor formation and occurrence of multidrug resistance during cancer chemotherapy. Many of the antineoplastic drugs are natural products or are derived from herbs. In future, search for apoptosis modulating novel agents from herbs would pave the way for development of highly efficacious therapeutic agents.

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