



# Lens culinaris agglutinin inhibits human hepatoma cell migration via mannose and fucose-mediated ERK1/2 and JNK1/2/3 signalling pathway

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## Abstract

**Background** Hepatocellular carcinoma (HCC) is the main types of primary liver cancer, which shows some abnormal glycosylation, such as the increase of fucose. Lens culinaris agglutinin (LCA), a natural plant lectin that can bind to mannose and fucose, has been reported to be antiproliferative to may tumors. However, the effect of LCA on the vitality and migration ability of human hepatoma cells is not demonstrated. Therefore, the aim of this study is to investigate the effects of LCA on vitality and migration in human hepatoma cells and its potential mechanisms.

**Methods and results** LCA had no significant effect on viability of human hepatoma cells (HCCLM3, MHCC97L and HepG2) and hepatocytes (L02) by CCK-8 kit, but it could inhibit human hepatoma cells migration significantly without affecting hepatocytes by Transwell method. Sugar inhibition assay was used to verify the possible binding site between LCA and human hepatoma cells. The result showed that Mannose- and fucose- related sites were associated with LCA inhibiting human hepatoma cells migration. Moreover, LCA could affect HCCLM3 migration by activating ERK1/2 and JNK1/2/3 signalling pathways. LCA did not affect MMP-2 and MMP-9 of HCCLM3 through gelatinase zymography. However, the results of immunofluorescence standing showed that LCA could reduce the F-actin formation in HCCLM3 via ERK1/2 and JNK1/2/3 signalling pathways.

**Conclusions** LCA might inhibit human hepatoma cell migration by reducing the F-actin formation via the mannose and fucose-mediated ERK1/2 and JNK1/2/3 signalling pathway. This result will deepen people's understanding on plant lectin as a drug in tumor glycobiology.

**Keywords** Lens culinaris agglutinin · Hepatocellular carcinoma · Cell migration · Sugar-binding · MAPK pathway

## Introduction

Hepatocellular carcinoma (HCC) is a malignant disease that seriously affects human beings. Its metastatic behavior makes its treatment and prognosis very complex and uncontrollable [1]. Some key steps occur in the process of liver cancer metastasis, for example, metastatic hepatoma cells can penetrate the basement membrane through

epithelial-mesenchymal-transition (EMT), pass through connective tissue through cell motility, can invade blood vessels and reach new tissues with blood flow through invasion or intravasation and extravasation [2]. In all the above metastatic processes, glycoprotein, as a protein with signal recognition function, plays an important role [3]. Most glycoproteins are located on the cytoplasmic membrane, and the functional region is often outward. Due to uncontrollable gene mutations, glycoproteins in HCC have been dysfunctional. Different from normal cells, the front-end synthesis and back-end modification of glycoproteins in some hepatoma cells have changed, which are related to glycosyltransferase and glycosylation process [4].

Plant lectin, a non-enzymatic protein derived from natural plants, are known for its ability to bind certain sugar chain specifically [5]. The difference of sugar-binding ability of

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various plant lectins is mainly reflected in the evolutionary conservation of sugar binding motif [6]. In previous decades, researchers have isolated and identified the sugar specificity of a large amount of plant lectins from different species and genera, and established a one-to-one correspondence between them [7]. For example, soy lectin has specific binding ability to galactose and galactosamine, Concanavalin A can bind to D-mannose or D-glucose, and Wheat germ lectin is sensitive to the combination of silicic acid [8–10]. Based on this capability of capture carbohydrates chain, plant lectin plays an important role in the targeted recognition of specific tumor cells, and even in the biological behaviors of tumor cells, such as signal regulation, division, immune defense and apoptosis [11]. Moreover, due to the specific sugar grasping ability of plant lectin, its application in cancer cells with different glycosylation variants can further deepen the understanding of the sugar-binding ability of plant lectin and glycosylation changes in cancer cells.

In some previous studies, researchers have made it clear that plant lectin has toxic effect on some malignant tumor cells. For example, *Dioclea violacea* lectin (DVL) is related to the autophagy of human glioma cell line U87 [12]. *Clematis montana* lectin (CML) is related to the apoptosis of human hepatoma cell line HepG2 [13]. A lectin called BEL  $\beta$ -trefoil is found to have a strong inhibitory effect on the viability and proliferation of A375 and MeWo melanoma cells [14]. In other related studies, based on the specific sugar binding ability of different lectins, researchers can accurately distinguish normal cells from cancer cells with glycoprotein mutations [15]. *Lens culinaris* agglutinin (LCA), a plant lectin that can bind mannose and fucose motif, has been reported to play a specific role in tumor detection and treatment [16–18]. Compared with normal liver tissue, the level of core and outer-arm fucose in HCC will increase [19, 20]. This changes of fucose have been used as a marker to detect the occurrence and development of human hepatoma cell in clinic [21]. Therefore, based on the ability of LCA to capture fucose motif specifically, it may play an important role in the recognition and treatment of metastatic HCC.

At present, the effect of LCA on the biological behavior of human hepatoma cells, especially the migration behavior, and its molecular mechanisms still not understood. The present investigation was aimed to verify the effects of LCA on the cell viability and migration ability of human hepatoma cells and its mechanisms.

## Materials and methods

### Cell culture

Three kinds of human hepatoma cells (HCCLM3, MHCC97L and HepG2) and one normal hepatocyte (L02) were obtained from Shanghai Hepatobiliary Research Institute (Shanghai, China). Human hepatoma cells and normal hepatocytes were cultured in high-glucose DMEM medium (Gibco, Carlsbad, CA, USA) and 1640 medium (Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS, HyClone, Logan, UT, USA), 100 U/ml streptomycin and 100 U/ml penicillin, respectively. Cells were cultured in 25cm<sup>2</sup> cell flask and in cell incubator with an atmosphere of 5% CO<sub>2</sub> at 37 °C after each passage and fluid exchange.

