

Plant lectins, from ancient sugar-binding proteins to emerging anti-cancer drugs in apoptosis and autophagy

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Abstract

Ubiquitously distributed in different plant species, plant lectins are highly diverse carbohydrate-binding proteins of non-immune origin. They have interesting pharmacological activities and currently are of great interest to thousands of people working on biomedical research in cancer-related problems. It has been widely accepted that plant lectins affect both apoptosis and autophagy by modulating representative signalling pathways involved in Bcl-2 family, caspase family, p53, PI3K/Akt, ERK, BNIP3, Ras-Raf and ATG families, in cancer. Plant lectins may have a role as potential new anti-tumour agents in cancer drug discovery. Thus, here we summarize these findings on pathway-involved plant lectins, to provide a comprehensive perspective for further elucidating their potential role as novel anti-cancer drugs, with respect to both apoptosis and autophagy in cancer pathogenesis, and future therapy.

Abbreviations

Apaf-1: apoptosis-associated factor-1

Atg: autophagy-related gene

BNIP3: Bcl2/E1B-19kDa protein-interacting protein 3

ConA: Concanavalin A

ConBr: Canavaliabrasiliensis

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cyto. c: cytochrome *c*

DISC: death-inducing signalling complex

DLA: Dalton's Lymphoma Ascites

ERK: extracellular-signal regulated kinase

FADD: Fas-associated death domain

FIP200: focal adhesion kinase family interacting protein of 200 kDa

MAPK: mitogen-activated protein kinases

MCL: *Momordica charantia* lectin

ML: Mistletoe lectin

MLL: mulberry leaf lectin

MMP-9: matrix metalloproteinase 9

MT1: membrane type-1

mTORC1: mammalian target of rapamycin complex 1

NMR: nuclear magnetic resonance

NO: nitric oxide

NPC: nasopharyngeal carcinoma

PCL: *P. cyrtonema* lectin

PI3K: phosphoinositide 3-kinase

PPI: protein-protein interaction

PTA: *Pinelliaternata* agglutinin

RBA: Rice bran agglutinin

ROS: reactive oxygen species

SFL: *S. flavescens* lectin

Introduction

Lectins are a ubiquitously distributed group of highly diverse plant, animal and fungus non-immune origin proteins, containing at least one non-catalytic domain (1). Their non-catalytic domain enables them to selectively recognize and reversibly bind to specific free sugars or glycans, present on glycoproteins and glycolipids, without altering structure of the carbohydrate (2). Lectins have already been recognised for many years, for their ability to agglutinate red blood cells (72), but their roles played in programmed cell death of apoptosis and autophagy, have not yet been fully elucidated. In the 1980s, first studies suggested that lectin-like molecules, constitutively expressed on surfaces of macrophages, selectively recognized changes to glycans decorating plasma membranes of apoptotic thymocytes (74). Some years later, more compelling evidence was obtained when Griffiths and co-workers identified apoptotic changes within lymphoid tissues after injection of plant lectins, *in vivo* (73), followed by additional studies documenting cell shrinkage and DNA fragmentation in lymphocytes exposed to plant lectins, *in vitro*. This approach set the basis for therapeutic strategies aimed at eliminating aberrantly glycosylated cancer cells (61). Plant lectins have been demonstrated that specifically bind to various sugar structures, and trigger several important cell processes. Regarding their biochemical properties, carbohydrate-binding specificities and biological functions, hundreds of plant lectins have been purified and characterized. Carbohydrate-binding specificities can be either polyspecific or monospecific, thus, plant lectins are classified into 12 families: *Agaricusbisporus* agglutinin homologues (3), amarantins (4), Class V chitinase homologues with lectin activity (5), cyanovirin family members, the EEA family (6), GNA family members, proteins with hevein domains (7), jacalins (8), proteins with legume lectin domains (9), LysM domain bearers (10), Nictaba family members (11), and Ricin-B family members (12).

On the one hand, according to their specific binding characteristics, plant lectins have already been used as recognition tools to study subtle distinctions between malignant and non-malignant cells (13). In microarrays, plant lectins have been used to analyze high throughput protein glycosylation, or profile global changes on surfaces of mammalian cells (14). In addition, activity of plant lectins has been demonstrated in different tissues and processes, demonstrating their widespread importance as potential therapeutic agents, especially for cancer (15). Some plant lectins are also able to reduce treatment-associated side effects as adjuvant agents during chemotherapy and radiotherapy.

On the other hand, plant lectins have been found to target apoptotic- and autophagy-related signalling

pathways in a variety of tumour cells. Apoptosis is a complex and highly defined type of programmed cell death, while autophagy is an evolutionarily conserved and multi-step lysosomal degradation process (16). In the context of cancer, apoptosis and autophagy may work together to jointly determine the destiny of cancer cells, thus, the ability of plant lectins to influence both apoptosis and autophagy could conceivably be used to treat cancer in the future. For example, mistletoe lectins (MLs) and ricin may exert both anti-proliferative and apoptosis-inducing influence on cancer cells (17). Concanavalin A (ConA) and *P. cyrtonema* lectin (PCL) lead to autophagy after internalization or binding to certain sugar-containing receptors on the surfaces of cancer cells (18,19). Accordingly, these recently reported data of anti-tumour activities of plant lectins and their molecular mechanisms need to be discussed to reveal their potential role in future cancer therapy.

