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Original Article

Epigenetic silence of lumican inhibits the motility of colon cancer via inactivating MAPK signaling *in vitro* and *in vivo*

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Abstract: Objective: To explore the effects of lumican on invasion and migration abilities of colon cancer cells and the relevant mechanism. Methods: The expression of lumican in four colon cancer cell lines (SW480, SW620, HCT-8 and LOVO) and the human colonic epithelial cells CCD-18Co was detected by western blotting. Lumican was silenced by constructing lentivirus and transfecting into SW480 and HCT-8 cells. The efficiency of silencing was detected by qRT-PCR and western blotting. The effect of lumican siRNA on invasion and migration of colon cancer cells was detected by transwell assay and wound scratch assay. The expression of metastasis-related proteins vascular endothelial growth factor (VEGF) and metal matrix proteinase (MMP)-9 was detected by western blotting. The level of ERK1, p-ERK1, JNK and p-JNK was also detected by western blotting. Finally, the effects of lumican on tumor growth and metastasis were detected in colon cancer xenograft. Results: The expression level of lumican was significantly increased in colon cell lines compared with CCD-18Co. Lumican was then silenced by transfecting siRNA into SW480 and HCT-8 cells. The decreased invasive cell number with reduced wound closing rate was detected in SW480 and HCT-8 cells transfected with lumican siRNA compared with control group. The decreased level of metastasis-related proteins VEGF and MMP-9 further convinced the inhibitory effect of lumican siRNA transfection on the motility of colon cancer. In addition, the expression of ERK/JNK pathway proteins was strongly suppressed under lumican siRNA treatment. Finally, the *in vivo* experiment revealed that lumican-siRNA strongly reduced the tumor growth and tumor volume. Besides, lumican-siRNA transfection inhibited tumor metastasis via suppressing the expression of metastasis-related proteins. The level of p-ERK1/2 and p-JNK was also reduced treated with lumican-siRNA. Conclusions: The inhibiting of lumican restrains the invasion and migration abilities of colon cancer and may through inhibiting ERK/JNK signaling pathway. Lumican may become a biological marker for predicting progression and prognosis of colon cancer.

Keywords: Lumican, colon cancer, invasion, migration, ERK

Introduction

Colon cancer is one of the most malignant tumors in China, with increasing incidence and mortality. According to relevant statistical data, the mortality of colon cancer has ranked the second among all cancer-related deaths, and half of the patients have distant metastasis [1, 2]. The onset of colon cancer is occult, without evident symptoms in the early stage. More severe, colon cancer in the middle and advanced stages was often accompanied by tumor metastasis. With rapid advances in the surgery-based comprehensive treatment, the prognosis of patients with colon cancer has been improved significantly, but the 5-year survival rate is still around 50% [3]. Therefore, investigation on mechanisms of invasion and migration and searching for the targets for early diagnosis and monitoring on the treatment effect are constantly the focus in studies on colon cancer.

The extracellular matrix (ECM) is closely related to tumor invasion and migration. Pathological changes can be observed during tumor infiltration and metastasis, including adhesion of the tumor cells to ECM and invasion into the matrix [4-6]. Lumican, a poly-membrane protein, is an essential component of ECM. The human Lumican gene is located at chromosome 12q21.3-

q22 [7]. Lumican plays an important role in regulating the balance of extracellular water. Lumican has also been suggested to regulate cell proliferation and metastasis in various tumors [8]. There is a relationship between lumican expression and growth of tumor cells in the interstitial tissues adjacent to cells; Lumican expression is possibly an important factor in invasion of pancreatic tumor cells [9]. Beyrau *et al.* has shown that lumican is involved in the transmission of tumor inflammation-related signals by binding to β2, αM and αL integrin subunits of polymorphonuclear leukocytes. Besides, inflammation response may destroy the body's immune system, leading to the occurrence of tumors [10]. However, the effect of lumican in colon cancer has been seldom reported, especially the effect of lumican on tumor invasion and migration and the related mechanisms.

In this study, we investigated the effect of lumican on the motility of the colon cancer and found that lumican was overexpressed in colon cell lines compared with CCD-18Co cells. The inhibiting of lumican effectively restrained the invasion and migration abilities of colon *cancer in vitro* and *in vivo* and may through inhibiting ERK/JNK signaling pathway.