### Cell viability

Cell viability was detected by CCK-8 kit. The cells were inoculated into 96 well plates at a density of  $5 \times 10^3$ /well. After adherence, the cells were serum-starved overnight and then treated with different concentrations of LCA (0, 1, 5, 10 and 30  $\mu$ g/mL) for 12 h, 24 h, or 48 h. 10 mg of LCA powder (Aladdin Industrial Corporation, Shanghai, China) was dissolved in 1 mL of sterile PBS to prepare a mother liquor with a concentration of 10  $\mu$ g/ $\mu$ L, and then the mother liquor was diluted into several 2  $\mu$ g/ $\mu$ L working solutions in the ratio of 1:4 through PBS and last stored at -20 °C. The 5 mg/mL CCK-8 reagent (0.5 mg/ml, Sigma-Aldrich, St. Louis, MO, USA) was added to each well after the indicated treatment time. After incubation at 37 °C for 2 h, the cell viability was measured by spectrophotometer (Molecular Devices, Shanghai, China) and the absorbance was measured at 450 nm.

### Cell migration

Cell migration was performed in 24-well plates by Transwell method (pore diameter: 8  $\mu$ m. Millipore, Billerica, MA). The cells were starved overnight in serum-free medium before the experiment. After the cell suspension was prepared by serum-free medium, the suspension was inoculated to the upper layer of the transwell chamber with a cell density of  $5 \times 10^4$ /100  $\mu$ L, 600  $\mu$ L serum medium was added to the lower layer of the transwell chamber to induce the upper cells with different concentrations of LCA (0, 1, 5, 10 and 30  $\mu$ g/mL). After 6 h of cell migration, the transwell chamber was transferred out of 24 well plate, and the liquid in the upper layer of the chamber was absorbed with cotton ball, and then the cells which is migrated unsuccessfully in the upper layer were slightly wiped out by cotton swab. Subsequently, the cells in the lower layer were fixed and stained

with 4% paraformaldehyde and crystal violet (Biyuntian Biotechnology, Shanghai, China), and then photographed by inverted microscope (Olympus, Tokyo, Japan).

### Sugar inhibition assay

In order to verify the binding motif of different kinds of carbohydrates on LCA and the effect of LCA after sugar binding on cell migration, we used D-mannose and L-fucose (Aladdin Industrial Corporation, Shanghai, China) in our experiment. 10 mg of two sugars powder were dissolved in 1 mL of sterile deionized water respectively to prepare a mother liquor with a concentration of 10  $\mu\text{g}/\mu\text{L}$ , and then the mother liquor was diluted into several 2  $\mu\text{g}/\mu\text{L}$  working solutions in the ratio of 1:4 through deionized water and last stored at  $-80\text{ }^\circ\text{C}$ . During the experiment, two sugars solutions mixed with LCA (10  $\mu\text{g}/\text{mL}$ ) at the concentration ratio of 1:1 in  $37\text{ }^\circ\text{C}$  for 30 min respectively before cell migration experiment.

### Western blotting

To assess the effect of LCA on MAPK pathways of HCCLM3, we used Western blot to detect protein expression of ERK1/2, JNK1/2/3 and p38. First, cells were seeded into 6 well plates at a density of  $2 \times 10^4/\text{well}$ . When the cell density reached about 70%, the cells were starved overnight, and treated with LCA (10  $\mu\text{g}/\text{mL}$ ) for 0, 15, 30, 60 and 120 min. After LCA treatment, the cells were washed with ice-cold PBS, and the total cell protein was collected with cell lysate. The protein concentration was determined by Lowry's method and using bovine serum albumin (BSA) as a standard. Total cell protein was electrotransferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA) after electrophoretic separation by SDS-polyacrylamide gel electrophoresis. Then, the PVDF membrane was blocked on a horizontal shaking table with Tris-buffered saline containing 0.1% Tween-20 (TBST) and 5% BSA at room temperature for 1 h. A ERK1 (pT202/pY204)+ERK2 (pT185/pY187) rabbit mAb (Abcam, Cambridge, MA, USA), ERK1+ERK2 rabbit mAb (Abcam, Cambridge, MA, USA), JNK1+JNK2+JNK3 (phospho T183+T183+T221) rabbit mAb (Abcam, Cambridge, MA, USA), JNK1+JNK2+JNK3 rabbit mAb (Abcam, Cambridge, MA, USA), p38 alpha/MAPK 14 (phospho Y322) rabbit pAb (Abcam, Cambridge, MA, USA), p38 alpha/MAPK14 rabbit mAb (Abcam, Cambridge, MA, USA) and GAPDH rabbit mAb (ZSGB-Bio, Beijing, China) were used according to the manufacturers' protocols, and these antibody dilutions and PVDF membranes were incubated under the condition of slight shaking at  $4\text{ }^\circ\text{C}$  overnight. After overnight, PVDF membrane was washed several times with

TBST and further incubated with HRP-conjugated antibody (MultiSciences, Beijing, China) for 1 h at room temperature. Finally, fluorescence signal detection was made by an enhanced chemiluminescence kit (Thermo, Waltham, MA, USA). A semiquantitative evaluation of the bands was performed by densitometry (Clix Science Instruments, Shanghai, China).

### Pathway protein inhibition assay

We used ERK1/2 inhibitor U0126 (Meilunbio, Dalian, China) and JNK1/2/3 inhibitor SP600125 (Beyotime, Shanghai, China) in our experiment. The concentrations of two inhibitors were set to 5  $\mu\text{M}$ , 10  $\mu\text{M}$  and 20  $\mu\text{M}$  to determine the optimal action concentration of inhibitors on HCCLM3. After that, cells were pretreated with U0126 or/and SP600125 for 60 min at  $37\text{ }^\circ\text{C}$  before the addition of LCA in western blot analysis and migration assay.

