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Original Article

Lycium barbarum polysaccharide inhibits gastric cancer cell proliferation, migration and invasion by down-regulation of MMPs and suppressing epithelial-mesenchymal transition

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Abstract: Objective: To evaluate the effect of *Lycium barbarum* polysaccharide (LBP) on gastric cancer (GC) cells and to explore the associated mechanism. Methods: Human GC SGC-7901 cells were divided into control, 10 μM LBP, 20 μM LBP and 50 μM LBP groups. CCK8 assay and Transwell assay were performed to evaluate the proliferation, migration and invasion of SGC-7901 cells. Western blotting was used to determine protein expressions. Results: The proliferation, migration and invasion decreased significantly in 20 μM LBP and 50 μM LBP groups. As the result of Western blotting, protein levels of MMP2, MMP9, Snail and vimentin decreased in 20 μM LBP and 50 μM LBP groups with different degrees. The expression E-cadherin significantly increased in all three experimental groups. The phosphorylation levels of AKT and PI3K in 20 μM LBP and 50 μM LBP groups were much lower than control group. Conclusion: LBP could inhibit the proliferation, migration and invasion of human GC cells bydown-regulation of MMPs and suppression of epithelial-mesenchymal transition (EMT).

Keywords: *Lycium barbarum* polysaccharide, gastric cancer, MMPs, epithelial-mesenchymal transition

Introduction

Gastric cancer (GC) is the second most frequent lethal cancer worldwide with approximately 50% cases in China [1, 2]. Most patients who are diagnosed with GC are in advanced stage of disease and predict extremely poor prognosis. The 5-year survival rate for GC patients varies between 5% in Stage IV and 90% in Stage I [3]. Although, in recent years, tremendous progress has been made in the development and advancement of GC research, the underlying molecular mechanisms involving migration and invasion are still poorly understood.

Lycium barbarum L. is a Solanaceous defoliated shrubbery that widely grows in arid and semi-arid regions of Northwestern China, Southeastern Europe and Mediterranean areas. *Lycium barbarum* L., also called Goji berry or wolfberry, is 1-2 cm long, bright orange-red ellipsoid berries [4]. *Lycium barbarum* L. has been used in East Asia as a traditional herbal medicine and functional food [5, 6], with a large variety of beneficial effects on reducing blood glucose and serum lipids, nourishing eyes, kidneys and liver, anti-radiation, immunity improvement, anti-aging, anticancer, anti-fatigue, enhancing hemopoiesis and male infertility and so on [7-10]. *Lycium barbarum* polysaccharides (LBP) are a group of water-soluble glycoconjugates isolated from the aqueous extracts of Lycium barbarum L., containing six monosaccharides (arabinose, rhamnose, xylose, mannose, galactose and glucose) [11]. As one of the major active ingredients responsible for above biological activities, LBP are estimated to comprise 5-8% of the dried fruits [5, 12]. LBP exerted beneficial effects on the animal models of ocular diseases, such as protecting retinal ganglion cells and retinal vasculature from acute ocular hypertension, preserving retinal function after partial optic nerve transaction, reducing

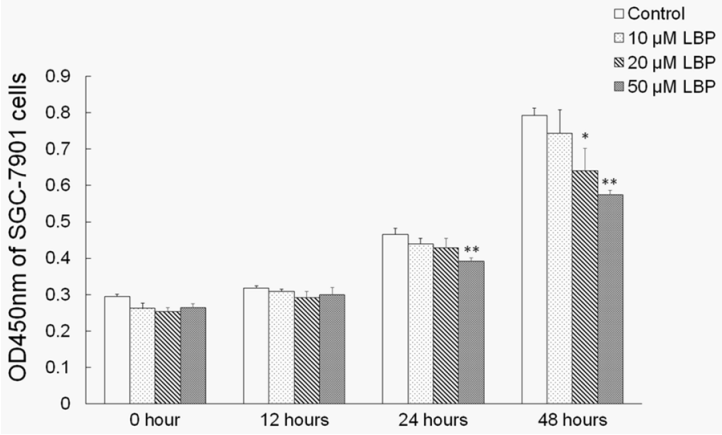

Figure 1. CCK8 assay was performed 0, 12, 24 and 48 h after adding LBP. To determine the amount of cells alive, absorbance was measured on 450 nm wavelength. *, $P<0.05$, vs control group; **, $P<0.01$, vs control group.