Materials and methods

Cell lines and culture

The human colon cancer cell lines SW480, SW620, HCT-8, LOVO and the human colonic epithelial cells CCD-18Co were purchased from Wuhan University Cell Collection Center. Cell culture conditions: SW480, SW620 and CCD-18Co were cultured in RPMI 1640 containing 10% fetal calf serum at 37°C, 5% CO_2; HCT-8 and LOVO were cultured in RPMI DMEM containing 10% fetal calf serum at 37°C, 5% CO_2. The fetal calf serum was purchased from Gibco. The RPMI 1640 and RPMI DMEM media were obtained from HyClone.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA from each cell line were extracted using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The extracted RNA was then eluted with RNase-free water and stored at -80°C. qRT-PCR was performed by using SYBR-green PCR Master Mix in a Fast Real-time PCR 7500 System (Applied Biosystems). The RT-PCR primers for lumican were purchased from GeneCopoeia (SanDiego, California, USA). The specific primers were as follows: lumican (5'-TCGAGCTTGATCTCTCCTAT-3' forward and 5'-TGGTCCCAGGTCTTACAGAA-3' reverse). GAPDH was used as the internal control of the mRNA. Fold change of lumican was calculated by the equation $2^{-\Delta\Delta Ct}$.

Western blot analysis

Cell samples were lysed in lysis buffer (Beyotime, China). The samples mixed with loading buffer were incubated in boiling water for 10 min. 20-30 μg protein was separated through SDS-PAGE and then transferred onto Polyvinylidene Fluoride (PVDF) membranes (Millipore, Massachusetts, USA). The membranes were blocked in PBS with 0.1% Tween 20 containing 5% nonfat milk for 2 h at room temperature, and then were incubated with the primary antibodies and the corresponding HRP-conjugated secondary antibodies. The primary antibodies of lumican, vascular endothelial growth factor (VEGF), metal matrix proteinase (MMP)-9, ERK1, p-ERK1, JNK and p-JNK were purchased from Abcam (ab168348, ab17942, ab50011, ab179461 and ab76572). Membranes were extensively washed several times. Proteins were detected using a ChemiDoc XRS imaging system and Quantity One analysis software (Bio-Rad, San Francisco, California, USA). U6 and GAPDH (Abcam) were used as endogenous references.

Cell transfection

SiRNA fragments targeting lumican were designed and purchased from Invitrogen (USA). The scramble fragments were designed as the negative control of lumican. The SW480 and HCT-8 cells were seeded in 24-well plates at 1×10^5 cells per well. lumican siRNA and siRNA scramble were transfected into SW480 and HCT-8 cells using Lipofectamine 2000 according to the protocol (Invitrogen, USA). Cells were harvested for subsequent experiments after transfection for 24 hours.

Wound-healing assay

Wound-healing assay was performed to evaluate the migration rate of SW480 and HCT-8

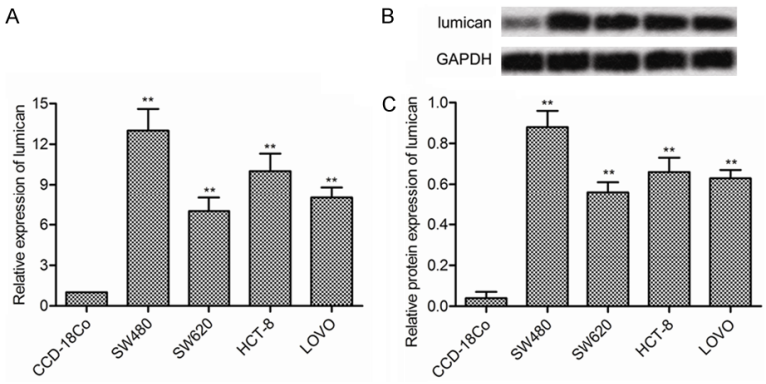

Figure 1. Lumican is highly expressed in colorectal cancer cell lines. Relative expression of lumican in colon carcinoma cell lines (SW480, SW620, HCT-8 and LOVO) and normal human colonic epithelial cells lines (CCD-18Co) was detected through qRT-PCR (A) and western blot (B) (**$P < 0.01$ versus CCD-18Co). (C) Relative protein expression of lumican in each cells was quantified using Image-Pro Plus 6.0 software and normalized to GAPDH. Data are represented as the mean ± SD of three experiments.

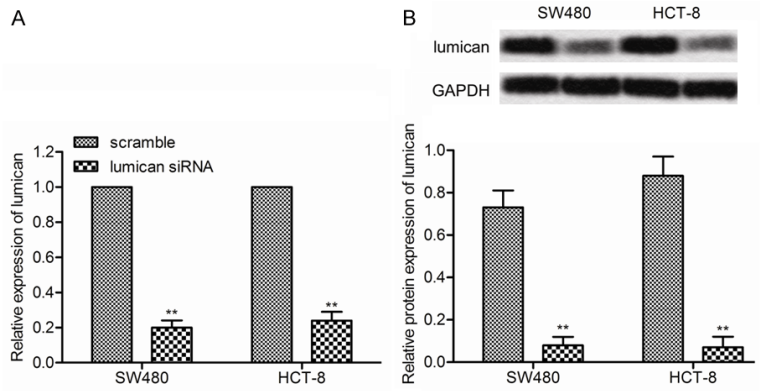

Figure 2. SiRNA transfection effectively reduces the level of lumican. Two colon cancer cell lines SW480 and HCT-8 were transfected with lumican siRNA or scramble, respectively. A. The expression of lumican in SW480 and HCT-8 cells was detected through western blotting. GAPDH was used as an endogenous reference. B. Histogram represents the statistical analysis of the changes in the expression of lumican (**$P < 0.01$ versus scramble group).