### Immunofluorescence staining

To assess the effect of LCA on F-actin, cells were seeded into 24 well plates at a density of  $1 \times 10^4/\text{well}$ . When the cell density reached about 50%, the cells were starved overnight, and then the cell treated with or without LCA (10  $\mu\text{g}/\text{mL}$ ). To block the ERK1/2 or JNK1/2/3 signal, the cells were incubated with U0126 or SP600125 for 60 min at  $37\text{ }^\circ\text{C}$  before treatment with LCA. After LCA treatment for 6 or 24 h, the cells were washed with ice-cold PBS and fixed with 4% formaldehyde for 30 min. Then cells were treated with permeabilization solution containing 0.25% Triton-X-100 (Solarbio Biotechnology, Beijing, China) for 10 min. After permeabilization, cells were treated with blocking solution containing 5% BSA (Solarbio Biotechnology, Beijing, China) for 60 min. Then FITC phalloidin (Biyuntian Biotechnology, Shanghai, China) was added to stain the F-actin overnight at  $4\text{ }^\circ\text{C}$ . The nuclei were labeled with DAPI (Biyuntian Biotechnology, Shanghai, China) for 10 min at room temperature. Finally, the cells were photographed using an inverted fluorescent trichromatic microscope (Olympus, Tokyo, Japan). Fluorescence results of F-actin were semi quantitatively evaluated by optical density analysis.

### Gelatin zymography

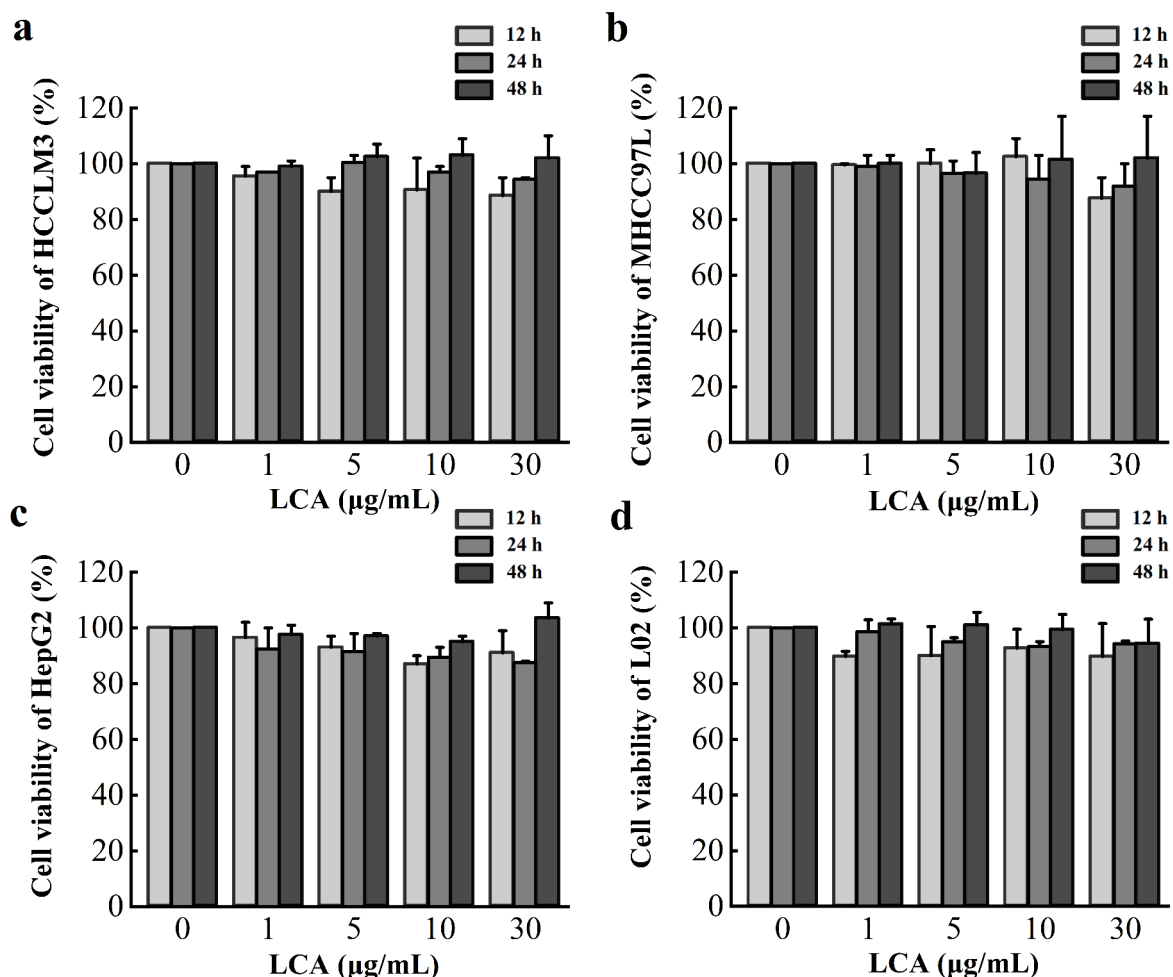
The matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) can reduce the components of extracellular matrix, which plays a key role in the development of HCC invasion and metastasis. To assess the effect of LCA on the activation of MMP-2 and MMP-9, the gelatin zymography was used. Briefly, the cells were seeded into 6

well plates at a density of  $3 \times 10^5$ /well. When the cell density reached about 90%, the cells were starved overnight, and then the cell treated with or without LCA (10  $\mu\text{g}/\text{mL}$ ). After LCA treatment for 48 h, supernatants were collected and centrifuged (8000 rpm, 5 min). The protein concentrations were determined by the BCA protein quantification kit (KeyGEN Biotech, Nanjing, China) and equal amounts of proteins were applied to the gel. The prestained protein molecular weight marker was used as size standards in protein electrophoresis (Fermentas, Waltham, USA). After the gelatinase was concentrated and separated by SDS-polyacrylamide gel containing pinkskin gelatin (Biyuntian Biotechnology, Shanghai, China) electrophoresis, the separated gelatin was removed and renatured at least 60 min in 2.5% Triton-X-100 solution, then incubated 16 h at 37 °C in a developing buffer (Tris 0.5 M, Brij35 0.2%, NaCl 2 M,  $\text{CaCl}_2$  50 mM, pH 7.6). After incubation, gelatin was stained by Coomassie brilliant blue (Solarbio Biotechnology, Beijing, China). A

semiquantitative evaluation of the bands was performed by densitometry (Clix Science Instruments, Shanghai, China).