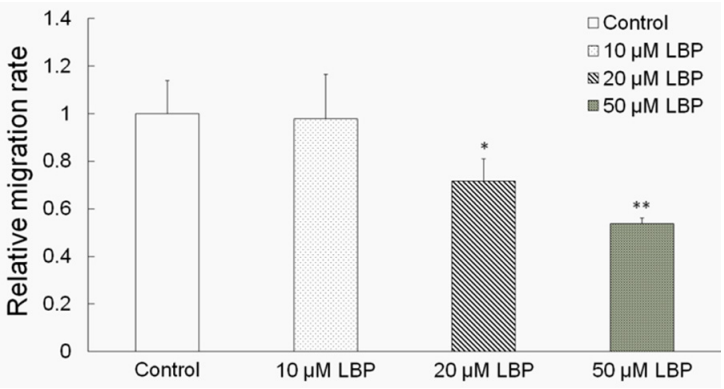

Figure 2. The relative migration rate of each group. *, $P<0.05$, vs control group; **, $P<0.01$, vs control group.

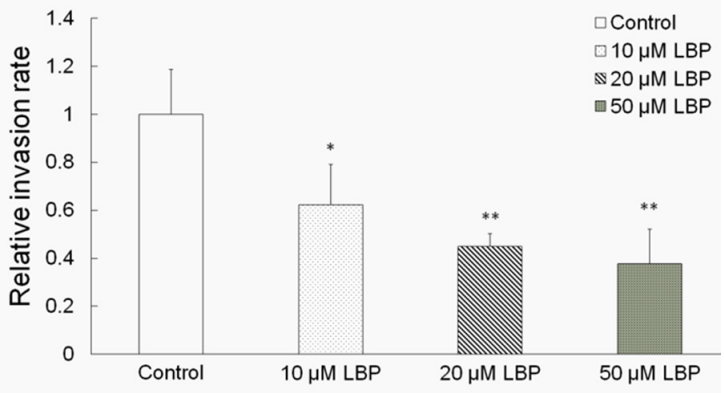

Figure 3. The relative invasion rate of each group. *, $P<0.05$, vs control group; **, $P<0.01$, vs control group.

neuronal damage, blood-retinal barrier disruption and oxidative stress in retinal ischemia/reperfusion injury [13-16].

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases which play an important role in the proteolytic destruction of extracellular matrix and basement membranes, thereby, they are essential for tumor invasion and metastasis [17]. MMPs, particularly MMP-2 and MMP-9 have been implicated in cancer invasion and metastasis [18].

Epithelial-mesenchymal transition (EMT) is a process in which epithelial cells lose their cell-cell contacts and undergo remodeling of the cytoskeleton to form a migratory phenotype [19]. The expression of epithelial marker proteins, such as E-cadherin and Keratin, are downregulated, while the expression of mesenchymal markers, such as vimentin and N-cadherin, are upregulated with EMT [20]. Therefore, it is widely accepted that EMT plays an important role in cancer metastasis of several human malignancies including GC [21]. The inhibition of EMT may improve GC, so EMT could be a very promising therapeutic target.

In this study, SGC-7901 cells were used to investigate the effects of LBP on human GC cells and the related mechanisms.

Methods

Cell line

A human GC SGC-7901 cell line (Beijing Dingguo Changsheng Biotech Co., Ltd, China) was cultured in RPMI1640 (Gibco, USA) containing 100 U/mL penicillin, 100 mg/L streptomycin and 10% heat-inactivated fetal bovine serum (Gibco, USA) at 37°C in humidified 5% CO_2 incubator.