cells transfected with lumican siRNA/siRNA scramble. To accomplish this, 1.5×10^6 cells/well were seeded in 6-well plates and cultured overnight until the cells reached 90% confluence. A straight scratch was created by a sterile pipette tip. The destroyed cells were rinsed off with PBS 3 times gently and cultured in medium for another 24 h. Cell migration was observed and imaged at 0 h and 24 h with a digital camera (Leica DFC300FX).

Trans-well invasion assays

Two transwell invasion chambers with Matrigel (1 mg/ml) (Becton-Dickinson, New Jersey, USA) were used in invasion assays of SW480 and HCT-8 cells. Firstly, 200 μl serum-free medium containing 1×10^5 cells/well was added into the upper chamber, and the lower chamber contained 0.6 ml medium containing 20% FBS. After incubation at 37°C for 24 h, non-invading cells on the upper membranes were removed with a cotton swab. The migrated or invaded cells were fixed in 95% ethanol, stained with hematoxylin. The cell numbers were counted by Image J software and photographed under an inverted microscope on 10 random fields in each well. Each experiment was independently repeated in triplicate.

Colon cancer xenografts

Specific pathogen-free (SPF) athymic nude mice (male, six to eight weeks of age) were housed and manipulated according to the protocols approved by the Experimental Animal Center of the Southwest Medical University. For researching tumorigenicity of lumican in vivo, SW480 cells were transfected with lumican siRNA or siRNA scramble, respectively. Each mouse was subcutaneously inoculated with 1×10^7 SW480 cells transfected with lumican siRNA or scramble (fluorescent-labeled) in 50% Matrigel (BD Biosciences). After the development of a palpable tumor, the tumor volume was monitored every 6 days and assessed by measuring the 2 perpendicular dimensions using a caliper and the formula $(a \times b^2)/2$, where a is the larger and b is the smaller dimension of the tumor. At 30 days after inoculation, the mice were killed and tumor weights were assessed. Tumors from each mouse were randomly selected for immunohistochemical (IHC) analysis. All the animal experiments were performed according to relevant national and international guidelines and were approved by the Animal Experimental Ethical Committee.

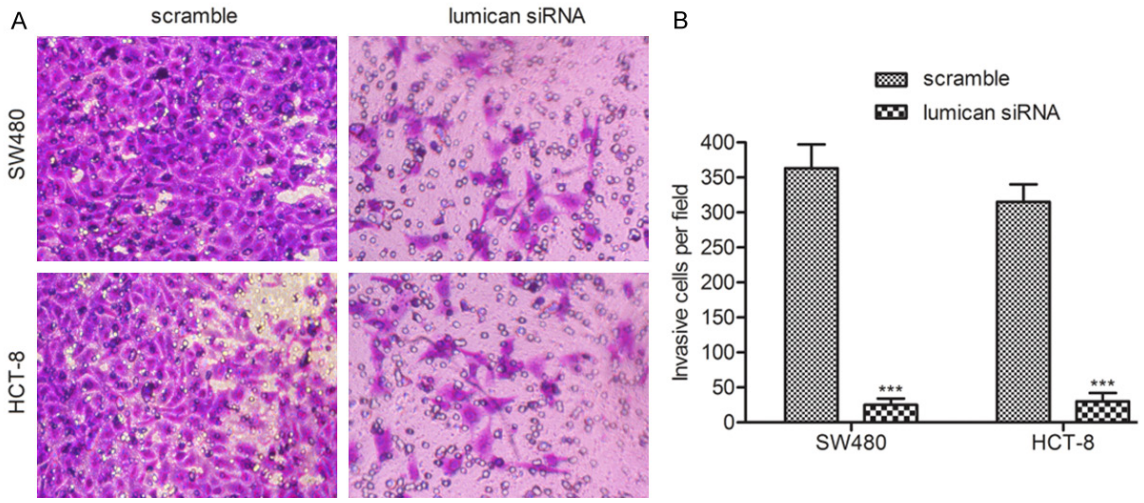

Figure 3. The inhibition of lumican reduces cell invasion ability of colorectal cancer. A. The invasive cells were detected by transwell invasion assays in SW480 and HCT-8 cells. B. Histogram represents the statistical analysis of Wound-healing assay. Data are represented as the mean ± SD of three experiments. $^{***}P < 0.001$ versus scramble group.