Here, we present an account of current modern research on plant lectins inducing cell death, by targeting apoptotic and autophagic signalling pathways involved in Bcl-2 family, caspase family, p53, PI3K/Akt, ERK, BNIP3, Ras-Raf and the ATG family, in different types of cancer cells. These important data help reveal the potential therapeutic role of plant lectins, which may shed new light on future cancer drug discovery.

Plant lectins and apoptosis in cancer

Apoptotic mechanisms

Apoptosis is an evolutionarily conserved process that can remove superfluous cells that have outlived their usefulness, or are dangerous for the survival of an organism. It has been demonstrated to occur generally *via* two major pathways, namely a death-receptor pathway triggered by Fas or other plasma membrane receptor ligation, and a mitochondria-dependent pathway releasing cytochrome *c* (cyto. *c*) (20).

Activation of cell surface death receptors induces the death-receptor pathway, by binding to specific ligands including TNF, TRAIL or FasL. After this intracellular domains of the receptors interact with Fas-associated death domain, resulting in assembly of a death-inducing signalling complex and recruitment of initiator caspase-8/caspase-10 (21).

Controlled by the Bcl-2 protein family, the mitochondrial pathway regulates mitochondrial membrane permeability, thereby releasing specific mitochondrial proteins, such as cyto. *c*, into the cytosol (22). Anti-apoptotic Bcl-2 proteins including Bcl-2, Bcl-X_L and Mcl-1 prevent permeabilization of the outer membrane

of mitochondria by inhibiting action of pro-apoptotic proteins such as cytosolic Bax and Bak, whereas other pro-apoptotic Bcl-2 family members including BH3-only proteins Bad, Bik, Bid, Bim, PUMA and NOXA, are cytosolic sensors of cell damage or stress (23) (Fig. 1).

Widely accepted as a crucial cellular molecular mechanism, apoptosis is targeted and regulated by numerous cell signalling pathways, leading to cell death, in normal as well as tumour cells. With identification of required apoptosis-related molecules and pathways that suppress cancer, modulation of apoptosis will emerge as a modern target for cancer therapy. Thus, targeting key molecular components of the cell death machinery induced by apoptosis is an attractive strategy for use of plant lectins (24).

Interaction between apoptosis and well-studied plant lectins

Various types of plant lectin modulate several apoptosis-related signalling pathways in different cancer cells. On the one hand, representative plant lectins such as ConA, mistletoe (*V. album*) lectins and PCL have been extensively studied. ConA, the first reported legume lectin, can lead to potential collapse of mitochondrial transmembrane, cyto.*c* release and caspase activation, eventually triggering mitochondria-mediated apoptosis, in both human melanoma A375 cells and human hepatocellular carcinoma HepG2 cells (25,26). This lectin directly binds to the extracellular region of SHPS-1, a multifunctional transmembrane glycoprotein, the interaction mediating ConA-dependent activation of AKT and secretion

of matrix metalloproteinase 9 (MMP-9). Thereby, both AKT and extracellular-signal regulated kinase (ERK) are required for increased secretion of MMP-9 by ConA (27). In addition, ConA up-regulates expression of COX-2 and down-regulates AKT expression *via* an IKK/NF- κ B-dependent pathway, in glioblastoma U87 cells (28). ConA selectively induces apoptosis by rendering the ratio of Bax/Bcl-2, inhibiting AKT expression, and activating the Foxo1a-Bim signalling pathway, in ovarian cancer SKOV3 cells (29).

As type II ribosome-inactivating proteins (RIPs II), MLs, can be divided into three distinct types including ML-I, ML-II and ML-III, among which ML-I and ML-II have been demonstrated to hold anti-proliferative and apoptosis-inducing mechanisms in different cancer cells. ML-I induces apoptosis by activating caspase-8 *via* the extrinsic apoptotic pathway, in Jurkat leukemic T cells, with sensitivity to apoptotic induction in cancer cell death and cooperating with TNF- α from the TNF-family death receptors through Bcl-2 down-regulation (30,31). ML-I can also break down MMP and activate caspase-3, thus inducing apoptosis through the apoptosis-associated factor-1 (Apaf-1)-dependent pathway, in human p53-mutated adenocarcinoma cells (31). Since ML-I alters levels of MMP, it releases cyto.*c* and increases reactive oxygen species (ROS), in hepatocellular carcinoma Hep3B cells (17). Contrary to ML-I, ML-II activates mitogen-activated protein kinase signalling involved in ERK and p38, then changes cell signalling pathways that modulate responses related to apoptosis. It has been reported that ML-II induces apoptosis by activating signalling pathways involved in SAPK/JNK and p38, or by inhibiting the