Cell proliferation assay

SGC-7901 cells proliferation was measured by Cell Counting Kit-8 detection kit (Dojindo, Japan). Cells were seeded at a concentration of

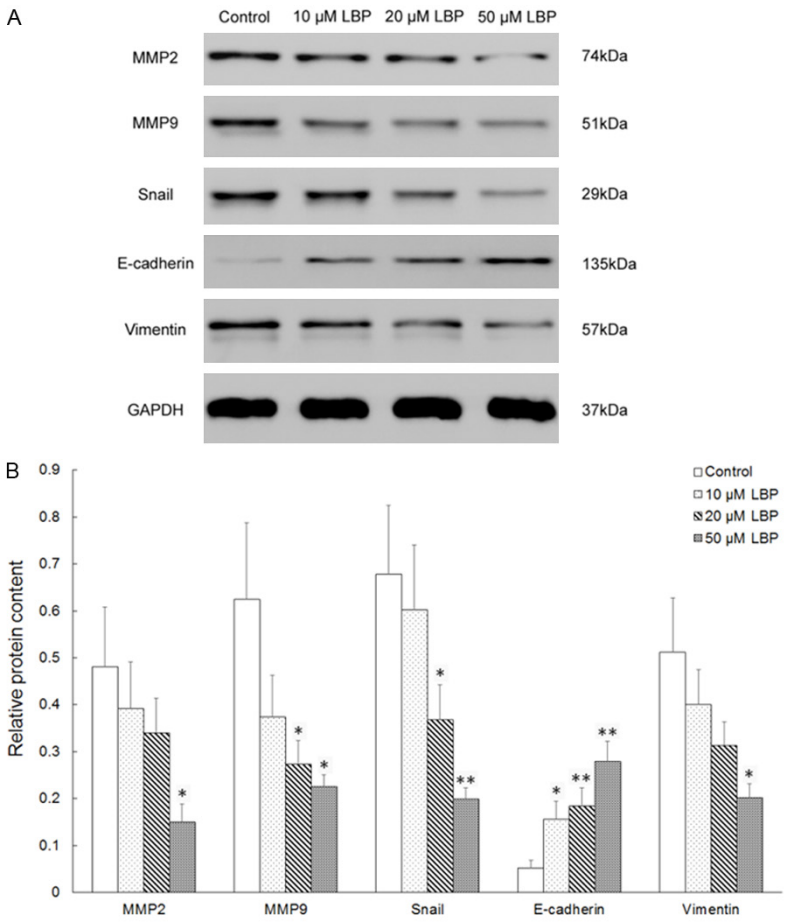

Figure 4. Western blotting. A. Western blotting of MMP2, MMP9, Snail, E-cadherin, Vimentin and their corresponding internal reference (GAPDH); B. The histogram for the content of proteins in each group. *, $P<0.05$, vs control group; **, $P<0.01$, vs control group.

5×10^3 cells per well in 96-well plates. All experiments were conducted in triplicate. After grown for 24 h resulted in about 70-80% confluence, cells were performed with different treatments as described above. At 0, 12, 24 and 48 h after transfection, CCK-8 solution was applied at 10 μL per well and followed by 2-h incubation at 37°C. Absorbance values of all wells were then determined at 450 nm in Microplate Reader (Bio-Rad, USA).

Migration assay

Migration assays were performed by seeding 1×10^4 cells in 100 μL of RPMI1640 on top of Transwell cell culture inserts consisting of a non-coated polyethylene terephthalate membrane (24-well inserts, 8.0 μm pore size; Corning, USA). The lower chamber was filled with 0.6 mL of RPMI1640. After incubation for 24 h, the non-migrating cells were scraped off, and the membranes were fixed and stained using the Diff-Quik™ stain kit (Sysmex, Japan). Cells that had migrated through the membranes were quantified by determination of the cell number in three randomly chosen visual fields at × 200 magnification.