Statistical analysis

The results are presented as the mean ± the standard error of the mean of 3 replicates. Differences between means were analyzed using Student's t test. The difference was considered statistically significant at $P < 0.05$.

Results

Lumican is highly expressed in colorectal cancer cell lines

To determine whether lumican was involved in the development of colon cancer, the expression of lumican in colorectal cancer tissues and cell lines was detected through qRT-PCR. As shown in **Figure 1A**, The RNA expression level of lumican was significantly increased (range from 7-fold to 13-fold) in a panel of human colon cancer cell lines compared with normal human colonic epithelial cells lines CCD-18Co ($^{**}P < 0.01$, **Figure 1A**). The western blot analysis further convinced the elevated expression of lumican in colon cancer cells compared with CCD-18Co group (**Figure 1B** and **1C**). These results suggest that lumican is highly expressed in colon cancer.

SiRNA transfection effectively reduces the level of lumican

Considering the high expression of lumican in colon cancer cells, we then silenced the expression of lumican by transfecting siRNA fragments specific for targeting lumican into SW480 and HCT-8 cells. The results indicated that the mRNA and protein expression level of lumican was significantly decreased in both SW480 and HCT-8 cells ($^{**}P < 0.01$, **Figure 2**).

The inhibition of lumican reduces cell motility of colorectal cancer

We next investigated the role of lumican siRNA in regulating the migration and invasion ability in colon cancer cells. The number of invasive cells was decreased over 7 times in SW480 and HCT-8 cells transfected with lumican siRNA compared with scramble group ($^{***}P < 0.001$, **Figure 3**). Besides that, the results of wound healing assays showed a large closure of the gap in scramble group, whereas, a negligible effect on the closing rate of scratch wounds was seen in the lumican siRNA group compared with scramble group ($^{***}P < 0.01$, **Figure 4**). Moreover, the expression level of tumor metastasis-related proteins (MMP-9 and VEGF) were largely decreased by lumican siRNA ($^{***}P < 0.01$, **Figure 5**). These results indicate that the inhibition of lumican reduces cell motility of colorectal cancer.

The inhibition of lumican inactivates ERK/JNK signaling

The related signaling was then investigated in colon cancer cells. No significant change was