### Statistical analysis

All the presented data and results were confirmed by at least three independent experiments. These data were expressed as mean  $\pm$  S.D. The one-way analysis of variance (ANOVA) was performed to analyze the differences between groups. Bonferroni multiple comparison were used when the  $p$  value indicated a significant difference between the groups. It was considered significant if the  $p$  value was less than 0.05 ( $p < 0.05$ ).



**Fig. 1** Cell viability of HCCLM3 (a), MHCC97L (b), HepG2 (c) and L02 (d) after treating with LCA (0, 1, 5, 10 and 30  $\mu\text{g}/\text{mL}$ ) for 12, 24, or 48 h. The data were expressed as the means  $\pm$  S.D.,  $n = 3$ . LCA: Lens culinaris agglutinin



## Results

### LCA had no significant effect on the cell viability of human hepatoma cells and hepatocytes

In order to evaluate whether LCA can inhibit human hepatoma cell growth, a CCK-8 assay was used to evaluate the viability of three kinds of human hepatoma cells (HCCLM3, MHCC97L and HepG2) and one normal hepatocyte (L02) after LCA treatment. As shown in Fig. 1, cell viability of three kinds of human hepatoma cells and one normal hepatocyte all had no significant change under the different concentration of LCA (0, 1, 5, 10 and 30  $\mu\text{g}/\text{mL}$ ) treatment for 12 h, 24 and 48 h.

### LCA inhibited the migration of three human hepatoma cells but had no effect on normal hepatocytes

After clarifying the effect of LCA on the viability of four kinds of cells, the effect of LCA on the cell migration was also evaluated. As shown in Fig. 2, LCA had a strong migration inhibition ability to three human hepatoma cells, and it had no significant effect on the migration of normal hepatocytes. These results suggested that LCA might have a specific inhibitory effect on the migration of human hepatoma cells.

### LCA affected the migration of human hepatoma cells through identifying the mannose and fucose site

When LCA concentration was 10  $\mu\text{g}/\text{mL}$  and treated cells for 6 h, the migration inhibition rate of LCA on three kinds of human hepatoma cells was strongly significant (Fig. 2 and Fig. 3). Then, we let the LCA (10  $\mu\text{g}/\text{mL}$ ) interact with the mannose (MAN, 10  $\mu\text{g}/\text{mL}$ ) or fucose (FUC, 10  $\mu\text{g}/\text{mL}$ ) for 30 min in advance. As shown in Fig. 3, the addition of MAN or FUC alone all had no effects on the migration of human hepatoma cells. After sugar inhibition assay, the migration rate of three human hepatoma cells in LCA+MAN groups and LCA+FUC groups was restored compared with the LCA groups (Fig. 3b-d). These results suggested that LCA might inhibit the migration of human hepatoma cells by binding to the receptors related to mannose or fucose on the cell membrane or in cells.

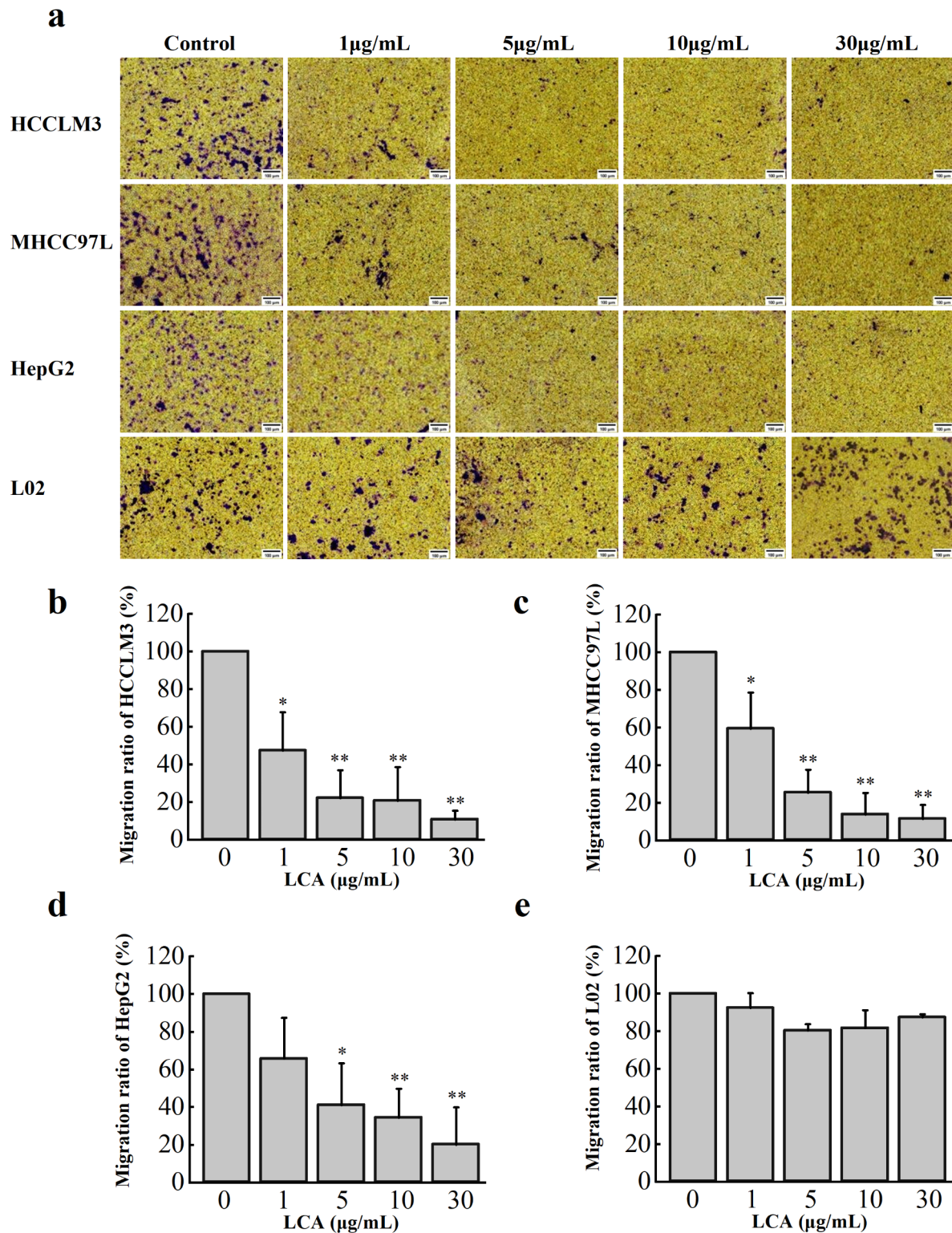
### LCA inhibited HCCLM3 migration by up regulating ERK1/2 and JNK1/2/3 signalling pathway