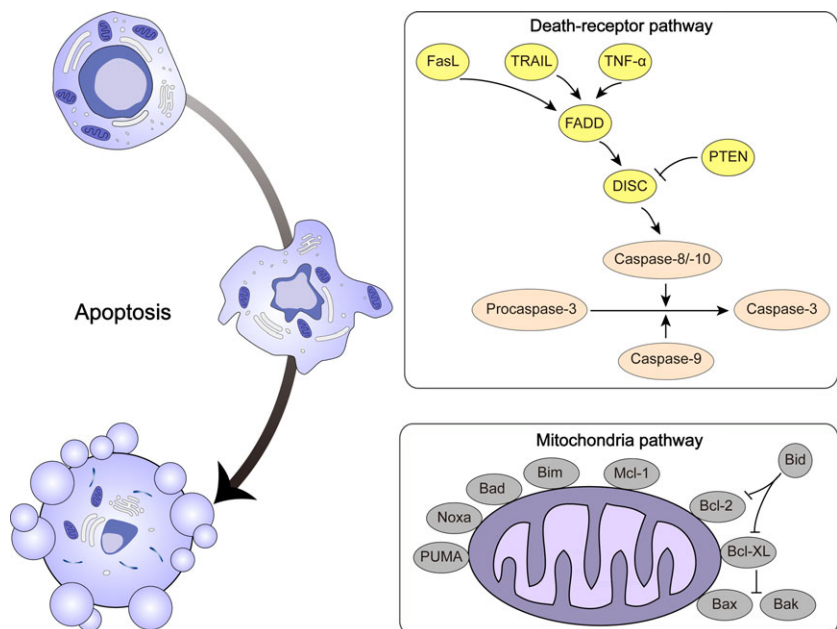


Figure 1. Mechanisms of apoptosis in cancer.

ERK1/2 pathway, in human monoblastic leukaemia U937 cells (32).

P. cyrtonema lectin, a mannose/sialic acid lectin belonging to the GNA family, was first isolated from *P. cyrtonema*, Hua (33). This lectin induces apoptosis in melanoma A375 cells, the mechanism of which involves regulation of Bax, Bcl-X_L and Bcl-2 proteins as well as collapse of MMP, leading to release of cyto.*c* and activation of caspases (25). PCL has anti-proliferative and apoptosis-inducing activities on cervical cancer HeLa cells, or induces human breast cancer MCF-7 cell apoptosis, *via* caspase activation (34,35). PCL also induces apoptosis accompanied with activation of caspases-3/-8/-9 in fibrosarcoma L929 cells, through a caspase-dependent apoptotic mechanism (36).

Interaction between apoptosis and new emerging plant lectins

On the other hand, other previously less studied plant lectins are now widely attracting more and more attention from researchers. *S. flavescens* lectin, a legume lectin, induces apoptotic tumour cell death through a death-receptor pathway, which is dependent on caspase activation (37). With specificity to sialic acid, a typical legume lectin purified from *Phaseolus coccineus* L. (*Phaseolus Multiflorus* wild) seed products activate caspase-dependent apoptosis in murine fibrosarcoma L929 cells (38). As a monocotyledonous mannose-binding lectin, *Pinelliaternata* agglutinin has been demonstrated, by nuclear staining assay and DNA fragmentation, to induce apoptosis in human hepatocarcinoma Bel-7404 cells, further revealing its biological and pharmacological activities (39). Pea (*Pisum sativum* L.) lectin treatment results in cell cycle arrest in the G2/M phase of Ehrlich ascites carcinoma cells. In addition, intensive increase of *Bax* gene expression with reduction in *Bcl-2* and *Bcl-X_L* gene expression, is observed in cells treated with Pea lectin (40). Apoptotic cell death induction by mulberry leaf lectin of human breast cancer MCF-7 cells and colon cancer HCT-15 cells, has also been verified to be in a caspase-dependent manner (41). VAA-I, a specific plant lectin found in *Viscum album*, induces the phosphoinositide 3-kinase (PI3K) pathway, thus leading to apoptosis in human hepatocellular carcinoma SMMC-7721 cells (4). Similar to ConA, Canavaliabrsiliensis (ConBr) inhibits tumour cell proliferation by inducing death mechanisms of apoptosis in both human leukaemia MOLT-4 and HL-60 cells (43). *Momordica charantia* lectin (MCL) increases cyto.*c* release, activates caspases-3/-8/-9 and enhances production of cleaved PARP, with regulation of mitogen-activated protein kinase phosphorylation and promotion of downstream nitric

oxide production. This results in apoptosis of nasopharyngeal carcinoma 69 cells (44). Purified from a Chinese herb, galactose-binding lectin AML induces apoptosis in a caspase-dependent manner in chronic myeloid leukaemia K562 cells (45). Belonging to the ricin-B family, ricin has been reported to lead to apoptotic cell death by up-regulating expression of caspase-8 and its downstream caspases-3/-7 in Hodgkin's lymphoma L540 cells (46). Rice bran agglutinin (RBA) is able to inhibit cell proliferation *via* cytotoxic mechanisms involved in caspase activation and apoptotic induction in human promyelocytic leukemia HL-60 cells (47). Abrin also has been found to increase caspase-3 expression and block Bcl-2, thereby inducing apoptosis in murine Dalton's lymphoma ascites cells (48). In addition, AMML, from the roots of *Astragalus mongholius*, has been reported to induce apoptosis in various kinds of cancer cells including HeLa cervical cancer cells, human osteoblast-like MG63 cells and human leukemia K562 cells (49) (Table 1).

Plant lectins and autophagy in cancer

Distinct from apoptosis, autophagy is an evolutionarily conserved, multi-step lysosomal degradation process, in which a cell degrades long-lived proteins and damaged organelles (50). In cancer cells, autophagy might act as a physiological mechanism of temporary survival, however, cell death may occur if cellular stresses result in continuously or excessively induced autophagy. Autophagy may play an important role in attacking cancer by regulating a limited number of autophagy-related gene (ATG) family members (51). The complete autophagic flow is divided into the following five stages: induction, vesicle nucleation, vesicle elongation and completion, docking and fusion, as well as degradation and recycling (52).

Induction of autophagy is initiated by the ULK complex composed of ULK1/2, mAtg13, focal adhesion kinase family interacting protein of 200 kDa (FIP200) and ATG101, which can be inhibited by a negative regulator, mammalian target of rapamycin complex 1 (mTORC1) (53).

Subsequently, proteins and lipids are recruited for construction of the autophagosomal membrane for vesicle nucleation, which is induced by activation of the class III PI3K/Beclin-1 complex (54). Numerous binding partners of this complex function as positive or negative regulators, including Bif-1, Atg14L, UVRAG, Ambr1 and Rubicon (55).

In vesicle elongation and completion, two unique ubiquitin-like conjugation systems may take place. The first pathway involves the covalent conjugation of

Table 1. Plant lectins targeting apoptotic/autophagic signalling pathways in cancer

Name	Apoptosis/autophagy	Target	Cancer cell type	Specific mechanism	References
Concanavalin A	Apoptosis	Caspase, Akt, MMP-9, ERK	Human melanoma A375 cell	Trigger mitochondria-mediated apoptosis in A375 and HepG2 cells	(25–29)
		–	Hepatocellular liver carcinoma HepG2 cell		
	Autophagy	COX-2, Bax/Bcl-2,	Glioblastoma U87 cell	Modulate IKK/NF- κ B-dependent pathway in U87 cells	(28,57–60)
		–	Human ovarian cancer SKOV3 cells	Activate Foxo1a-Bim signaling	
Mistletoe lectin	Apoptosis	BNIP-3,	Glioblastoma U87 cell	Induce autophagy via the interplay between JAK2/STAT3 and MT1-MMP signaling pathway	(17,30–32)
		Atg3, Atg12, PI3K/AKT/mTOR, MEK/ERK	Cervical cancer HeLa cell	Activate autophagy by lowering PI3K/AKT/mTOR expression and induce the MEK/ERK pathway	
		Caspase-3, caspase-8, TNF- α , Bcl-2,	Leukemia U937 cell	Induce apoptosis via the extrinsic apoptotic pathway in Jurkat leukemic T cells	
	Autophagy	MMP, MAPK, p38	Adenocarcinoma	Induce apoptosis through Apaf-1-dependent pathway	(63)
–		Human hepatic carcinoma Hep3B cells	Release cyto. <i>c</i> and increase ROS		
<i>P. cyrtonema</i> lectin	Apoptosis	–	Leukemia U937 cell	Activating SAPK/JNK and p38 pathways, or inhibiting ERK1/2 pathway	(25,33–36)
		mTORC1, PI3K/CIII, Beclin-1, Atg12	hepatocellular liver carcinoma SK-Hep-1 & Hep 3B cell	Reduce cell death by increasing expressions of phosphorylated mTORC1, PI3K/CIII, Beclin-1, Atg12 and active LC3 form	
		Bax, Bcl-X _L , Bcl-2, MMP	Melanoma A375 cell	Induce apoptosis cyto. <i>c</i> release and caspase activation	
	Autophagy	Caspase-3/-8/-9	Cervical cancer HeLa cell	Induce apoptosis via activation of caspase	(61,62)
–		Breast cancer MCF-7	–		
<i>S. flavesces</i> lectin	Apoptosis	–	Murine fibrosarcoma L929 cell	induce apoptosis via a caspasedependent pathway	(37)
		P38, p53, Ras-Raf, PI3KCI/Akt	Melanoma A375 cell	Induce autophagy via a ROS-p38-p53 pathway	
<i>Phaseolus Multiflorus</i> willd	Apoptosis	–	Murine fibrosarcoma L929 cell	Induce autophagy by inhibiting Ras-Raf signaling pathway or PI3KCI/Akt signaling pathway	(38)
		–	Hepatocarcinoma Bel-7404 Cell	Induce apoptosis	
<i>Pinellia ternata</i> agglutinin	Apoptosis	–	Hepatocarcinoma Bel-7404 Cell	Induce apoptosis	(39)
Pea (<i>Pisum sativum</i> L.) lectin	Apoptosis	Bax, Bcl-2, Bcl-X _L	Ehrlich ascites carcinoma (EAC) Cell	Result in cell cycle arrest at G2/M phase of EAC cells	(40)
Mulberry leaf lectin	Apoptosis	Caspase	Breast cancer MCF-7 Cell Colon cancer HCT-15 Cell	Induce apoptotic cell death via a caspase-dependent manner	(41)

(continued)

Table 1 (continued)

Name	Apoptosis/autophagy	Target	Cancer cell type	Specific mechanism	References
VAA-I	Apoptosis	PI3K	Hepatocellular carcinoma SMMC7721 cell	Lead to apoptosis by inducing PI3K pathway	(42)
Canavalia brasiliensis	Apoptosis	–	Leukemia U937 cell	Inhibit tumor cell proliferation by inducing apoptosis	(43)
<i>Momordica charantia</i> lectin	Apoptosis	Caspase-3/-8/-9, MAPK	Nasopharyngeal carcinoma NP-69 cell	Increase cyto. c release, activate caspases-3/-8/-9, enhance production of cleaved PARP, regulate MAPK phosphorylation and promote downstream NO production	(44)
		Caspase-8/-9	Human hepatocellular carcinoma Hep G2 cell	Induce apoptosis through caspase-8 regulated extrinsic and caspase-9 regulated intrinsic caspase cascades	(45)
AML	Apoptosis	Caspase	Chronic myeloid leukemia cell	Induce apoptosis in a caspase-dependent manner	(46)
Ricin	Apoptosis	Caspase-3/-7/-8	L540 human Hodgkin's lymphoma-derived cell	Induce apoptosis by up-regulating caspase-8 and caspase-3/-7	(47)
Rice bran agglutinin	Apoptosis	Caspase	Promyelocytic leukemia U937cell	Inhibit cell proliferation via cytotoxic mechanisms involving caspase activation and apoptosis	(48)
Abrin	Apoptosis	Caspase-3, Bcl-2	Murine Dalton's Lymphoma Ascites cells	Induce apoptosis by increasing caspase-3 expression and blocking Bcl-2 expression	(49)
AMML	Apoptosis	–	Human cervical carcinoma HeLa cell line Leukemia Human osteoblast-like MG63 cell Human leukemia K562 cell	Induce apoptosis	(50)

ATG12 to ATG5 in a reaction that requires ATG7 and ATG10, respectively, while the other occurs via activation of LC3/ATG4.

Docking and fusion of the autophagosome may cause autolysosome maturation, eventually leading to the breakdown of autophagosomal contents (56) (Fig. 2).

Compared to plant lectins involved in apoptosis, there are limited numbers of plant lectins demonstrated to target autophagic signalling pathways in different types of cancer cells. For example, ConA induces autophagy in a Bcl2/E1B-19kDa protein-interacting protein 3 (BNIP3)-mediated way (57). ConA also induces autophagy in hepatoma cells through a pathway mediated by internalization and mitochondria, which also involves mitochondrial interacting protein BNIP3 (58). In addition, ConA-treated glioblastoma U87 cells have shown up-regulation of BNIP3, ATG3 and ATG12, but this induction can be reversed when membrane type-1 matrix

metalloproteinase (*MT1-MMP*) gene is silenced (28). Since the cytoplasmic domain of MT1-MMP is necessary for transducing STAT3 phosphorylation, ConA-induced autophagy by BNIP3 calls for interplay between JAK2/STAT3 and MT1-MMP signalling pathways in U87 cells (59). Moreover, ConA treatment reduces expression of PI3K/AKT/mTOR then induces the MEK/ERK pathway. This activates autophagy in human cervical cancer HeLa cells (60).

Compared to mechanisms involved in PCL-induced apoptosis, PCL also induces autophagy by a similar mitochondria-related ROS-p38-p53 pathway, in human melanoma A375 cells (61). In fibrosarcoma L929 cells, PCL induces autophagy by inhibiting the Ras-Raf and PI3KCI/Akt signalling pathways (62). It is interesting to note that PCL-induced autophagy and PCL-induced apoptosis may connect with each other to participate in determining the type of cancer cell death, since PCL

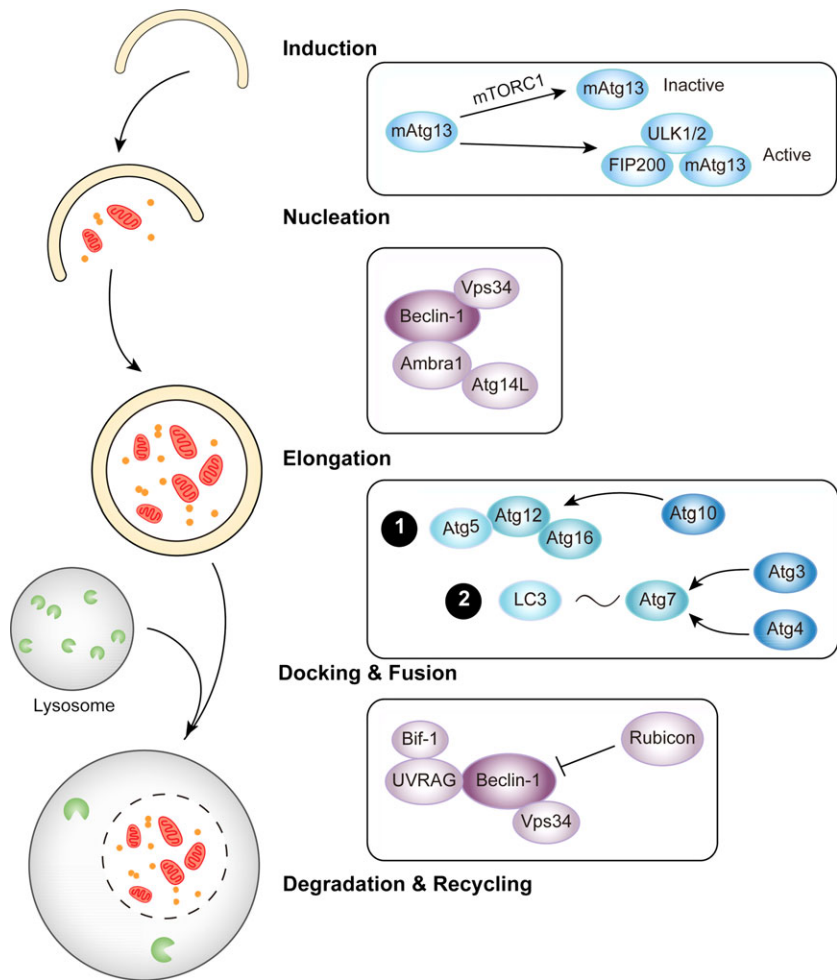


Figure 2. Mechanisms of autophagy in cancer.

promotes the mitochondria-mediated ROS-p38-p53 pathway or blocks Ras-Raf and PI3K-Akt pathways in both these types of phenomena.

Until recently, there has been only one report indicating that ML can also induce autophagy. *Viscum album* L. var. *coloratum* agglutinin (VCA), an agalactose- and *N*-acetyl-D-galactosamine-specific lectin isolated from Korean mistletoe, increases expression of phosphorylated mTORC1, PI3KCIII, Beclin-1, ATG12 and active LC3. Thus, VCA can induce survival factors and reduce cell death by autophagy modulation in human hepatocellular carcinoma HepG2 cells (63) (Table 1).

Plant lectins as potential new emerging anti-cancer drugs

Hitherto, most types of plant lectin have properties of binding distinct sugar-containing receptors on surfaces of various cancer cells, and this special characteristic has enabled them to determine the ultimate fate of cancer

cells. For example, it has recently been shown that PCL binds most of unbound EGFR. It is a key sugar-containing receptor on surfaces of cancer cells. In this way PCL can block EGFR-mediated survival pathways involved in BCR-ABL, PI3K-AKT-mTORC1 and Ras-Raf signalling pathways (64). Thus, due to their specific structures and abilities of binding sugar receptors, plant lectins can induce death in many kinds of cancer cell by targeting apoptotic or autophagic cell death involved in many key signalling pathways. Moreover, there are various connections between apoptotic and autophagic processes that can jointly seal the fate of cancer cells. As mentioned above, with deepening research on mechanisms of plant lectins in cancer, they are currently regarded as candidate anti-cancer drugs for humans, some of which have also been further applied up to pre-clinical or clinical trials for cancer treatment (Fig. 3, 4).

Recent studies have shown that ConA can be used as a therapeutic anti-hepatoma agent since it induces autophagic and immuno-modulatory activities *in vivo*; thus

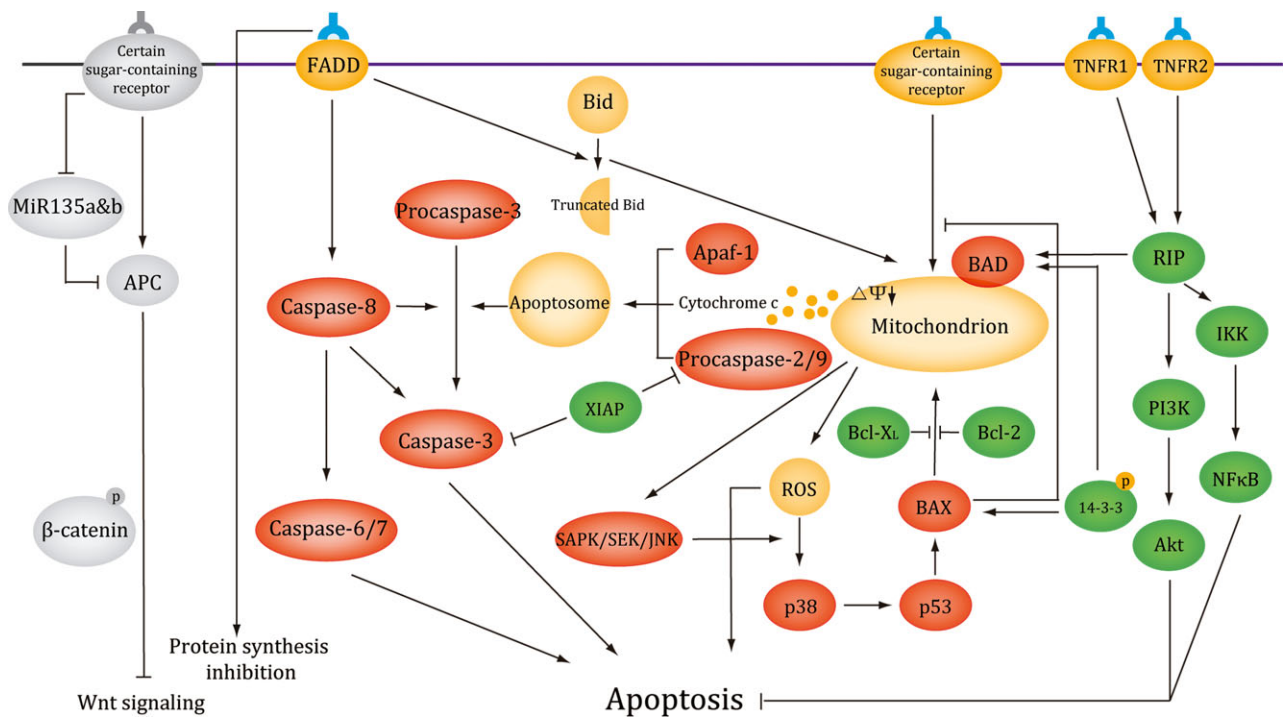


Figure 3. Signalling pathway of plant lectins involved in apoptosis.

ConA can inhibit tumour nodule formation in the liver (65). It has also been found that chemical modification of ConA (PEG-ConA) significantly enhances anti-tumour cytotoxicity of peripheral lymphocytes against B16 melanoma cells (66). In addition, *Phaseolus vulgaris* lectin (PHA), a lectin present in kidney beans remarkably reduces growth of murine non-Hodgkin lymphoma (67). As a typical chitin-binding lectin composed of hevein domains, wheat germ agglutinin is also effective in controlling tumour growth by activating the host immune response (68). It has been shown that alterations exist in carbohydrate structures of cell glycol-conjugates, and these differences can be related to goblet-cell differentiation in normal, benign and malignant tissues. Accordingly, these results and information offer guidance for subsequent clinical trials.

With regard to clinical trials, ML-I has widely been utilized as a potential anti-neoplastic drug or as an adjuvant therapeutic agent during chemotherapy and radiotherapy, and has been used to reduce treatment-associated side effects (69). *Viscum fraxini-2* has been used to treat hepatocellular carcinoma. Fourteen of 23 patients (median age of 54) were reported to have survived treatment; tumour status having been bilateral lobe 8, 34.8%; unilateral lobe 15, 65.2%; distant metastasis 4, 17.4%; hepatic portal vein thrombosis 2, 8.7%; ascites 4, 17.4%, when European mistletoe had been used to treat malignant melanoma. In a further study,

two hundred and twelve of 329 patients (median age of 51.4) survived from with no evidence of tumour enhancement, neither indication of increased frequency nor earlier onset of brain metastases. Recently, further researchers have tested safety and efficacy of European MLs, and European mistletoe (*V. album* L.) extract Iscador (FME) has been tested to be safe during postsurgical aftercare of primary intermediate to high-risk malignant melanoma (UICC/AJCC stage II–III) patients (70). MLs are also used in aqueous injectable solution, benefiting survival rates of patients, anti-tumour activities and low toxicity profiles in clinical trials (71). Further studies in combination with other active agents would be required to determine any the survival rate, tumour remission, overall quality of life and quality of life correlated to side effects, during cyto-reductive therapies.

Previously, plant lectins have been considered to be toxic, however, they have recently been demonstrated to possess inhibitory effects on cancer development. Currently, researchers are working to switch use of plant lectins from detection to actual use for fighting cancer. Hitherto, plant lectins, formerly regarded as simple recognition tools for identifying malignant tumours, have become crucial biomarkers and potential agents for cancer diagnosis and prognosis. Accumulating lines of evidence have revealed that targeting important key apoptotic and autophagic signalling

