cDNA cloning of a novel lectin that induce cell apoptosis from *Artocarpus hypargyreus*

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[ABSTRACT] We isolated a novel lectin (AHL) from *Artocarpus hypargyreus* Hance and showed its immunomodulatory activities. In this study, the amino acid sequence of AHL was determined by cDNA sequencing. AHL cDNA (875bp) contains a 456-bp open reading frame (ORF), which encodes a protein with 151 amino acids. AHL is a new member of jacalin-related lectin family (JRLs), which share high sequence similarities to KM+ and Morniga M, and contain the conserved carbohydrate binding domains. The antitumor activity of AHL was also explored using Jurkat T cell lines. AHL exhibits a strong binding affinity to cell membrane, which can be effectively inhibited by methyl-α-D-galactose. AHL inhibits cell proliferation in a time- and dose-dependent manner through apoptosis, evidenced by morphological changes, phosphatidylserine externalization, poly ADP-ribose polymerase (PARP) cleavage, Bad and Bax up-regulation, and caspase-3 activation. We further showed that the activation of ERK and p38 signaling pathways is involved for the pro-apoptotic effect of AHL.

[KEY WORDS] Lectin; cDNA cloning; Apoptosis; Antitumor drug; *Artocarpus hypargyreus*

[Introduction] Lectins are carbohydrate-binding proteins existing in many species [1]. Because of their carbohydrate-binding specificities, lectins are involved in important biological processes, including tissue development, cellular communication, embryogenesis, and pathogen recognition [2]. In addition, many lectins have been used in biological and biomedical research [3]. Recently, lectin microarray has been developed as a powerful tool for cell biology [4-5]. Plant lectins are classified into 12 carbohydrate-binding domains (families) based on their molecular structures and carbohydrate-binding specificities. Members belonging to each family share several common properties. Jacalin-related lectin (JRL) is a sub-group of lectins widely present in plants.

Jacalin, the prototype of JRLs, was first isolated from seeds of jack fruit (*Artocarpus integrifolia*; Moraceae) [6]. Subsequently, JRLs have also been isolated and characterized from various plant families of angiosperms, including Convolvulaceae, Asteraceae, Gramineae, Musaceae, Fagaceae, and Mimosaceae [7-9]. Moreover, some jacalins were also found in algae (*Boodlea coacta*, *Oscillatoria agardhii*), cyanobacteria, and sea worm (*Serpula vermicularis*) [10]. A majority of these JRLs with low sequence similarities among each other are secreted into the extracellular compartments. Only three and four JFRs genes have been identified in human and mouse, respectively. For example, zymogen granule protein 16 (ZG16), a secretory jacalin-related lectin, mediates condensation-sorting of pancreatic enzymes to the zymogen granule membrane in pancreatic acinar cells [11]. According to the sugar specificities, JRL family is divided into two types: galactose-binding jacalins (gJRLs) and mannose-binding jacalins (mJRLs) [12]. While the documented occurrence of gJRLs is confined to the family Moraceae, mJRLs are widespread in the plant kingdom.

The genus *Artocarpus* (Moraceae) contains about 50 species widely distributed in tropical and subtropical regions [13]. It is known as a rich source of flavonoids and other phenolic compounds with different physiological activities [14]. It has been used as traditional folk medicine against a number of diseases, including inflammation, cirrhosis, hypertension, malarial fever, dysentery, tuberculosis and diabetes mellitus [14-19]. *Artocarpus hypargyreus* Hance, an evergreen tree growing in South China, is a Chinese herb used for treatment of headache, jaundice and rheumatism. We previously...
found that *Artocarpus hypargyreus* lectin (AHL) was isolated and purified from the seeds of *Artocarpus hypargyreus*. It is a hetero-tetrameric glycoprotein with a molecular weight of 65.2 kDa [16]. Furthermore, we described its physicochemical properties, including its thermostability, pH stability, and carbohydrate specificity, which indicate that AHL is able to bind to *O*-linked or *N*-linked glycoproteins which contain methyl-α-D-galactose (Me-Gal), N-acetyl-D-galactosamine, methyl-α-D-mannose or galactose [18]. However, the genetic sequence of AHL has yet to be determined.

In the study, we reported the cloning of cDNA encoding a novel lectin from *Artocarpus hypargyreus* Hance and its effect on tumor cells. The cDNA sequence and the corresponding amino acid sequence of AHL showed high homology with two JRL members (*KM*+ and *Morniga M*), revealing that AHL is a novel JRL member. In addition, we also found that AHL displayed anti-proliferative effect on Jurkat T leukemia cells by inducing apoptosis, involving p38 and JNK phosphorylation. The anti-tumor effects of AHL suggested its potential medicinal applications.

**Materials and Methods**

**Materials**

The seeds and fully developed leaves of *Artocarpus hypargyreus* Hance were collected and identified by Professor YU Li-Ying from Guangxi Botanical Garden of Medicinal Plants in August 2015. The voucher specimens were deposited in Guangxi Key Laboratory of Biological Molecular Medicine Research.

**Isolation of total cellular RNA from Artocarpus hypargyreus Hance leaves and cDNA synthesis**

The fresh soft leaves were powdered with pestle in liquid nitrogen. Total cellular RNA was isolated using EasyPure Plant RNA Kit (Trans Gen Biotech, Beijing, China) according to the manufacturer’s protocol. The quality of total cellular RNA was evaluated on 1% agarose gel electrophoresis.

cDNA was reversely transcribed by TransScript First-Strand cDNA Synthesis kit (Fermentas, Vilnius, Lithuania) according to the manufacturer’s protocol. The synthesized cDNA was used as a template for PCR cloning.

**Cloning and sequencing of full-length cDNA**

Initially, gradient PCR was performed using the cDNA prepared above as template, with the Primers (AHL-F: 5′-AT-GGGTCCTTACTGGAAA-3′; and AHL-R: 5′-TGAAAGCGCAATVGCATCC-3′) designed according to the mRNA sequence of the conservative domains of Jacalin-related lectins (*KM*+ or *Morniga M*) according to the conservative region from AHL. The obtained PCR products were separated by electrophoresis on 1% agarose gel, and band was then excised and purified with DNA purification kit. The purified PCR product was ligated with pMD18-T vector (TaKaRa, China) using a standard protocol and then the positive clones were selected and sequenced by Sangon Biotech (Shanghai, China).

Having isolated a partial sequence, the 5′ and 3′ ends of mRNA were obtained by rapid amplification of cDNA ends (RACE) methods, using gene-specific primers according to the sequence of the conservative region of AHL. The gene-specific primers used for 5′-RACE were *AHL*5-1 (5′-TACAGGTCTCCTCACCCCAAGGGA-3′) and *AHL*5-2 (5′-GGTCTCTTTCACACCCCAAGGGA-3′) and for 3′-RACE were *AHL*3-1 (5′-GGCAGACCTTCTTCAACTC-3′) and *AHL*3-2 (5′-GCCCTGGGGAAGGAGGATTAA-3′) and for 3′-RACE were *AHL*3-1 (5′-GGCAGACCTTCTTCAACTC-3′) and *AHL*3-2 (5′-GCCCTGGGGAAGGAGGATTAA-3′). 5′-cDNA and 3′-cDNA were synthesized using the SMART™ RACE kit (Clontech, USA). PCR was performed with *AHL*5-1, *AHL*3-1 and a universal primer mix ([UPM] as supplied with the SMART RACE cDNA Amplification Kit) and the PCR amplifications used the previous PCR product as templates for a subsequent nested PCR with the NUP primer supplied in the kit together with *AHL*5-2 and *AHL*3-2. The PCR fragments were cloned into the pMID18-T vector (TaKaRa, China) using a standard protocol and then the positive clones were selected and sequenced by Sangon Biotech (Shanghai, China).

The full-length cDNA of *AHL* was obtained by combining the conserved sequence with the 5′-terminal and 3′-terminal sequences. On the basis of this assembled sequence, the sequence of the open reading frame (ORF) was obtained from the completed cDNA using Vector NTI, a Bioinformatics software package.

**Sequence analysis**

Similar studies were carried out with BLASTn and BLASTp algorithms at the NCBI server (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi), as well as FASTA3 and PSI-BLAST programs at the EBI server (http://www.ebi.ac.uk/Tools/similarity.html). The deduced amino acid sequence of AHL was analyzed using online proteomics tools at the ExPASy server (http://www.expasy.org/tools/). A Neighbor Joining (NJ) tree was constructed using the MEGA5.0 software package and the CLUSTAL algorithm in conjunction with the amino acid sequences of known jacalin-related lectins [17].

**Purification of Artocarpus hypargyreus lectin**

AHL was isolated from the seeds of *Artocarpus hypargyreus* according to the method described previously by Zeng et al. [16]. AHL was conjugated with fluorescein isothiocyanate (FITC) as previously described by Cui et al. [18].

**Cell culture**

The human leukemic cell line Jurkat T was purchased from the Cell Bank of the Chinese Academy of Sciences, and maintained in RPMI 1640 medium (Hyclone, USA) containing 10% fetal cow serum (FCS), 100 unit/mL penicillin, and 100 μg mL⁻¹ streptomycin in cell culture incubator at 37 °C in 5% CO₂ before use.

**Analysis of lectin binding**

Binding assay was also detected by confocal microscopy. Cells were incubated in FITC-AHL for 1 h at 4 °C, followed by fixing with 2% para-formaldehyde for 10 min. Samples were stained with 4′,6-diamidino-2-phenylindole (DAPI) and...
visualized with confocal laser scanning microscopy (Zeiss LSM 510, Göttingen, Germany).

**Cell proliferation assay**

Cells (2.5 × 10^4 cells/mL) were plated in a 96-well plate and incubated with different concentrations of AHL (0–150 μg mL⁻¹) at 37 °C in a CO₂ incubator for 48 h. For examining its competitive effect on binding to carbohydrate, AHL (25 μg mL⁻¹) was pretreated with 20 mmol L⁻¹ Me-Gal for 1 h as the described above. We then used cell counting kit-8 (CCK-8) (Sigma, St. Louis, MO, USA) to assay cell proliferation and calculated the proportion of viable cells and expressed the value as the percentage of control group, which was taken as 100%. Finally, we calculated the half maximal inhibitory concentration 50 (IC₅₀) with Probit regression analysis program using SPSS 13.0 software (SPSS Inc., USA).

**Annexin-V and PI staining**

After being treated with AHL at different concentrations for 24 h, cells were stained with Annexin V-fluorescein isothiocyanate (V-FITC)(BD Phamingen, CA, USA) and propidium iodide (PI) at room temperature for 15 min. Apoptotic cells were analyzed with a FACS Calibur flow cytometer (Becton Dickinson, Mountain View, CA, USA).

**Western blotting**

Cell lysates were prepared and analyzed by Western blotting. Primary antibodies specific for Poly-ADP-ribose polymerase (PARP), Bax and Bad (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), caspase-3, extracellular signal-regulated kinase (ERK) 1/2, c-jun N-terminal kinase (JNK) 1/2 and p38 (Cell Signaling Technology Inc., Danvers, MA, USA) in combination with horseradish peroxidase conjugated secondary antibodies (Cell Signaling Technology Inc., USA). The horseradish peroxidase activity was detected using SuperSignal West Femto Maximum Sensitivity Substrate (Pierce Biotechnology, Waltham, MA, USA).

**Statistical analysis**

GraphPad Prism 3.0 was applied to analyze the data. One-way or two-way ANOVA was applied to compare the mean values between groups with Bonferroni’s post hoc tests. P < 0.05 were regarded as statistically significant.

**Results**

**Cloning and sequencing of AHL-cDNA**

A partial sequence with 379-bp of a novel lectin was obtained from *Artocarpus hypargyreus* by reverse transcription-polymerase chain reaction (RT-PCR) using primers based on the conserved regions of lectins of other plants (see methods). BlastX analysis showed that the sequence exhibited a close homology with known lectins. Subsequently, a 392-bp fragment of the 5′-terminal and a 493-bp fragment at the 3′-terminal were obtained by 5′ and 3′ rapid amplification of cDNA ends (RACE), respectively. After analysis of the extended sequence, an 875-bp full-length cDNA containing a 456-bp open reading frame (ORF) from 109 to 564 bp was identified (Fig. 1A). The ORF encodes a peptide bearing 151 amino acid residues which theoretical isoelectric point (pI) of 5.61 and a calculated molecular mass of 16.4 kDa, respectively. The 151 amino acid residues include a jacalin domain (amino acids 16–149) with sugar-binding site according to NCBI BLAST (Fig. 1B, sugar-binding site were indicated by triangles).

Using the BLAST program, similarity searches were performed using the *AHL* cDNA sequence as the bait, which showed a 71% to 82% identity to other lectins. BLASTp searches of the non-redundant protein database in GenBank also showed that *AHL* had the highest sequence similarity with KM+ (*Artocarpin*, 68% identity) and *Morgina M* (66% identity) (Fig. 2). The high sequence similarity between these jacalin-related lectins strongly suggests that *AHL* belongs to the same superfamily of lectins. A total sequence analysis indicated that these proteins contain the potential sugar-binding site (Fig. 2). All the features mentioned above are consistent with the suggestion that this gene encodes a novel member of the jacalin-related lectin family. We therefore designated this gene *AHL* (GenBank accession No. KY924610).

**Phylogenetic analysis of AHL**

JRLs are classified as galactose-specific (gJRLs) and mannose-specific (mJRLs) according to their sugar-binding specificities, which are related to the carbohydrate recognition regions of jacalin-related lectins [19,20]. To analyze the evolutionary relationship of *AHL* with other jacalin-related lectins, we constructed a phylogenetic tree of *AHL* homologs based on a Neighbor-Joining (NJ) method by MEGA5.0 (Fig. 3). In the clade, KM+ (AAY35063.1) and *Morgina M* (AAL10685.1), two mannose-binding lectins formed a separate cluster together with *AHL* (ASK06165.1), indicating that *AHL* is a novel member of the JRLs and contains mannose recognition regions. Furthermore, it was found that *AHL* and...
Fig. 2  Alignment of the putative amino acid sequences of AHL with those of galactose-specific and mannose-specific JRLs. Multiple alignment was achieved using the software ClustalX and DNAMAN. AAY35063.1 and AAY35064.1 are the GeneBank accession numbers of KM+ including two isololcits from Artocarpus integer in NCBI. AHW81907.1 and AAL10685.1 are the GeneBank accession numbers of Mulberry and Moriga M from Morus alba var. atropurpurea and Morus nigra, respectively. AAA32678.1, AAA32680.1, AAA32679.1, AAA32771.1 and AAL09163.1, AAM90088.1 are the GeneBank accession numbers of all four isololcits of jacinil from Artocarpus integer and two isololcits of Moringa from Morus nigra deposited in NCBI, respectively. The inverted boxes indicate identical amino acid residues. The asterisks denote the carbohydrate-recognition site of the jacinil-related lectins. KM+ from Artocarpus integrifolia were clustered in the same group, indicating that they are closely related.

Binding of AHL to Jurkat T cells
We performed cell-based assay to evaluate the binding of
AHL on Jurkat T lymphocytes. AHL binding to Jurkat T cells stained with FITC-AHL was visualized under confocal microscopy (Fig. 4). These cells were also analyzed with flow cytometry, and the result also indicates that AHL strongly bonds to Jurkat T cells, and the binding of AHL to these cells was significantly reduced by pre-treatment with Me-Gal at 20 mmol·L⁻¹ (data not shown). These results demonstrated that AHL receptors were expressed at high-level on the surface of Jurkat T cells, where the function of AHL may be mediated by increasing with the glycans.

**AHL inhibited proliferation of Jurkat T cells**

To examine the effect on the growth of Jurkat cell, we treated cells with AHL and found that AHL significantly inhibited the proliferation of these cells in a time- and dose-dependent manner as revealed by CCK-8 tests (Figs. 5A, 5B), and the IC₅₀ of AHL on Jurkat cells was estimated to be 56.75 μg·mL⁻¹. However, in the presence of 20 mmol·L⁻¹ Me-Gal, AHL-mediated inhibition of proliferation was significantly attenuated (Fig. 5C) (*P < 0.05*). These results suggest that AHL induces cell death via apoptosis in a time- and dose-dependent manner by binding with gal-specific glycoproteins on cell surface.

**AHL-induced apoptosis in Jurkat cells**

For gaining further insight into the mechanism underlying AHL-mediated inhibition of proliferation, first we examined the changes in the nuclear morphology in AHL-treated cells stained with Hoechst 33258 and observed significant and clear nuclear condensation and nuclear fragmentation in AHL-treated cells (Fig. 6A). We then used Annexin V/PI double staining to further evaluate AHL-induced apoptosis and found that increased apoptosis was induced by AHL in a dose-dependent manner. The graph presented the per-

**Fig. 3** Dendrogram indicating the phylogenetic relationships of AHL with other jacalin-related lectins. Jacalin is a galactose-binding lectin from Artocarpus integer; MPA, galactose-binding lectin from Maclura pomifera; Morniga G, galactose-binding lectin from Morus nigra; KM+, mannose-binding lectin from A. integr; Morniga M, mannose-binding lectin from M. nigra; Oryzata, mannose-binding lectin from Oryza sativa; Heltuba, mannose-binding lectin from Helianthus tuberosus; Calsepa, mannose-binding lectin from Calyxpetea sepium; Parkia, mannose-binding lectin from Parkia platycephala; PALb, mannose-binding lectin from Phlebodium aureum; PALa, mannose-binding lectin from Phlebodium aureum

**Fig. 4** Binding of FITC-AHL to Jurkat T cells. Jurkat T cells were stained with both DAPI and FITC-AHL and then visualized under confocal microscopy. Magnification 40×

**Fig. 5** Effect of AHL on the proliferation of Jurkat T cells. (A) Jurkat T cells were incubated with AHL (25 μg·mL⁻¹) for indicated time points. Cell viability was measured with CCK-8 test. *P < 0.05, **P < 0.01 vs control. (B) Jurkat T cells were incubated with AHL at the indicated concentrations (0–150 μg·mL⁻¹) for 48 h. Cell viability was assayed with CCK-8 assays. *P < 0.05, **P < 0.01 vs control. (C) Cells were incubated with AHL (25 μg·mL⁻¹) alone or pre-incubated with Me-Gal (20 mmol·L⁻¹), and then cell viability was analyzed with CCK-8 test. *P < 0.05 vs control
Fig. 6  AHL-induced apoptosis of Jurkat T cells. (A) Changes in nuclear morphology in AHL-treated Jurkat T cells. Jurkat T cells were incubated with AHL (25 μg·mL$^{-1}$) for 48 h, and then stained with Hoechst 33258. Control cells (left panel) and AHL-treated cells (right panel). Apoptotic nuclei were shown by arrows. (B) AHL-induced dose-dependent apoptosis. Jurkat T cells were incubated with AHL at the indicated concentrations for 48 h and then stained with Annexin V and PI. X-and Y-axis are Annexin V-positive cells and PI-positive cells, respectively. The data were presented as mean ± SD (n = 3) (right panel). *P < 0.05, **P < 0.01 vs control cells. (C) Jurkat T cells were incubated with AHL (25 μg·mL$^{-1}$) for the indicated time points and the expression levels of Bax and Bad were measured by Western blot. (D) Cell lysates of Jurkat T cells were incubated with AHL for 24 h and the cleavage of PARP and Caspase-3 was detected by western blot. (E) AHL activated ERK and p38. Cells were incubated with AHL (25 μg·mL$^{-1}$) at the indicated time points and the phosphorylation levels of JNK, ERK and p38 were assayed by western blot.
residues of AHL, consisting mainly of aspartic, nonpolar and polar residues, were important for constructing the sugar binding, which was the common structural feature of this family. The phylogenetic tree indicated that AHL and KM formed a separate cluster comparing to other species. This may be due to the reason that they belong to the most primitive group. KM is a mannose-binding JRL. By analyzing the phylogenetic relationships of sugar-binding site of AHL, we demonstrate that AHL belongs to JRLs similar to KM from the seed of jack fruit (*Artocarpus integifolia*) [22].

Lectins are a group of proteins with specific sugar-binding specificity. Their multiple functions are tightly correlated with carbohydrate-binding types, and their activities are usually suppressed by mono- and oligo-saccharides [23]. This study presents a novel lectin in seeds of *Artocarpus hypargyreus*, a moraceae with important applications in traditional folk medicine. Our previous study showed that the AHL activity was suppressed by carbohydrates, such as methyl-galactose, methyl-mannose, N-acetyl-D-galactosamine, and D-galactose [16], which possibly impeded the binding of lectins with the plasma membrane via competition. This is supported by the finding that AHL exhibits the binding to the cell surface and its binding is inhibited in the presence of Me-Gal. We further used the FITC-conjugated AHL and observed the cellular localization of AHL, and found that intense fluorescence appeared in the cytoplasm (data not shown), demonstrating that AHL can enter the cells by passing through the plasma membrane mediated by mannose-specific glycoprotein(s).

Lectins are able to bind to the specific carbohydrate and regulate cell proliferation. The different amino acid sequence may explain their different biological activities. Recent reports showed that Jacalin could reduce the preneoplastic lesions in carcinogen-exposed mice, and that this anti-carcinogenic activity was associated with decreased proliferation of colonic epithelial cells and stromal COX-2 expression and also with the increased intestinal production of TNF-α [24]. Jacalin also polarizes macrophages toward the antitumor phenotype through binding to macrophage surface and inducing the release of proinflammatory cytokines via NF-κB signaling [25]. Some other lectins have also been reported to show the antitumor activity [26], but the underlying mechanisms remain unclear and need to be elucidated. *Rhizoctonia bataticola* lectin induces apoptosis of leukemia cells in caspase-8-dependent manner [27]. Pea lectin inhibits proliferation of human colon cancer SW480 and SW48 cells by inducing apoptosis in intrinsic mitochondrial pathway [28]. However, peanut agglutinin was reported to be able to mimics the actions of endogenous galectin-3 and promotes the metastatic spread of cancer cells by interaction with cancer-associated TF/MUC1 [29]. Significant efforts have been made in screening and finding out promising anti-tumor drugs capable of inducing apoptosis of cancer cells and in elucidating the underlying mechanisms of their actions.

In this study, AHL displayed a dose-and time-dependent anti-proliferation activity by inducing apoptosis in Jurkat cells. As shown in Fig. 6E, phosphorylation levels of ERK, p38 and JNK in AHL-treated cells. As shown in Fig. 6E, phosphorylation levels of ERK1/2 and p38 peaked after 30 min and 60 min of treatment, respectively, whereas no change in JNK phosphorylation level was observed in Jurkat cells. These results indicate that both phosphorylated ERK and p38 but not JNK may be involved in AHL-induced apoptotic signaling.

**Discussion**

Plant lectins are known for their significant potentials in medical and clinical applications. So far, JRLs have been found extensively in plants and have been classified as galactose-specific (gJRLs) and mannose-specific (mJRLs) according to their sugar specificities [19-20]. In our previous study, AHL has been reported as a lectin isolated from the seeds of *Artocarpus hypargyreus* [16]. In the present study, we obtained the completed cDNA sequence of AHL, which shows high similarity with the known sequence of JRLs, indicating that AHL is a new member of JRLs. The deduced amino acid sequence indicated no signal sequence using the SignalP 4.1 server (http://www.cbs.dtu.dk/services/SignalP/). Furthermore, the analysis of the putative amino acid sequence showed that its partial structure harbored the sequences of JRLs. The bioinformatics analysis found that AHL is highly identical to known JRLs, such as KM+ and Morniga M. These results strongly indicate that AHL is a new member of JRLs.

Multiple sequence alignment of AHL with previously isolated JRLs indicated that there were extensive sequence homologies with KM+ and Morniga M. Six key amino acid residues of AHL, consisting mainly of aspartic, nonpolar and polar residues, were important for constructing the sugar binding, which was the common structural feature of this family. The phylogenetic tree indicated that AHL and KM formed a separate cluster comparing to other species. This may be due to the reason that they belong to the most primitive group. KM is a mannose-binding JRL. By analyzing the phylogenetic relationships of sugar-binding site of AHL, we demonstrate that AHL belongs to JRLs similar to KM+ from the seed of jack fruit (*Artocarpus integifolia*) [22].

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cells. However, AHL activity can be inhibited in the presence of Me-Gal likely due to the competition for binding, indicating that AHL activity depends on lectin-carbohydrate interaction of glycoprotein, presenting a good strategy to modulate AHL activity by introducing or getting rid of specific carbohydrates.

Bel-2 family has been well known to be involved in the mitochondrial-dependent intrinsic apoptosis pathway. Bel-2 family is generally divided into three subgroups: (1) the pro-apoptotic proteins (e.g. Bak and Bax); (2) the anti-apoptotic proteins (e.g. Bel-xL, Bel-2, Bcl-2); (3) the BH3-only proteins (e.g. Noxa, PUMA and Bid). Bax, the firstly identified pro-apoptotic protein member of the Bel-2 family, is capable of translocating to the mitochondria, where it induces release of cytochrome c, causing apoptosis. Our results showed that AHL activated caspase-3, leading to up-regulation of Bad and Bax and the cleavage of PARP, indicating that AHL is able to induce a caspase-dependent apoptosis.

Mechanisms underlying lectins’ anti-tumor activities are diverse and multi-faced. For example, ConA was found to accumulate within mitochondria after passing through the plasma membrane, and sequentially to decrease Akt phosphorylation. While lectin of Polygonatum cyronema was found to induce mitochondrial permeability transition pore (MTP) collapse by regulating the expression of members of Bel-2 family, resulting in a caspase-dependent apoptosis via inhibition of ERK, PI3K-Akt ROS-mediated p38-p53 pathways. Furthermore, activation of JNK and p38 enhanced apoptotic and activation of ERK was associated with both pro-tumor and anti-tumor activities. Our studies showed that AHL potently induced ERK and p38 activation but did not induce JNK phosphorylation, indicating that MAPK signaling is likely involved in AHL-induced apoptosis.

In summary, we determined the full-length cDNA sequence encoding a lectin from Artocarpus hypargyreus Hance for the first time. Analysis of the amino acid sequence of AHL revealed that it is a novel member of JRLs family. Our cell assay experiments demonstrated that AHL is capable of enhancing anticancer efficacy by inducing caspase-dependent apoptosis. These findings pave the foundation for further evaluation and potential application of AHL for anti-tumor therapy.

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