In order to further explore the molecular mechanism of LCA affecting human hepatoma cells migration, we studied

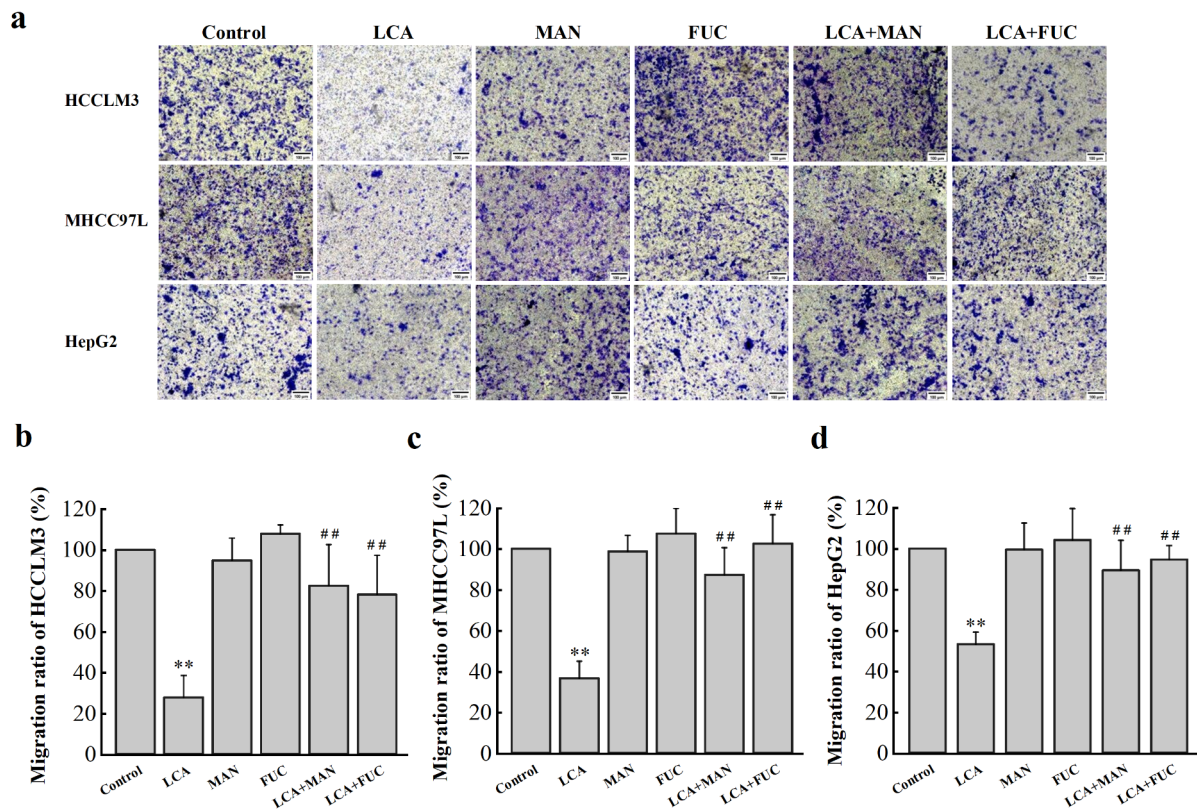
the MAPK classical pathway (ERK1/2 pathway), JNK/MAPK pathway (JNK1/2/3 pathway) and p38/MAPK pathway (p38 pathway) in HCCLM3. Figure 4a showed phosphorylated proteins and total proteins of ERK1/2, JNK1/2/3 and p38. The internal reference protein was GAPDH. Cells were treated with LCA (10  $\mu\text{g}/\text{mL}$ ) for 0, 15, 30, 60 and 120 min. The content of phosphorylated ERK1/2 in HCCLM3 increased significantly after 30 min of LCA treatment, and reached the maximum. After LCA stimulation for 120 min, the content of phosphorylated ERK1/2 began to decrease (Fig. 4b). The content of total ERK1/2 protein remained unchanged throughout the process. Similarly, the content of phosphorylated JNK1/2/3 in HCCLM3 increased significantly after 30 min of LCA treatment, and reached the maximum at 60 min. After LCA stimulation for 120 min, the content of phosphorylated JNK1/2/3 began to decrease, too (Fig. 4c). The content of total JNK1/2/3 protein also remained unchanged throughout the process. In p38/MAPK pathway, both phosphorylated p38 and total p38 were consistent with the control group, which had no significant change (Fig. 4d). Then we used ERK1/2 inhibitor U0126 and JNK1/2/3 inhibitor SP600125 and screened their optimal concentration. As shown in **Supplementary Fig. 1**, when the concentration of U0126 and SP600125 reached 5  $\mu\text{M}$ , they could restore the level of phosphorylated ERK1/2 and JNK1/2/3 to the control group. As shown in Fig. 4e and f, LCA (10  $\mu\text{g}/\text{mL}$ ) could inhibit the migration of most HCCLM3. After combined addition of ERK1/2 inhibitor U0126 (5  $\mu\text{M}$ ) and JNK1/2/3 inhibitor SP600125 (5  $\mu\text{M}$ ), the cell migration rate was restored significantly compared with the LCA group. These results suggested that LCA might affect the migration of HCCLM3 through ERK1/2 and JNK1/2/3 pathway.

### LCA decreased the polymerization of F-actin through ERK1/2 and JNK1/2/3 pathway in HCCLM3

As shown in Fig. 5a, the F-actin was displayed in red fluorescence, the nucleus was displayed in blue fluorescence. The result showed that LCA could reduce the polymerization of F-actin in HCCLM3 in 6 h. ERK1/2 inhibitor U0126 (5  $\mu\text{M}$ ) and JNK1/2/3 inhibitor SP600125 (5  $\mu\text{M}$ ) suppressed the LCA-promoted F-actin depolymerization respectively. Combined addition of two inhibitors would restore F-actin affected by LCA more significantly (Fig. 5b). As shown in **Supplementary Fig. 2**, the polymerization degree of F-actin in HCCLM3 decreased more significantly after increasing the action time of LCA to 24 h.



**Fig. 2** Cell migration of HCCLM3, MHCC97L, HepG2 and L02 after treating with LCA (0, 1, 5, 10 and 30  $\mu$ g/mL) for 6 h. (a) Microscopic image of four kinds of cells in Transwell Boyden Chamber (scale bar = 100 nm); Quantification of HCCLM3 (b), MHCC97L (c), HepG2 (d), and L02 (e) in transwell chamber was performed by counting cells. The data were expressed as the means  $\pm$  S.D.,  $n=3$ , \* $p<0.05$  and \*\* $p<0.01$  compared with the control group. LCA: Lens culinaris agglutinin



**Fig. 3** Cell migration of HCCLM3, MHCC97L and HepG2 treating with LCA after coincubation with mannose or fucose for 6 h. (a) Microscopic image of three kinds of hepatoma cells migration in Transwell Boyden Chamber (scale bar = 100 nm); Quantification of HCCLM3 (b), MHCC97L (c), HepG2 (d) in transwell chamber was performed by counting cells. The data were expressed as the means  $\pm$  S.D.,  $n = 6$ , \*\* $p < 0.01$  compared with the control group, ## $p < 0.01$  compared with the LCA group. LCA: Lens culinaris agglutinin (10  $\mu\text{g}/\text{mL}$ ); MAN: mannose; FUC: fucose

### LCA had no effect on the activation of MMP-2 and MMP-9 in HCCLM3

The activation of MMP-2 and MMP-9 were examined by gelatin zymography. As shown in Fig. 5c and d, LCA had no significant effects on the activation of MMP-2 and MMP-9 in HCCLM3.

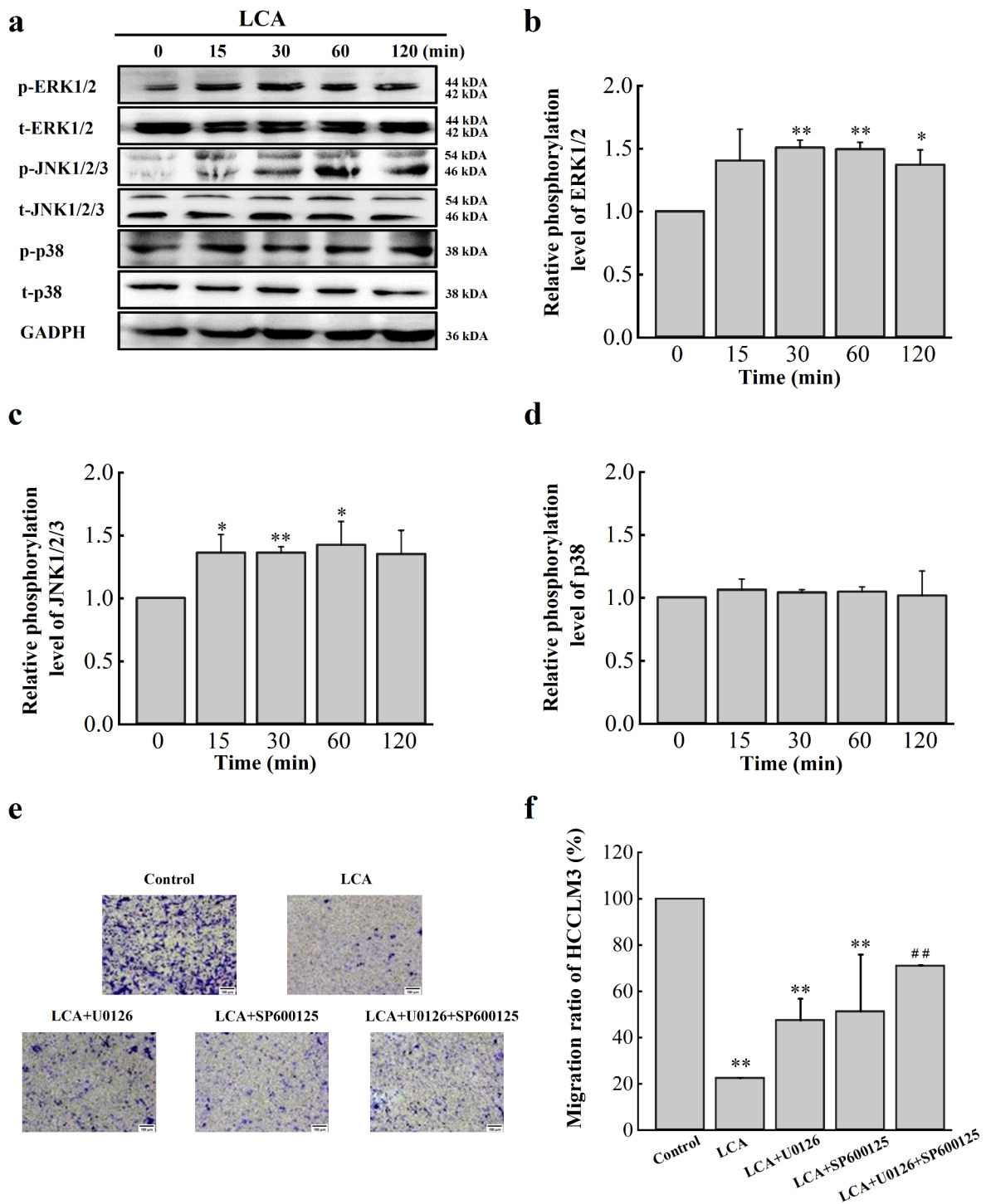
### Discussion

Recently, a series of experiments have demonstrated that the variation of some glycoproteins in hepatoma cells could affect the behavior of cell invasion or migration directly, and then turn the tumor into malignancy [3]. Although the cause and process of the variation of a glycoprotein could not be accurately determined, specific molecules and biological behavior of some mutations have been preliminarily

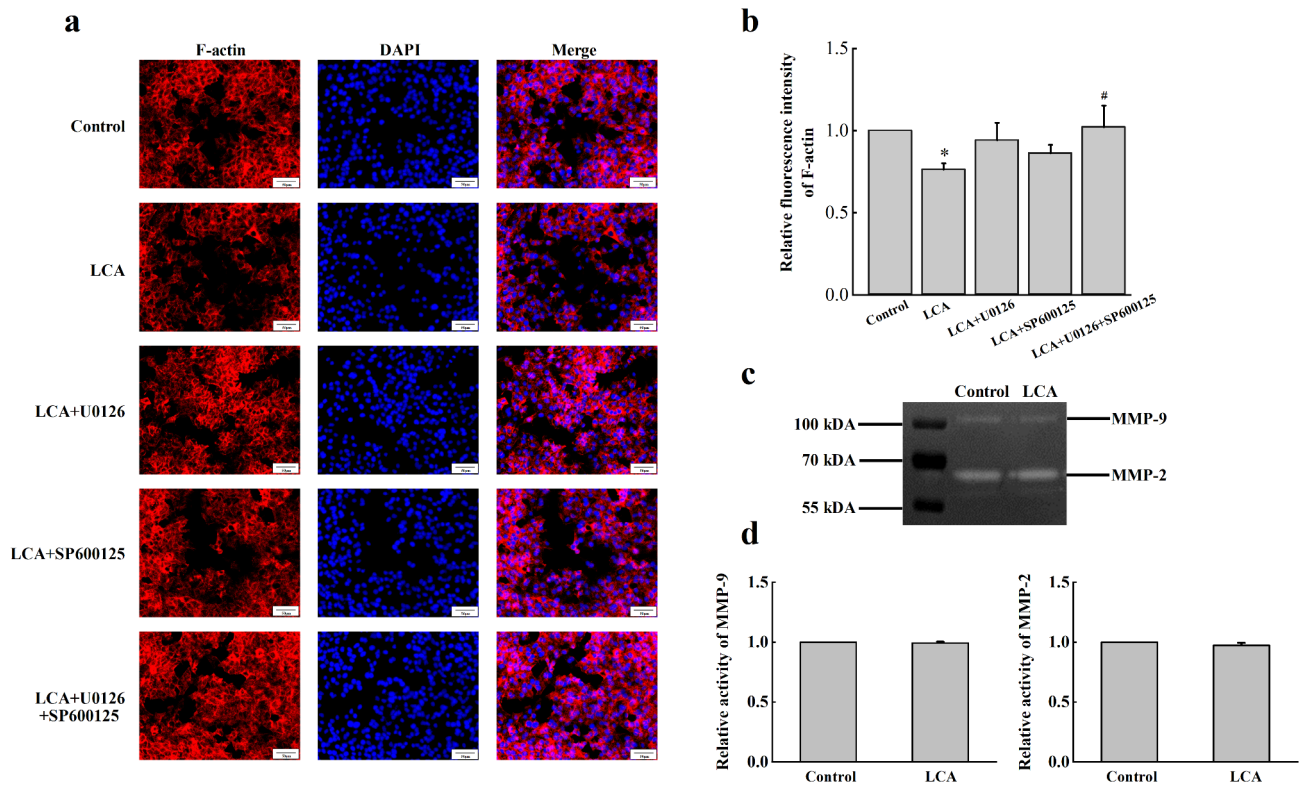
understood. In our study, we found that LCA had no significant toxic effect on three kinds of human hepatoma cells, but LCA might inhibit human hepatoma cell migration by reducing the F-actin formation via the mannose and fucose-mediated ERK1/2 and JNK1/2/3 signalling pathway.

In order to explore the similarities and differences of the action mode of LCA on human hepatoma cells with different malignant degrees, three kinds of human hepatoma cells were selected for preliminary experiments. Results showed that there was no significant difference in the response of the three human hepatoma cells to LCA in cell viability, cell migration and sugar inhibition assay. These results suggested that LCA might have same action mode on the three human hepatoma cell lines. Considering HCCLM3 and MHCC97L come from the same kind of hepatoma cell line MHCC97, so they are likely to have highly similar regulation modes of biological behavior when exposed to exogenous LCA [22]. HepG2 and MHCC97 are human





**Fig. 4** LCA inhibited HCCLM3 migration by up regulating ERK1/2 and JNK1/2/3 signalling pathway. (a) The expression levels of ERK1/2, JNK1/2/3 and p38 in HCCLM3 was analyzed by Western blot; Relative phosphorylation level of ERK1/2 (b), JNK1/2/3 (c), p38 (d) in HCCLM3; (e) Microscopic image of HCCLM3 migration after adding U0126 or/and SP600125 in Transwell Boyden Chamber (scale bar = 100 nm); (f) Quantification of HCCLM3 in transwell chamber was performed by counting cells after adding U0126 or/and SP600125. The data were expressed as the means  $\pm$  S.D.,  $n=4$ , \* $p < 0.05$  and \*\* $p < 0.01$  compared with the control group, ## $p < 0.01$  compared with the LCA group. LCA: Lens culinaris agglutinin (10  $\mu\text{g}/\text{mL}$ ); GADPH: Internal reference protein