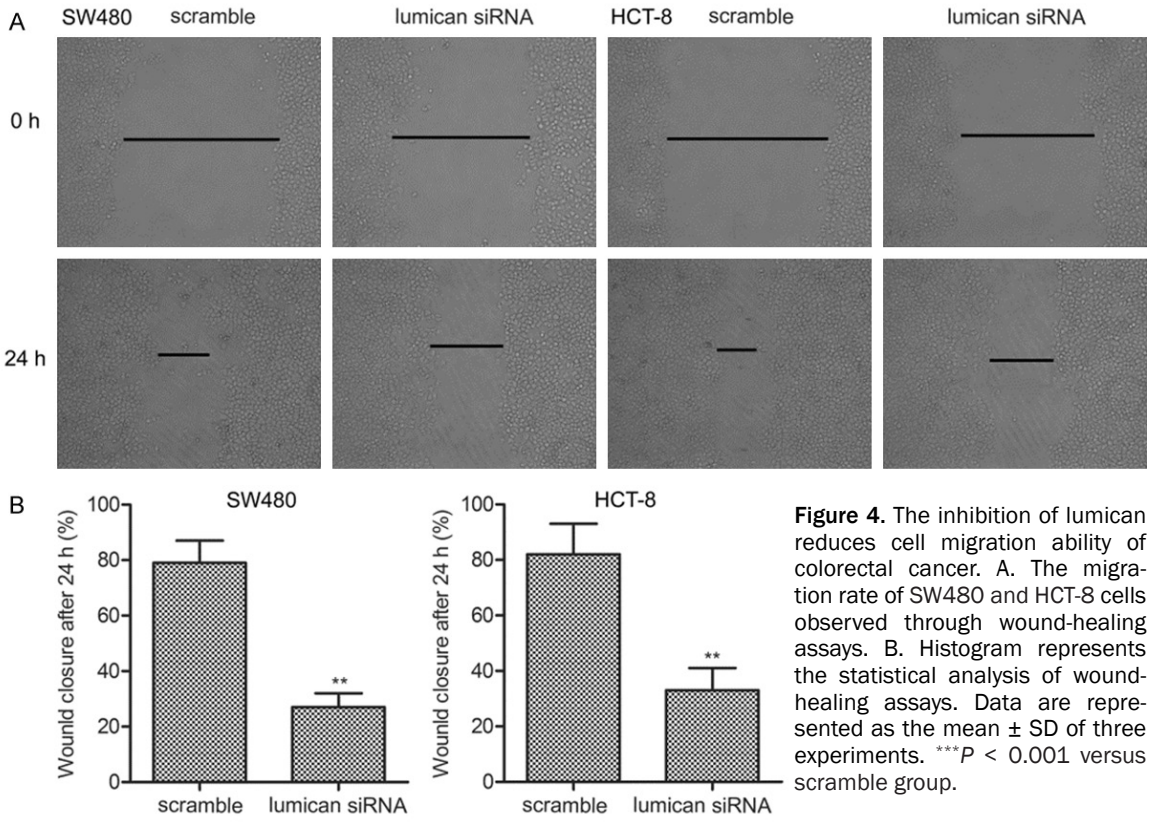

Figure 4. The inhibition of lumican reduces cell migration ability of colorectal cancer. A. The migration rate of SW480 and HCT-8 cells observed through wound-healing assays. B. Histogram represents the statistical analysis of wound-healing assays. Data are represented as the mean ± SD of three experiments. $^{***}P < 0.001$ versus scramble group.

observed in the expression of ERK1 and JNK ($P > 0.05$). However, the level of p-ERK1 and p-JNK was obviously down-regulated in SW480 and HCT-8 cells transfected with lumican siRNA compared with scramble group ($^{**}P < 0.01$, **Figure 6**). The results above indicate that the inhibition of lumican inactivated ERK/JNK signaling in colon cancer cells.

The inhibition of lumican suppresses tumor growth and metastasis in colon cancer xenografts

To investigate the effect of lumican siRNA on colon cancer growth and metastasis *in vivo*, xenograft mouse model was created by subcutaneous injection of SW480 cells pretreated with lumican siRNA or scramble to SPF nude mice. Lumican siRNA effectively suppressed tumor formation and tumor volume compared with scramble group ($^{*}P < 0.05$, **Figure 7A**, **7B**). The level of tumor metastasis-related proteins MMP-9 was significantly decreased in lumican siRNA treated mice tissue compared with scramble group (**Figure 7C**). Besides that, the level of lumican, p-ERK1 and p-JNK was strongly decreased in lumican siRNA group mice compared with scramble group ($^{**}P < 0.01$, **Figure 7D**, **7E**). These results indicated that the lumican siRNA restrained the growth and metastases of colon cancer in *vivo* and may inactivate the ERK1/JNK signaling pathway.

Discussion

Tumor infiltration and metastasis is a complicated process with multiple steps. To achieve metastasis, tumor cells must first infiltrate the ECM. Then, tumor cells can bind to ECM components, move to the infiltration area, pass through the matrix and result in metastasis [11-14]. Thus, ECM plays an essential role in tumor metastasis. Lumican is an important component of ECM. Many studies have shown that the leucine-rich proteoglycan family plays a major role in regulating cellular activities, including cell movement, tissue repair and tumor proliferation, as well as in ECM tissue hydration and collagen formation, etc [15, 16]. In recent years, several studies have demonstrated that lumican plays an critical part in tumor proliferation, apoptosis, infiltration and metastasis [17-

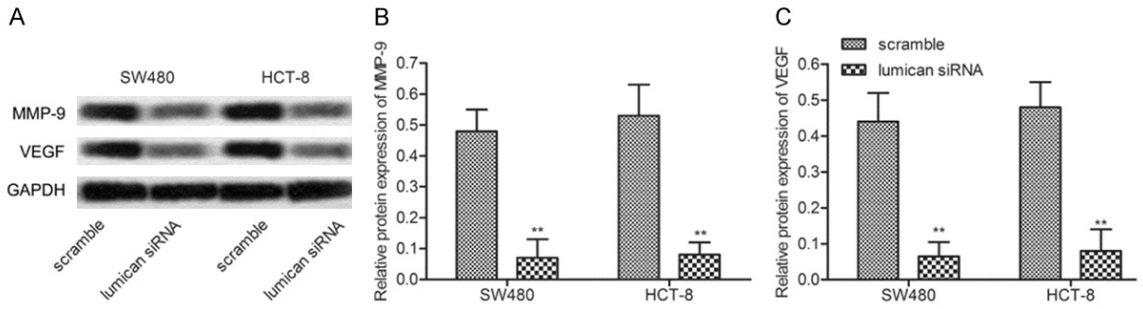

Figure 5. The inhibition of lumican suppresses the expression of metastasis proteins. (A) The expression of MMP-9 and VEGF in SW480 and HCT-8 cells were detected through Western blotting. GAPDH was used as an endogenous reference. Relative protein expression of MMP-9 (B) and VEGF (C) in SW480 and HCT-8 cells was quantified using Image-Pro Plus 6.0 software and normalized to GAPDH. Data are represented as the mean ± SD of three experiments. **P < 0.01 versus scramble group.

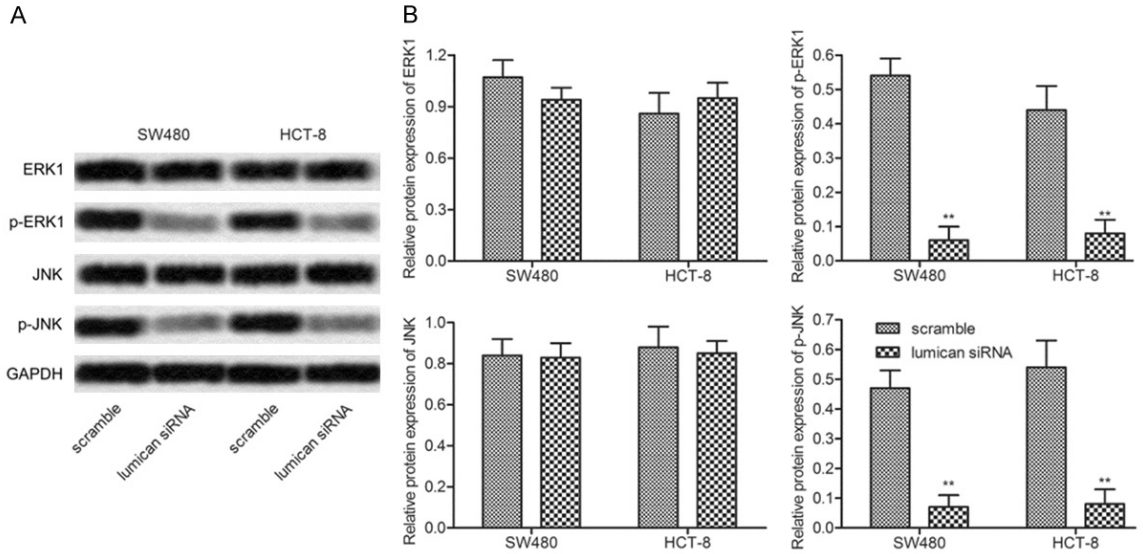

Figure 6. The inhibition of lumican inactivates ERK/JNK signaling. A. The expression of ERK1, p-ERK1, JNK and p-JNK in SW480 and HCT-8 cells was detected through Western blotting. GAPDH was used as an endogenous reference. B. Relative protein expression of each protein in SW480 and HCT-8 cells was quantified using Image-Pro Plus 6.0 software and normalized to GAPDH. Data are represented as the mean ± SD of three experiments. $^{**}P < 0.01$ versus scramble group.

19]. Our study investigated the effect of lumican on the motility of colon cancer.

As a member of a small leucine-rich proteoglycan family, the overexpression of lumican has been reported in breast [20], colorectal [21], uterine cervical [22] and pancreatic cancers [9]. Seya T et al. indicated that lumican expression was a potential prognostic factor in patients with advanced colorectal cancer with nodal metastasis [21]. Reports also has shown that lumican overexpression is associated with poor prognosis of pancreatic cancer [9]. In accordance with theses reports, high expression of lumican was detected in colon cancer cell lines compared with human colonic epithelial cells CCD-18Co, suggesting the tumorigenesis-promoting role of lumican.

The expression of lumican also correlates with the metastasis of various malignancies. Report indicated that lumican in stromal tissues plays an important role in the invasion of pancreatic cancer [9]. Report indicated that the increasing invasion of colorectal cancer with higher positive rate of lumican was observed in colon cancer tissues compared with healthy tissues [23], suggesting that lumican is closely related to the

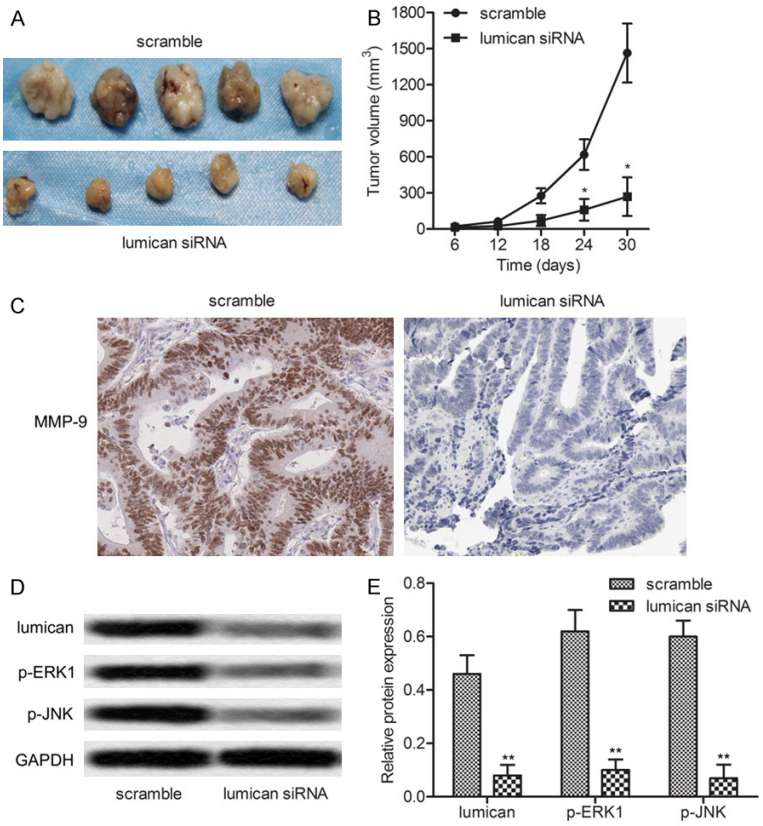

Figure 7. The inhibition of lumican suppresses tumor growth and metastasis in colon cancer xenografts. SW480 cells pretreated with lumican siRNA or scramble were injected into BALB/c nude mice. A. Representative tumors from two groups were shown (n = 5). B. Tumor growth trend in SW480-lumican siRNA mice and SW480-scramble mice was showed. C. The production of migration markers MMP-9 in formalin-fixed, paraffin-embedded tumors from SW480-lumican siRNA mice and SW480-scramble mice was detected through IHC analysis. D. The expression of lumican, p-ERK1 and p-JNK in each group was detected through Western blotting. GAPDH was used as an endogenous reference. E. Relative protein expression of each protein was quantified using Image-Pro Plus 6.0 software and normalized to GAPDH. Data are represented as the mean ± SD of three experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ versus scramble group.

metastasis of colorectal cancer. Consistent with these results, the would closing rate and invasive cell numbers was decreased with restrained expression of VEGF and MMP-9 were measured in SW480 and HCT-8 cells transfected with lumican siRNA compared with scramble group. These results indicate that the inhibition of lumican effectively suppresses the motility of colon cancer.

MAPK (mitogen-activated protein kinases) is a class of intracellular serine/threonine protein kinase, playing an important role in cell proliferation, apoptosis and intercellular function synchronization [24]. The phosphorylation of MAPK induces the activation of some nuclear transcription factors and thereby regulating the biological behaviors and functions of cells [25]. Studies have suggested that the invasion and metastasis of breast cancer is associated with abnormal activation of ERK/MAPK signaling pathways [26]. In gastric cancer cells, protease-activated receptor-2 induces the expression of VEGF and cyclooxygenase-2 by activating the ERK1/2 and p38 signaling pathways, thereby promoting angiogenesis and metastasis of gastric cancer cells [27]. In this study, the expression levels of p-ERK1/2 and p-JNK was significantly decreased after silencing lumican, suggesting that reduced level of lumican down-regulated the expression of p-ERK1/2 and p-JNK, and lumican could influence the functions of colon cancer cells through inactivating the MAPK signaling pathway.

Taken together, our study investigated the role of lumican in the metastasis of colon cancer and found that lumican was overexpressed in colon cancer cell lines. After silencing lumican by siRNA, the invasion and migration abilities of colon cancer was significantly inhibited with reduced expression level of ERK1/JNK pathway. Finally, the *in vivo* experiment revealed that lumican siRNA strongly reduced the tumor growth and metastasis via suppressing the expression of metastasis-related proteins and ERK1/JNK pathway. These findings suggest that lumican may be involved in invasion and migration of colon cancer, and lumican may become a biological marker for predicting progression and prognosis of colon cancer.

Acknowledgements

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Disclosure of conflict of interest

None.

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References

[1] Wang ZX, Cao JX, Liu ZP, Cui YX, Li CY, Li D, Zhang XY, Liu JL and Li JL. Combination of chemotherapy and immunotherapy for colon cancer in China: a meta-analysis. World J Gastroenterol 2014; 20: 1095-1106.

[2] Mao Q, Zhang Y, Fu X, Xue J, Guo W, Meng M, Zhou Z, Mo X and Lu Y. A tumor hypoxic niche protects human colon cancer stem cells from chemotherapy. J Cancer Res Clin Oncol 2013; 139: 211-222.

[3] Wang Y, Lu P, Zhang W, Du Q, Tang J, Wang H, Lu J and Hu R. GEN-27, a newly synthetic isoflavonoid, inhibits the proliferation of colon cancer cells in inflammation microenvironment by suppressing NF-kappaB pathway. Mediators Inflamm 2016; 2016: 2853040.

[4] Lu P, Weaver VM and Werb Z. The extracellular matrix: a dynamic niche in cancer progression. J Cell Biol 2012; 196: 395-406.

[5] Bonnans C, Chou J and Werb Z. Remodelling the extracellular matrix in development and disease. Nat Rev Mol Cell Biol 2014; 15: 786-801.

[6] Willis AL, Sabeh F, Li XY and Weiss SJ. Extracellular matrix determinants and the regulation of cancer cell invasion stratagems. J Microsc 2013; 251: 250-260.

[7] Saika S, Shiraishi A, Liu CY, Funderburgh JL, Kao CW, Converse RL and Kao WW. Role of lumican in the corneal epithelium during wound healing. J Biol Chem 2000; 275: 2607-2612.

[8] Nikitovic D, Papoutsidakis A, Karamanos NK and Tzanakakis GN. Lumican affects tumor cell functions, tumor-ECM interactions, angiogenesis and inflammatory response. Matrix Biol 2014; 35: 206-214.

[9] Ishiwata T, Cho K, Kawahara K, Yamamoto T, Fujiwara Y, Uchida E, Tajiri T and Naito Z. Role of lumican in cancer cells and adjacent stromal tissues in human pancreatic cancer. Oncol Rep 2007; 18: 537-543.

[10] Beyrau M, Bodkin JV and Nourshargh S. Neutrophil heterogeneity in health and disease: a revitalized avenue in inflammation and immunity. Open Biol 2012; 2: 120134.

[11] Fink K and Boratynski J. [The role of metalloproteinases in modification of extracellular matrix in invasive tumor growth, metastasis and angiogenesis]. Postepy Hig Med Dosw (Online) 2012; 66: 609-628.

[12] Hsiao KC, Shih NY, Fang HL, Huang TS, Kuo CC, Chu PY, Hung YM, Chou SW, Yang YY, Chang GC and Liu KJ. Surface alpha-enolase promotes extracellular matrix degradation and tumor metastasis and represents a new therapeutic target. PLoS One 2013; 8: e69354.

[13] Oskarsson T. Extracellular matrix components in breast cancer progression and metastasis. Breast 2013; 22 Suppl 2: S66-72.

[14] Tanis T, Cincin ZB, Gokcen-Rohlig B, Bireller ES, Ulusan M, Tanyel CR and Cakmakoglu B. The role of components of the extracellular matrix and inflammation on oral squamous cell carcinoma metastasis. Arch Oral Biol 2014; 59: 1155-1163.

[15] Xi HQ, Cai AZ, Wu XS, Cui JX, Shen WS, Bian SB, Wang N, Li JY, Lu CR, Song Z, Wei B and Chen L. Leucine-rich repeat-containing G-protein-coupled receptor 5 is associated with invasion, metastasis, and could be a potential therapeutic target in human gastric cancer. Br J Cancer 2014; 110: 2011-2020.

[16] Glinskii OV, Sud S, Mossine VV, Mawhinney TP, Anthony DC, Glinsky GV, Pienta KJ and Glinsky VV. Inhibition of prostate cancer bone metastasis by synthetic TF antigen mimic/galectin-3 inhibitor lactulose-L-leucine. Neoplasia 2012; 14: 65-73.

[17] Liu XJ, Kong FZ, Wang YH, Zheng JH, Wan WD, Deng CL, Mao GY, Li J, Yang XM, Zhang YL, Zhang XL, Yang SL and Zhang ZG. Lumican accelerates wound healing by enhancing alpha-2beta1 integrin-mediated fibroblast contractility. PLoS One 2013; 8: e67124.

[18] Nikitovic D, Berdiaki A, Zafiropoulos A, Katonis P, Tsatsakis A, Karamanos NK and Tzanakakis GN. Lumican expression is positively correlated with the differentiation and negatively with the growth of human osteosarcoma cells. FEBS J 2008; 275: 350-361.

[19] Zhou K and Ge M. [Effect of lumican gene overexpression on proliferation of lung adenocarcinoma cell A549 and its mechanism]. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 2013; 29: 492-495, 497.

[20] Kelemen LE, Couch FJ, Ahmed S, Dunning AM, Pharoah PD, Easton DF, Fredericksen ZS, Vierkant RA, Pankratz VS, Goode EL, Scott CG, Rider DN, Wang X, Cerhan JR and Vachon CM. Genetic variation in stromal proteins decorin and lumican with breast cancer: investigations in two case-control studies. Breast Cancer Res 2008; 10: R98.

[21] Seya T, Tanaka N, Shinji S, Yokoi K, Koizumi M, Teranishi N, Yamashita K, Tajiri T, Ishiwata T and Naito Z. Lumican expression in advanced colorectal cancer with nodal metastasis corre-

lates with poor prognosis. Oncol Rep 2006; 16: 1225-1230.
[22] Naito Z, Ishiwata T, Kurban G, Teduka K, Kawamoto Y, Kawahara K and Sugisaki Y. Expression and accumulation of lumican protein in uterine cervical cancer cells at the periphery of cancer nests. Int J Oncol 2002; 20: 943-948.
[23] Radwanska A, