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A nuclear ligand MRG15 involved in the proapoptotic activity of medicinal fungal galectin AAL (*Agrocybe aegerita* lectin)

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ABSTRACT

Background: We have previously reported a novel fungal galectin *Agrocybe aegerita* lectin (AAL) with apoptosis-induced activity and nuclear migration activity. The importance of nuclear localization for AAL's apoptosis-induced activity has been established by mutant study. However, the mechanism remains unclear.
Methods: We further investigated the mechanism using a previously reported carbohydrate recognition domain (CRD) mutant protein H59Q, which retained its nuclear localization activity but lost most of its apoptotic activity. The cell membrane-binding ability of recombinant AAL (rAAL) and H59Q was analyzed by FACS, and their cellular partners were identified by affinity chromatography and mass spectroscopy. Furthermore, the interaction of AAL and ligand was proved by mammalian two-hybrid and pull down assays. A knockdown assay was used to confirm the role of the ligand.
Results: The apoptotic activity of AAL could be blocked by lactose. Mutant H59Q retained comparable cell membrane-binding ability to rAAL. Four cellular binding partners of AAL in HeLa cells were identified: glucose-regulated protein 78 (GRP78); mortality factor 4-like protein 1 (MRG15); elongation factor 2 (EEF2); and heat shock protein 70 (Hsp70). CRD region of AAL was required for the interaction between AAL/mutant AAL and MRG15. MRG15 knockdown increased the cells' resistance to AAL treatment.
Conclusion: MRG15 was a nuclear ligand for AAL in HeLa cells. These data implied the existence of a novel nuclear pathway for the antitumor activity of fungal galectin AAL.
General significance: These findings provide a novel explanation of AAL bioactivity and contribute to the understanding of mushroom lectins' antitumor activity.

1. Introduction

Medicinal mushrooms have long been used in traditional therapies in Asian countries [1,2]. Over the past 30 to 40 years, the pharmaceutical value of medicinal mushroom extracts has prompted studies on the potent and unique properties of their bioactive compounds, which include polysaccharides, proteins, nucleic acids, and trace elements [1,2].

Among the proteins, the largest family are the lectins, which are purified from a wide variety of medicinal mushrooms [3,4]. Mushroom lectins are di- or multivalent carbohydrate binding proteins or glycoproteins. Many of them display immunomodulatory and antitumor/cytotoxic activities [5], including *Grifola frondosa* lectin (GFL) [6], *Agaricus bisporus* lectin (ABL) [7], *Volvariella volvacea* lectin (VVL) [8], *Kurokawa* lectin [9], and *Clitocybe nebularis* lectin (CNL) [10] etc. Although this largely untapped fungal resource has already been reported to contain hemagglutinins [11], research focusing on the medicinal function of mushroom lectins is still limited.

Furthermore, the antitumor mechanism of lectins remains unclear. Most lectins are implicated in cell regulation through binding to the cell membrane [12,13], and few are reported to be able to translocate into the cytosol and exert their function. ABL, the one lectin whose antiproliferation mechanism is well defined, was reported to accumulate in the perinuclear region of HT29 colon cancer cells, blocking the nuclear protein import via the cytoplasmic ligand oxygen-regulated protein 150 (Orp150) [14,15]. Previously, we reported that *Agrocybe aegerita* lectin (AAL) strongly inhibited the growth of human tumor cell lines HeLa, SW480, SGC-7901, MGC80-3, BGC-823, HL-60 and mouse sarcoma S-180. *In vivo* studies showed that AAL inhibited S180 tumor growth in tumor-bearing mouse model mainly through inducing apoptosis proved by TUNEL analysis [16]. Moreover, AAL could migrate into the nucleus of HeLa cells and induce cell apoptosis [5].

Galectins are a sub-family of lectins with an affinity for β-galactosides, and membership in the galectin family requires

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fulfillment of following criteria: affinity for β-galactosides, significant sequence similarity and seven conserved residues in CRD region [17,18]. We have cloned the cDNA of AAL and revealed that it belonged to galectin family for the conserved CRD motif and lactose binding character [19]. The hemagglutination inhibition assay indicated that AAL bound specifically to lactose, bovine submaxillary mucin, glycophorin A, and hog gastric mucine but not to D-galactose, D-fucose and D-xylose etc. [20]. To exploit the antitumor activity of AAL, several mutant proteins were generated based on crystallographic analysis [21], including CRD mutant proteins H59Q and R63H used in this paper. Mutagenesis assays revealed that mutant proteins that were unable to localize to the nucleus lost the apoptotic activity, indicating the existence of intracellular partners mediating the biological process.

Here, we employed mutant protein H59Q to investigate the mechanism of AAL's proapoptotic activity and to identify the nuclear ligand(s). Using affinity chromatography and mass spectroscopy, several interaction partners were identified, and of these, MRG15 was shown to be one of AAL's nuclear ligands. This paper provided a novel mechanism of mushroom lectins' proapoptotic activity.

2. Materials and methods

2.1. Antibodies

Rabbit anti-MRG15 polyclonal antibody was a gift from Professor Olivia Pereira-Smith of the University of Texas Health Science Center at San Antonio (UTHSCSA). GRP78 (P641) and EEF2 (T56) antibodies were obtained from ABZOOM (ABZOOM BIOLABS, Dallas, TX, USA). Hsp70 antibody was purchased from Boster Biological Technology (Wuhan, Hubei, China).

2.2. Annexin V staining and FACS analysis

Expression and purification of rAAL and mutant proteins were performed as described previously [5]. rAAL and lactose (120 μM) were applied exogenously in the HeLa cells' culture medium for 36 h. The HeLa cells were then trypsinized, washed twice with ice-cold PBS (pH 7.4) and assayed for apoptosis using the Annexin V/PI apoptosis kit as described previously [5].

2.3. rAAL and H59Q binding assay

Cells (6×10^5) were suspended by PBS containing 2% EDTA. The cells were washed ten times by PBS, and then incubated with the indicated amount of rAAL or H59Q at 4 °C for 3 h. After washing, the cells were incubated with rabbit AAL antisera (1:100) for 45 min and then washed five times with PBS. Cells that had not been treated with either protein were used as controls. FITC-conjugated Goat Anti-Rabbit IgG (Pierce, Rockford, IL, USA) was added to the sample and incubated for 30 min. After washing, the cells were analyzed by flow cytometry.

2.4. Preparation of rAAL, H59Q, BSA-sepharose, and affinity chromatography

rAAL, H59Q and BSA were separately coupled to CNBr-activated Sepharose 4B (Amersham Pharmacia Biotech, Piscataway, New Jersey, USA) according to the manufacturer's instructions. The amount of proteins bound to the Sepharose 4B was estimated by testing the protein concentration remaining in the coupling buffer. HeLa cell lysates were added to pre-equilibrated protein-sepharose. After incubation and exhaustive washing (phosphate buffer containing 0.05 M NaCl), retained proteins were eluted by a high-salt solution (phosphate buffer containing 0.5 M NaCl).

2.5. Mass spectroscopy

After separation on a polyacrylamide gel, selected Coomassie-stained bands were subjected to in-gel trypsin digestion. The resultant peptides were analyzed using a capillary column liquid chromatography (LC)-microelectrospray mass spectrometry (MS) system using a QTRAP3200 mass spectrometer (Applied Biosystems) with TEMPO nano LC Systems. The peptides were analyzed by the information dependent acquisition method acquiring full scan mass spectra to determine peptide molecular weights (MS) and tandem MS (MS/MS) to determine amino acid sequence in successive instrument scans.

2.6. Mammalian two-hybrid system

The mammalian two-hybrid system was a gift from Professor Jianjie Ma in the University of Medicine and Dentistry of New Jersey and further reconstructed by replacing the pG5-CAT reporter gene with the pG5-Luciferase in the State Key Laboratory of Virology, Wuhan University.

AAL and truncated AAL were PCR amplified and cloned into the pM vector. MRG15 was PCR amplified and cloned into a VP16 vector. The primers for AAL were: forward, 5′-CCGGAATTC-CAGGGCGTCAACATCTATAAC-3′; reverse, 5′-CGGGATCCCGCTACGC-CAAACCCGTG-3′. The primers for truncated AAL were: AAL-Δ28 forward, 5′-CCGGAATTCTTCTTCCTCAGCGCTCA-3′; AAL-Δ58 forward, 5′-CCGGAATTCCTCCACATCGCCTTCCGC-3′; AAL-Δ85 forward, 5′-CCGGAATTCCGTGTATCTGACGTCGCA-3′; and AAL-Δ123 forward, 5′-CCGGAATTCTACACGAAGCAAATCTCGGGTC-3′. The MRG15 gene (GenBank accession no. BC002936) was purchased from Wuhan Sanying Biotechnology, and the primers of for MRG15 were: MRG15 forward, 5′-CGCGGATCCGTATGGCGCCGAAGCAGGACCCG-3′; and MRG15 reverse, 5′-AAACTGCAGCACAGCTTTCCGATGGTAC-3′. The PCR products were digested by *Eco*RI and *Bam*HI for AAL and *Bam*HI and *Pst*I for MRG15. We used pM-53 and pVP16-T as positive control vectors, and pVP16 and pM as negative control vectors. All recombinant plasmids were verified by sequencing. HeLa cells were transfected with the indicated DNAs in each set of experiments using Lipofectamine 2000 Transfection Reagent (Invitrogen, Carlsbad, CA, USA). Equal amounts of total DNA (0.8 μg) within each set of experiments were used by adding unrelated DNA to the transfection mixture when necessary. 48 h after the transfection, the cells were harvested and the firefly-luciferase activity was detected to indicate the interaction activity of the proteins, using the *Renilla* Luciferase as the transfection control.

2.7. Pull down assays

HeLa cells were lysed with lysis buffer [pH 7.4, 20 mM Tris–HCl, 1 mM EDTA, 250 mM NaCl, 0.5% Nonidet P-40, and protease inhibitors]. Cell lysates were centrifuged at 16,000 *g* for 10 min at 4 °C, and the supernatant was collected. The supernatant was incubated with recombinant His-AAL, His-H59Q, His-R63H and His-K99A bound to His-Mag Agarose Beads in interaction buffer (PBS with 1% [v/v] Triton X-100, and 1% [w/v] BSA) for 2 h at room temperature with shaking. The beads were then washed in washing buffer (0.5 M NaCl, 60 mM imidazole, 20 mM Tris–HCl, pH 7.9). Proteins were eluted with elution buffer (4 M imidazole, 2 M NaCl, 80 mM Tris–HCl, pH 7.9) and separated by SDS-PAGE. The lysate was incubated with His-Mag Agarose Beads without His-AAL as the negative control.

2.8. MRG15 knockdown and AAL inducing activity

Constructs expressing three interfering RNAs against MRG15 were generated in the pSUPER.neo+gfp vector as described previously [22]. They contained the target sequences RNAi14: 5′-GGACCCGAAGCC-TAAATTC-3′, RNAi1203: 5′-GAGGGATATGCTGTAGAGT3-3′, and

RNAi1370: 5′-CACACTCATCCATTTGTGC-3′, which are common to MRG15 mRNA isoforms and are 100% conserved in human MRG15. Transient transfections were performed with the Lipofectamine 2000 Transfection Reagent. Knockdown of MRG15 was determined by western blotting using anti-MRG15 antibodies after transfecting the plasmids for 72 h. Different concentrations of rAAL were then added and incubated for 24 h. The apoptosis-inducing activity was measured by an Annexin V/PI apoptosis kit.

3. Results

3.1. Lactose blocks the proapoptotic activity of AAL

Carbohydrate binding is an essential property of galectins. To test whether the proapoptotic effect of AAL was carbohydrate-dependent, HeLa cells were treated with rAAL in the presence or absence of lactose. As shown in Fig. 1A and B, when an excessive amount of

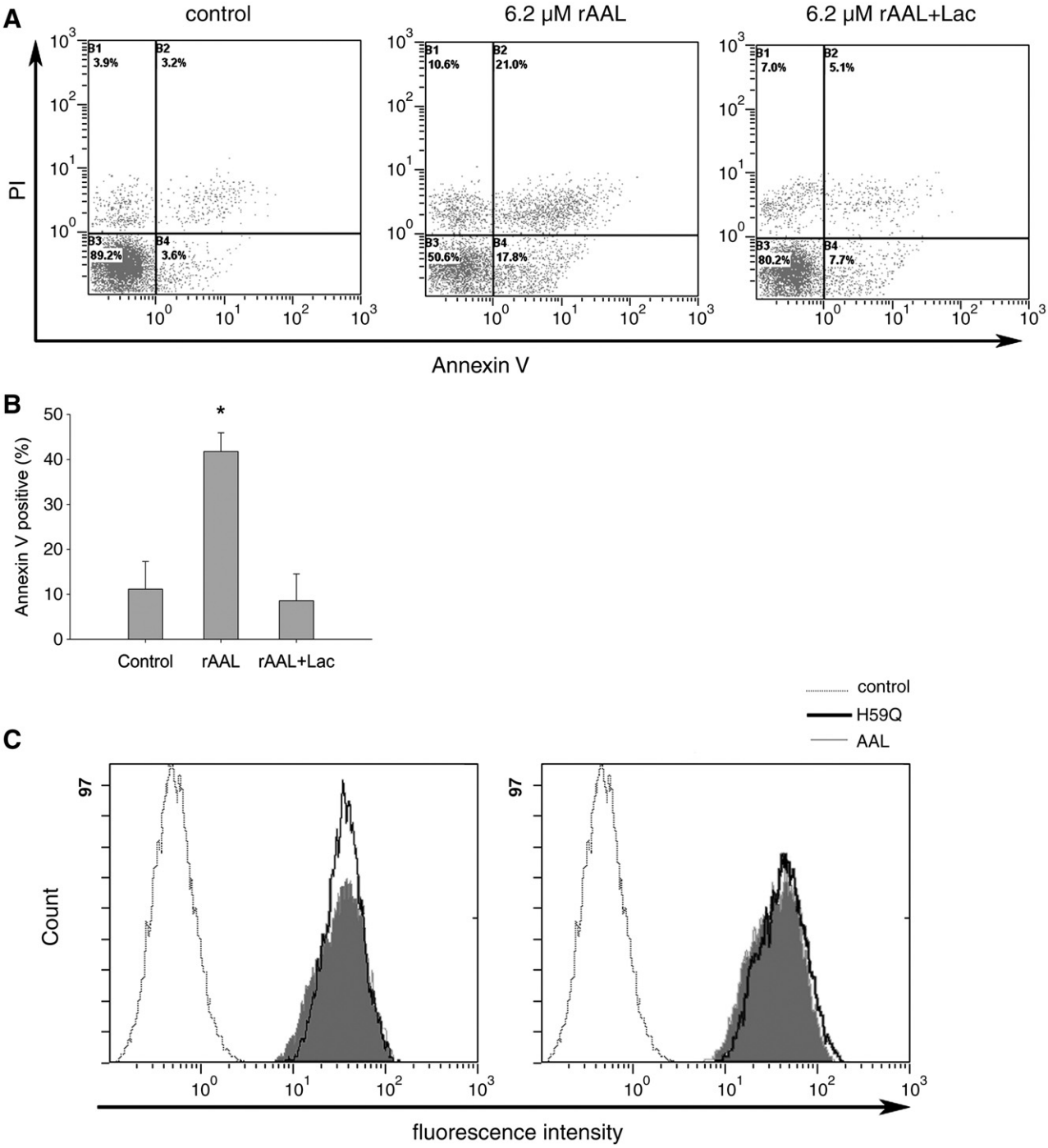

Fig. 1. Lactose blocked the apoptosis-inducing activity of rAAL. A. HeLa cells were treated with rAAL (6.2 μM) or rAAL (6.2 μM) and lactose (120 μM). After 36 h, cells were collected and the apoptotic events were stained by annexin V and PI, and analyzed by FACS. B. Quantification of the graphical results of A. The experiments were repeated three times, values represent mean ± S.D. * represents a significant difference. C. The cell membrane-binding ability of rAAL and H59Q were analyzed by immunofluorescence. Cells were treated with rAAL (closed histogram) or H59Q (black line) at 3.1 or 6.2 μM. After 4 h, cells were collected and analyzed by immunofluorescence staining followed by FACS. Dashed line represented the cells without rAAL or H59Q treatment.

lactose was added, the annexin V-positive cell ratio was reduced dramatically from 41.8% to 8.6%, which was comparable to the control group (11.1%). The result demonstrated lactose could inhibit rAAL-induced apoptosis of HeLa cells, which indicated that the CRD might contribute to the AAL apoptotic inducing activity.

We then constructed AAL mutants H59Q and R63H in the conserved CRD. In our previous work, AAL was found to be internalized in HeLa cells and localized to the nucleus [5]. CRD mutant protein H59Q could also translocate to the cell nucleus, but R63H lost the cell membrane-binding activity. Galectins are likely to bind oligosaccharides in the extracellular matrix or on the cell surface for their physiological function [23–25]. Therefore, we analyzed the cell membrane-binding ability of AAL and H59Q using an immunofluorescence assay and by FACS analysis. As shown in Fig. 1C, the MFI (Median Fluorescence Intensity) values of rAAL and H59Q were 39.4 and 40.6 at 3.1 μM, and 39.6 and 45.7 at 6.2 μM respectively, while the MFI value of the control group was 3.6. This result indicated that the mutation of the conserved CRD residue His59 to Gln did not significantly affect the cell membrane-binding ability.

3.2. Interaction partners of AAL and H59Q

rAAL and CRD mutant protein H59Q could both enter HeLa cells, but H59Q had a lower apoptosis-inducing activity. As there were no significant differences between the cell membrane-binding abilities of rAAL and H59Q, We hypothesized that the H59Q mutant had lost the ability to interact with one or more AAL interaction factors, leading to loss of the proapoptotic activity.

Sepharose-conjugated rAAL, H59Q and BSA affinity columns were prepared for affinity chromatography, and the coupling efficiency of rAAL, H59Q and BSA were 6.79, 7.01 and 6.21 mg protein/mL Sepharose, respectively. Affinity matrices were incubated with HeLa cell lysate, and the bound proteins were eluted with high-salt solution and separated by SDS-PAGE (Fig. 2A). Several protein bands were detected in the elution fraction of rAAL-sepharose but were weak in that of H59Q and BSA-sepharose. These bands were excised and analyzed by Liquid Chromatography Coupled with Tandem Mass Spectrometry (LC/MS/MS), and many peptides were identified (see Table 1). These peptides were found to belong to four proteins, glucose-regulated protein 78 (GRP78), mortality factor 4-like protein 1 (MRG15), elongation factor 2 (EEF2), and heat shock protein 70 (Hsp70) respectively. These four proteins were validated by western blotting and the results showed that GRP78 was the common binding partner of rAAL and H59Q, while MRG15, EEF2 and Hsp70 bound only to rAAL (Fig. 2B).

Table 1
Peptide sequences identified by tandem mass spectrometry following in-gel trypsin digestion.

Accession number	Protein description	Peptide sequences identified by MS/MS
P08107	Heat shock 70 kDa protein 1 (Hsp70)	TTPSYVAFTDTER NQVALNPQNTVFDAK FGDPVVQSDMK IINEPTAAAIAYGLDRT AGDTHLGGEDFDNR SINPDEAVAYGAAVQAAILMGDK QTQIFTTYSDNQPGVLIQVYEGER
P11021	78 kDa glucose-regulated protein precursor (GRP78)	AGDTHLGGEDFDNR FEELNMDLFR IINEPTAAAIAYGLDK NEPTAAAIAYGLDKR NQLTSNPENTVFDAK SDIDEIVLVGGSTR SINPDEAVAYGAAVQAAILSGDK SQIFSTASDNQPTVTIK TFAPEEISAMVLTK TWNDPSVQQDIK VTHAVVTVPAYFNDAQR IEIESFYEGEDFSETLTR SQIFSTASDNQPTVTIK DNHLLGTFDLTGIPPAPR DAGTIAGLNVMR ELEEIVQPIISK
P13639	Elongation factor 2 (EEF2)	AYLPVNESFGFTADLR VFSGLVSTGLK WLPAGDALLQMITIHLPSPVTAQK
Q9UBU8	Mortality factor 4-like protein 1 (MRG15)	PQYAEILADHPDAPMSQVYGAPHLLR TPGNGDGGSTSETPQPPR VDPTVENEETFMNR

3.3. Interaction between AAL and MRG15

Hsp70 and EEF2 was reported to be localized in cytoplasm [26,40], GRP78 was in endoplasmic reticulum lumen [27,28], and MRG15 was in nucleus [29]. As we reported before, nuclear migration was important for the AAL's proapoptotic activity [5], so the nuclear protein MRG15 seemed a likely candidate for the "lost" H59Q interacting partner. To further verify the interaction between AAL and MRG15, a mammalian two-hybrid assay was used. pM-AAL, pVP16-MRG15, pG5-firefly luciferase and control (pRenilla) luciferase were transfected into HeLa cells, and the luciferase activity was quantified by a luminometer. As shown in Fig. 3A, compared with the positive control (the interaction between mouse p53 and SV40 large T-antigen), AAL was found to have strong binding activity with MRG15 *in vivo*. These

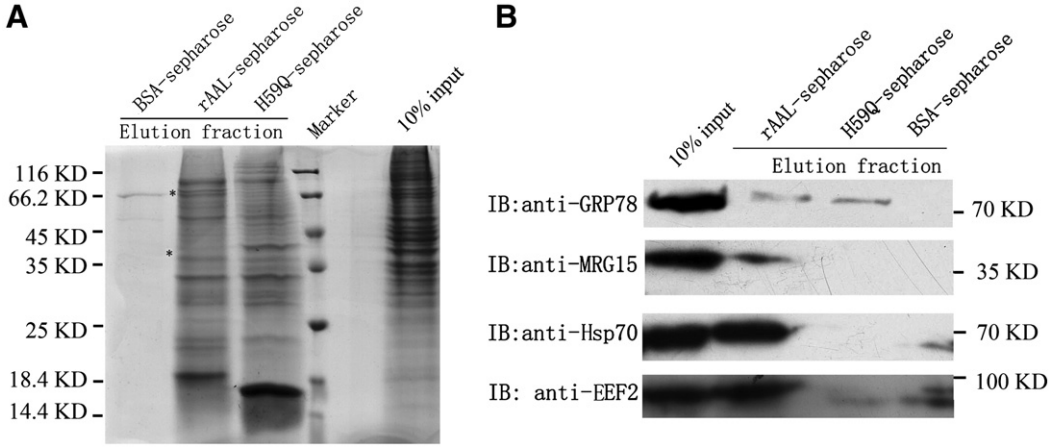

Fig. 2. rAAL and H59Q had different binding partners in HeLa cells. Three affinity chromatography columns (rAAL, H59Q, and a BSA-column) were prepared using CNBr-activated Sepharose 4B, incubated with HeLa cell lysate, and then eluted with high-salt solution. A. The eluted fractions were collected and separated by 15% SDS-PAGE. Several protein bands (marked by asterisks), which were apparent in the elution fraction of rAAL-sepharose but weaker in that of H59Q and BSA-sepharose, were cut and analyzed by MS. B. Four proteins (GRP78, MRG15, Hsp70, and EEF2) were further identified by western blotting.

data indicated that AAL could interact with MRG15, which was confirmed by the following pull down assay (Fig. 3C).

To further investigate the binding region of AAL and MRG15, we tested the interaction of a series of truncated AALs and MRG15 using the mammalian two-hybrid assay (Fig. 3B). Several truncated mutants of AAL were constructed in vector pM, and co-transfected with pVP16-MRG15 into the HeLa cell. The luciferase activity was quantified by a luminometer. Fig. 3B shows that as more amino acids sequence were deleted, the interaction between AAL and MRG15 became weaker. On the assumption that interaction of AAL with MRG15 was 100, truncation of the N-terminal 28 residues and 58 residues (AAL-Δ28 and AAL-Δ58) retained the 80% and 60% of interacting ability, respectively, while ability of the truncation of the N-terminal 85 residues (AAL-Δ85) to bind to MRG15 was dramatically reduced to 15%. The interaction between the truncation of the N-terminal 123 residues (AAL-Δ123) and MRG15 was as weak as AAL-Δ85. These results suggested that the sequence 58–85 (the CRD region) was required for the interaction between AAL and MRG15.

In addition, we used a pull down assay to further confirm the AAL-MRG15 interaction region using as baits, two CRD mutant proteins, H59Q and R63H, a non-CRD mutant protein K99A, and rAAL (Fig. 3C). rAAL/mutant proteins bound to Nickel beads were incubated with the HeLa cell lysates, and the eluted fraction was tested by anti-MRG15 antibodies. MRG15 existed in the eluted fraction of rAAL and K99A, but not in that of H59Q and R63H (CRD mutant proteins). This result confirmed that the CRD region was essential for the interaction between AAL and MRG15.

3.4. MRG15 knockdown assay

We established three different MRG15 RNAi expression vectors to determine the effect of MRG15 in HeLa cells on AAL's apoptosis-inducing activity. Compared with mock (control vector) transduced cells, MRG15 expression was dramatically down-regulated by RNAi14, RNAi1203, and RNAi1370 (Fig. 4A). MRG15 knockdown had no effect on cell viability (Fig. 4B). When cells were exposed to AAL (3.1, 6.2, and 12.5 μM) for 24 h, the percentage of cells in the sub-G1 peak of the control vector transduced cells increased from 10% to 47.6%. On the other hand, the MRG15 knockdown cells were resistant to AAL at a concentration of 12.5 μM, showing a significant decrease of AAL's apoptosis-inducing activity (from 47.6% to 9.6%). Taken together, the results indicated that MRG15 was a nuclear ligand of AAL in HeLa cells, and was essential for AAL's proapoptotic activity.

4. Discussion

We previously found that fungal galectin AAL could penetrate HeLa cells, migrate to the nucleus, and induce cellular apoptosis [5].

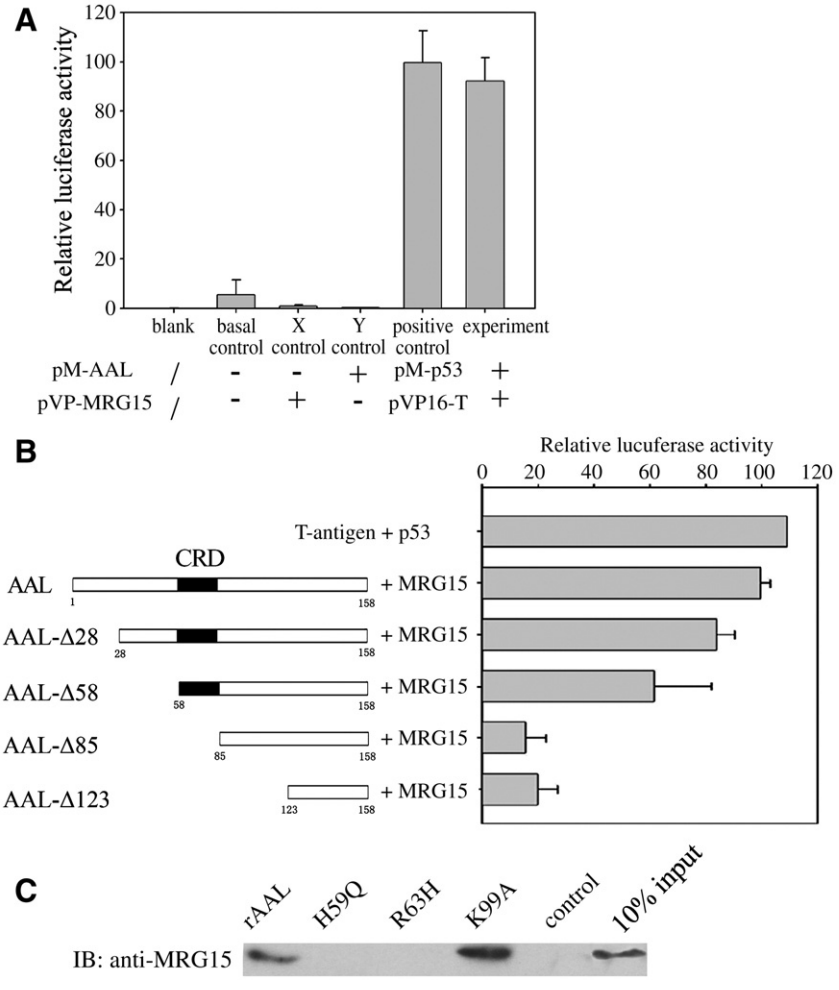

Fig. 3. The interaction between AAL and MRG15. A. AAL and MRG15 were incorporated into mammalian two-hybrid constructs and their interaction was detected by dual-luciferase assays after transfection into HeLa cells. The interaction between mouse p53 and the T-antigen was set as 100. The data shown are the mean percentages ± S.D. from triplicate measurements. B. Interaction between N-terminal truncations of AAL (28/58/85/123 amino acids deleted) and MRG15 were tested in the mammalian two-hybrid system. The interaction between AAL and MRG15 was set as 100. C. A pull down assay was performed to test the interaction between AAL/mutant AAL proteins and MRG15. rAAL and three mutant proteins (H59Q, R63H, and K99A) with His tags were coupled to nickel beads as bait proteins. The columns were incubated with the HeLa cell lysates, and the eluted fraction was collected and probed by anti-MRG15 antibodies. The experiments were repeated three times.

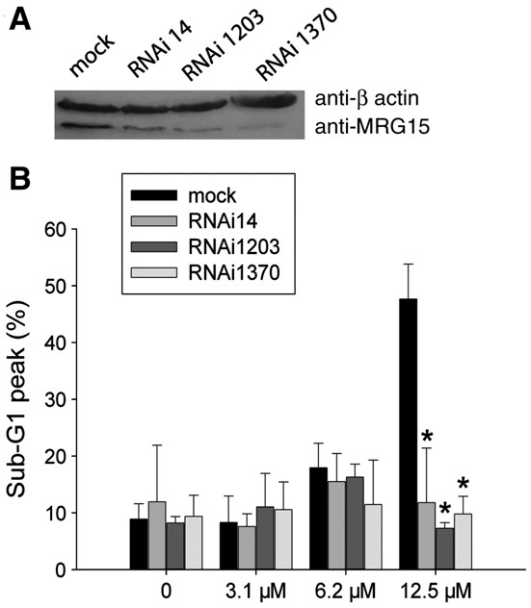

Fig. 4. MRG15 knockdown assay. A. The knockdown of MRG15 was detected by western blotting after transfection with RNAi plasmids for 72 h. B. 72 h after RNAi transfection, different concentrations of rAAL were added to the culture medium and incubated for 24 h. The apoptosis-inducing activity was measured by PI staining and FACS analysis, * significant difference, $P<0.05$. The experiments were repeated three times, values represents mean ± S.D.

Moreover, the nuclear localization was important for AAL's proapoptotic activity, as shown by mutants and immunofluorescence assay, which indicated that there might be some nuclear molecules involved in AAL's bioactivity. In this paper, we identified one of these nuclear factors as MRG15. Knockdown of MRG15 rendered the cell resistant to the AAL, which provided a partial explanation for the mechanism of AAL's proapoptotic activity. MRG15 is the first nuclear ligand for AAL to be identified, implying that a novel nuclear pathway is involved in mushroom lectins' antitumor activity. MRG15 is highly conserved in yeast, *C. elegans*, *Drosophila*, plants, and mammals, and has been implicated in chromatin remodeling and cell proliferation [30–32]. However, knockdown or deletion in mice was found to result in DNA repair defects and embryonic lethality, which hampered further investigation of its function. Our study on the ligands of AAL or the AAL interaction complex in HeLa cells will provide useful information for the research on MRG15.

The CRD is critical for the function of galectins. Although most studies have focused on its carbohydrate binding characteristics, others have proposed that the CRD region is also involved in the galectin–protein ligand interaction [23,25,33–36]. Here, we demonstrated that the CRD mutant protein H59Q retained the cell membrane-binding activity, but its proapoptotic activity was largely impaired. Therefore, H59Q can be regarded as a unique material for exploring the role of the CRD in protein interaction and the proapoptotic activity of AAL. Using affinity chromatography and SDS-PAGE, several different interacting proteins of H59Q and rAAL were identified, including MRG15. In the study of the interaction between AAL and MRG15, the truncation of amino acids 58–85 of AAL significantly disrupted the interaction, and CRD mutant proteins H59Q and R63H lost the ability to bind to MRG15, which revealed that the CRD was essential for the interaction between AAL and MRG15. Our results supported the hypothesis that the CRD initially functions in carbohydrate recognition outside of the cells and then contributes to (or is responsible for) protein–protein interactions inside cells [33]. There is limited information about the glycosylation status of MRG15 and other three cellular ligands Hsp70, GRP78 and EEF2; whether the interaction takes place by way of galectin–carbohydrate or galectin–protein interaction remains to be further investigated.

We developed a method to investigate function-related binding partners, which we called mutation comparison affinity chromatography (MCAC). In this paper, through MCAC coupled with MS, we quickly identified the protein ligands related to the proapoptotic activity of AAL. Using the CRD mutant protein H59Q as a comparator, four proteins related to the proapoptotic activity and the CRD region were specifically identified, while the hundreds of other potential AAL binding partners could be ignored. MCAC has proved to be an effective approach for the identification of the ligands and the role of the CRD domain in the protein's function. However, affinity chromatography does not allow us to distinguish between the binary interactions of protein pairs or multiprotein complexes. Therefore, some of the protein associations we detected may be indirect.

Hsp70, GRP78, and EEF2, the other possible ligands of AAL, have been found to be the potential targets of the antitumor fungal galectin AAL. Hsp70, a member of stress or heat shock protein family, is abundantly expressed in malignant cells, and strategies targeting Hsp70 could be especially effective in overcoming tumor cell resistance [37]. GRP78, the ER homolog of Hsp70 proteins, might serve as a biomarker of tumor behavior and treatment response due to its promotion of tumor proliferation, survival, metastasis, and resistance to a wide variety of therapies [38]. EEF2 could cause loss of anti-apoptosis proteins enhancement of sensitivity to TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) and could be used as a sensor by lowering the apoptotic threshold of an affected cell [39]. These data suggest that other pathways may be involved in AAL's proapoptosis activity.

5. Conclusions

Among all the fungal lectins, only the cytoplasmic ligand Orp150 of ABL has been identified [15]. Our work has identified one nuclear ligand, MRG15, and several other ligands, which will contribute to the understanding of fungal lectins' activity and their downstream signaling pathways.

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