**Fig. 5** LCA decreased the polymerization of F-actin through ERK1/2 and JNK1/2/3 pathway. (a) Microscopic images of F-actin and nucleus excited by fluorescence (scale bar = 50 nm); (b) Fluorescence intensity diagram of F-actin after quantitative analysis; (c) MMP-2 and MMP-9 under white light transmission treat with LCA; (d) The activation of MMP-2 and MMP-9 after optical density analysis. The data were expressed as the means  $\pm$  S.D.,  $n = 3$ , \* $p < 0.05$  compared with the control group, # $p < 0.05$  compared with the LCA group. LCA: Lens culinaris agglutinin (10  $\mu$ g/mL); F-actin: fibrous-actin; DAPI: 2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride; MMP: matrix metalloproteinase

liver cancer tissues from different races, given that they may have different genetic mutations, some of their biological behaviors and response patterns may be different. In addition, the metastatic attributes of these three cells are also different. Although HCCLM3 and MHCC97L are derived from MHCC97 cell line with high metastatic ability, while HCCLM3 has higher metastatic attributes than MHCC97L after additional inoculation and passage. HepG2 belongs to human hepatoblastoma with high differentiation and low metastasis. In short, the metastatic potential of three hepatoma cell lines in this study from high to low is HCCLM3, MHCC97L and HepG2. In some research on microRNAs of HepG2 and MHCC97, researchers found that there might be differences in gene expression and transcription between the two kinds of cells, which could directly affect their proliferation or migration behavior [23, 24]. In our study, the responses of three human hepatoma cells to LCA tended to be consistent, suggesting that HCC from different sources

and different degrees of malignancy might have the similar abnormal glycosylation patterns in cell proliferation and migration.

In the cytotoxicity experiment, we revealed that LCA had no significant killing ability human hepatoma cells. In other studies, researcher found that LCA could induce apoptosis of nasopharyngeal carcinoma cell lines CNE1 and CNE2, which was related to its mannose binding activity [25]. Moreover, another study showed that concanavalin A (Con A) had anti-proliferative activity against liver cancer, and Con A could cause liver cancer death through autophagy pathway [26]. These results suggested that different plant lectins might have different biological effects on cancer cells from different tissues. And these changes in biological effects were usually related to the combination of plant lectin and specific glycoproteins.

In our study, we found that LCA could inhibit the migration of HCCLM3, MHCC97L, HepG2 significantly, but had

no effect on L02. We speculated that the difference between human hepatoma cells and normal hepatocytes in response to LCA was caused by the change of glycosylation. The binding sites between plant lectin and tumor cells could be proved by exogenous addition of sugars indirectly that could compete with plant lectin [27]. Therefore, based on previous studies on the sugar grasping ability of LCA, mannose and fucose were added to understand the relationship between these two sugars and the binding sites of LCA in our study [16, 17]. We found that the inhibitory effect of LCA on the migration of three human hepatoma cells would be greatly reduced once LCA combined any of these two sugars in advance. These results suggested that the three human hepatoma cells all had binding sites of mannose or fucose, and the downstream biochemical reactions triggered by these sites were directly related to the behavior of cell migration. However, since blocking mannose or fucose could not completely restore the migration ability of cells, the LCA may combined with other glycoproteins to inhibit cell migration. From the above cell viability assay, cell migration assay and sugar inhibition assay, we found that the response of three kinds of hepatoma cells to LCA tends to be the same basically. Therefore, in the subsequent western blotting and immunofluorescence staining assay, we selected HCCLM3 cell line with relatively high metallic attributes to carry out the experiment.

MAPK pathway is an important intracellular signaling chain in the study of tumor biology. It is closely related to the occurrence, development, apoptosis and proliferation of many different types of tumor cells [28–30]. Xie et al. found that activation of ERK/MAPK signaling pathway might be involved in the occurrence and invasion of HCC [31]. J. Min et al. showed that the expression of MMP-9 could be regulated through p38/MAPK pathway, which affected the metastasis and invasion of HCC [32]. In our study, we found that exogenous LCA could up-regulate HCCLM3 phosphorylated ERK1/2 and JNK1/2/3 expression in a short time significantly, but had no effect on phosphorylated p38. This result indicated that LCA might activate part of the MAPK pathway to affect the migration of HCCLM3. When added inhibitors of ERK1/2 or JNK1/2/3, the LCA-inhibited cell migration was restored. This result indicated that LCA might activate part of the MAPK pathway to affect the migration of HCCLM3.

Numerous studies have demonstrated that the migration and invasion of tumor cells depended on the polymerization and dynamic assembly of actin filaments [33, 34]. At the same time, some studies showed that MAPK pathway was closely related to the formation of actin filaments in tumor cells [35]. In our study, we found that LCA could reduce the polymerization of F-actin in HCCLM3. ERK1/2 inhibitor U0126 and the JNK1/2/3 inhibitor SP600125 could suppress

the LCA-promoted F-actin depolymerization respectively. These results indicated that LCA might inhibit HCCLM3 migration by reducing the F-actin formation via ERK1/2 and JNK1/2/3 signalling pathway.

MMPs are a family of neutral endopeptidases and participate in ECM components degradation, which is involved in metastasis of tumor cells. Among them, MMP-2 and MMP-9 are being paid considerable attentions as high levels of those MMPs are correlated with invasive phenotypes [36]. MMP-2 and MMP-9 were usually modified and added with various glycosyl when they were released to the outside of the cell in a soluble form. Therefore, plant lectins with different sugar binding ability might affect the activities of MMP-2 and MMP-9 through different sugar binding site, which in turn affected the metastatic of tumor cells [37]. In our study, LCA showed no significant effect on the activation of MMP-2 and MMP-9 in HCCLM3. However, Jian et al. showed that BS-I could inhibit the hepatoma cells line MHCC97 migration and invasion by down-regulating the expression of MMP-2 and MMP-9 [38]. On the one hand, these results suggested that different plant lectins might have different sugar binding site for MMP-2 and MMP-9. On the other hand, the increased expression levels of MMP-2 and MMP-9 don't indicate that activity levels of MMP-2 and MMP-9 were increased [39, 40].

Various abnormal behaviors of tumors are closely related to their abnormal glycosylation. Nevertheless, the abnormal glycosylation processes are very complex, which is not only related to the synthesis of glycoproteins and the influence of various glycosylation enzymes, but also concerned with the different modifications of glycoproteins. In our study, we reported the effects of LCA on human hepatoma cells and normal hepatocytes, and explored the molecular mechanism of its effect on the migration behavior of HCCLM3 preliminarily. The sugar-binding motif was used as a motivation to link LCA with specific sugar grasping ability and HCC with aberrant glycosylation, which will deepen people's understanding on plant lectin as a drug in tumor glycobiology.

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## Declarations

**Conflict of interest** The authors declared no potential conflicts of interest with respect to research, authorship, and/or publication of this article.

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