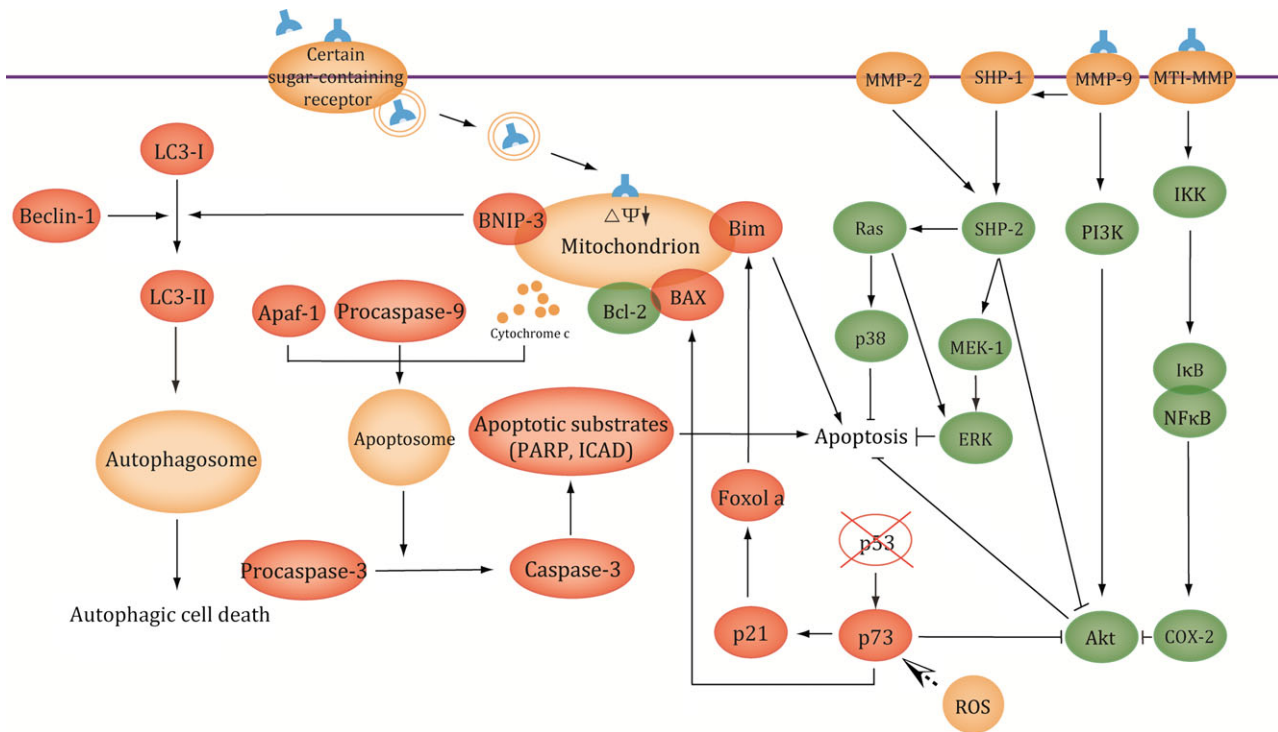


Figure 4. Signalling pathway of plant lectins involved in autophagy.

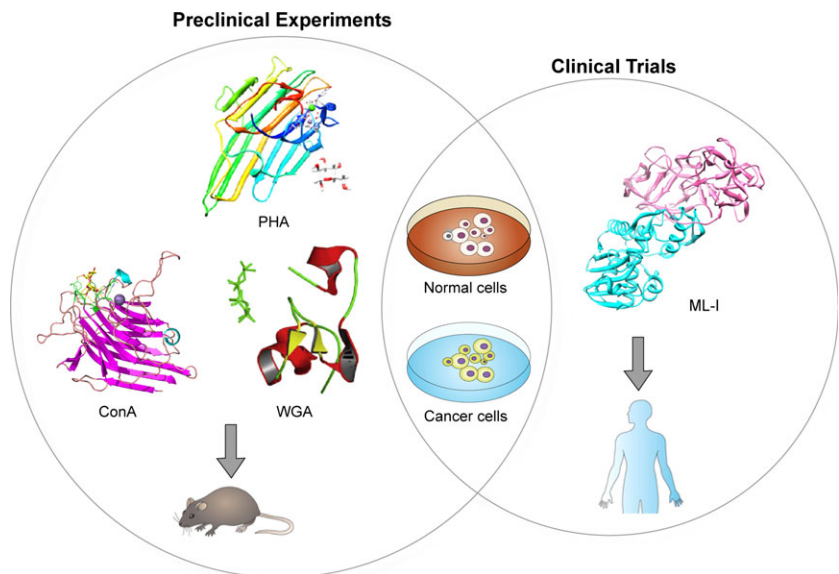


Figure 5. Potential therapeutic roles of plant lectins in cancer.

pathways may be a promising avenue for potential therapeutic purposes. Interestingly, some plant lectins such as PCL have been shown to induce apoptosis and autophagy *via* the same mechanisms, thus dealing with matters related to carcinogenesis. Due to plant lectins

having natural toxicity and drug-resistant production, direct mutations could be used to modify them, designing them into synthetic ‘ideal’ candidate anti-tumour drugs with higher efficiency and lower toxicity for their therapeutic purposes (Fig. 5).

Conclusions

Plant lectins, highly diverse carbohydrate-binding proteins of non-immune origins, are widely distributed in different plant species. They have previously been used as simple recognition tools for identifying malignant tumours, but have gradually developed into important biomarkers for cancer diagnosis and prognosis and potential agents for cancer therapeutics. Moreover, due to their widely accepted anti-proliferative activities, plant lectins may exhibit complicated mechanisms, especially involved in apoptotic and autophagic signalling pathways. Plant lectins may also determine the fate of cancer cells mainly in three ways: (i) inactivate ribosomes of cancer cells; (ii) selectively localize to organelles in cancer cells; (iii) bind certain sugar-containing receptors on surfaces of cancer cells. Thus, plant lectins are able to deal with many types of cancer cells by targeting several core apoptotic and autophagic pathways according to these three possible avenues. However, our understanding of how plant lectins play their important roles in cancer cells still remains limited, and thus there is an urgent need for more additional information. Further discoveries will be prompted by new methods such as X-ray crystallography and nuclear magnetic resonance, which could harness plant lectins for cancer drug discovery. In addition, protein–protein interaction can be used to screen potential candidate drugs. The best hopes for targeting cancer-related pathways in potential therapeutic applications lie in the discovery of numerous useful agents to target physiological effects of altered key pathways and the whole cancer network rather than their individual genes or proteins.

Thus, based on their specific structures and binding sugar receptor abilities, plant lectins can kill many types of cancer cells by targeting autophagic death involved in many key signalling pathways. With biochemical and molecular complexities of apoptotic and autophagic pathways becoming better understood, new therapeutic strategies will be developed and initiate other therapeutic strategies. Additional research, such as clinical trials into mechanisms of action at the molecular level, will help cancer scientists and clinicians to further understand the therapeutic effects, nutritional benefits and toxic consequences of plant lectins. Together, these findings provide a comprehensive perspective for further elucidating the role of plant lectins that may target apoptotic and autophagic cell death pathways as potential agents in cancer pathogenesis and therapeutics.

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