Matrigel invasion assay

Tumor cells in serum-free RPMI1640 were seeded on top of transwell inserts with 8 μm pore-size polyethylene terephthalate membrane coated with Matrigel™ basement membrane matrix (BD Biosciences, USA), whereas the lower chamber was filled with RPMI1640 with 1% FBS as chemoattractants. Cells were cultured for 24 h before the non-migrating cells in the inserts were scraped off; membranes were fixed and stained using Diff-Quik™ stain kit (Sysmex, Japan). The cells that had migrated through the membrane were quantified by determination of the cell number in three randomly chosen visual fields at × 200 magnification.

Western blotting

Whole cell lysates were harvested and samples (50 μg protein/lane) were fractionated by SDS-PAGE and transferred to PVDF membranes. The membranes were incubated in 5% skimmed milk for 1 h at room temperature, and overnight at 4°C with primary antibodies against MMP2 (1:1000), MMP9 (1:500), Snail (1:1000), E-cadherin (1:1000), vimentin (1:1000) p-AKT (1:1000), AKT (1:1000), p-PI3K (1:1000), PI3K (1:1000) or GAPDH (1:2000) (Santa Cruz, USA). GAPDH was used as a loading control. And then secondary antibody (1:1000) was added and incubated for 2 h at room temperature. Bands were visualized using an ECL chemiluminescence kit (Genview, USA) and quantitated by Quantity One (Bio-Rad, USA).

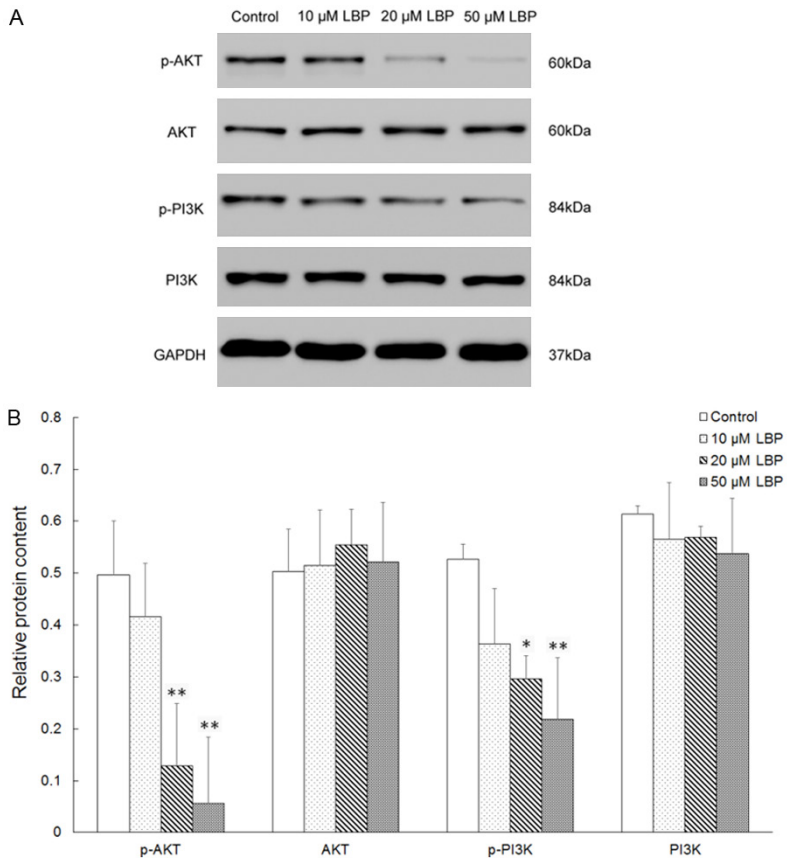

Figure 5. Western blotting. A. Western blotting of p-AKT, AKT, p-PI3K, PI3K and their corresponding internal reference (GAPDH); B. The histogram for the content of proteins in each group. *, $P<0.05$, vs control group; **, $P<0.01$, vs control group.

Statistical analysis

All data were expressed as mean ± SD (±s), and the statistical differences between two different groups were assessed by Student's t-test. $P<0.05$ indicated a significant difference, $P<0.01$ indicated that there was a very significant difference. Prism 5.0 (Graphpad Software, Inc., Canada) was used to calculate statistical differences between groups.

Results

LBP inhibited the proliferation of SGC-7901 cells

As the result of CCK8 assay, the cell proliferation decreased very significantly in 50 μM LBP group after 24 and 48 h ($P<0.01$, $P<0.01$). The cell proliferation also decreased in 20 μM LBP group after 48 h ($P<0.05$). There was no difference between 10 μM LBP group and control group at any time point ($P>0.05$) (**Figure 1**). It demonstrated that LBP with high concentration could inhibit the proliferation of human GC cells.

LBP inhibited the migration of SGC-7901 cells

Migrated cells through the membrane in each group were counted in three random fields and then the relative migration rate was calculated (relative migration rate = the number of migrated cells/the number of migrated cells in control group). **Figure 2** showed that the relative migration rate of 20 μM LBP and 50 μM LBP groups decreased significantly ($P<0.05$, $P<0.01$). It indicated that LBP could inhibit GC cell migration.

LBP inhibited the invasion of SGC-7901 cells

The relative invasion rate of each group was shown in **Figure 3**. The invasion of SGC-7901 cells could be suppressed by LBP even with a low concentration.

LBP affected the protein expression in SGC-7901 cells

SGC-7091 cells in 50 μM LBP group showed a significant decrease protein expression of MMP2 ($P<0.05$), MMP9 ($P<0.05$), Snail ($P<0.01$) and vimentin ($P<0.05$) (**Figure 4**). While 20 μM LBP could significantly reduce the expression of MMP9 ($P<0.05$) and Snail ($P<0.05$). An increase expression of E-cadherin was observed in all three experimental groups ($P<0.05$; $P<0.01$; $P<0.01$).

As shown in **Figure 5**, AKT and PI3K were expressed at a similar level in control group and experimental groups, however LBP reduced the phosphorylation of these proteins in a concentration-dependent manner.

Discussion

LBP was a kind of polysaccharide-protein complex, which has anticancer and immunologic enhancement activities [22, 23]. This study showed that LBP inhibited the proliferation, migration and invasion of human GC cell line SGC-7901.

One potential mechanism by which LBP could inhibit the invasion and migration of GC cells is the down-regulation of MMPs levels [24]. It is well established that secretion of MMPs with the capacity for extracellular matrix (ECM) degradation is a feature of metastatic cancer cells [25]. MMP2 and MMP9 are two of the most well-characterized MMPs and are closely associated with cancer invasion and metastasis due to their strong proteolytic activity of ECM [26].

EMT plays key roles in the pathogenesis of cancer and other human diseases [27, 28]. During EMT, expression levels of the adhesion molecule E-cadherin are decreased, whereas vimentin levels are increased. These molecular alterations possibly cause dysfunctional cell-cell adhesion and loss of cell-cell junctions, thereby allowing dissemination of tumor cells from the primary sites. According to our experimental results, LBP had certain effects on inhibiting the migration and invasion of GC cells by the suppression of EMT.

PI3K signaling plays a key role in inducing and maintaining EMT. Cells expressing a constitutively active form of PKB/AKT, the most important downstream effector of PI3K signaling, induces the expression of Snail, which in turn represses E-cadherin gene transcription and induces EMT [29]. In the present study, it was shown that LBP reduced the expression of Snail and the phosphorylation of PI3K and AKT in cells. These results suggested that LBP could inhibit the PI3K/AKT/Snail signaling pathway which was involved in EMT of human GC cells.

Conclusion

In conclusion, LBP with certain concentration could inhibit the proliferation, migration and invasion of human GC cells by down-regulation of MMP2 and MMP9 and suppression EMT in GC cells.

Disclosure of conflict of interest

None.

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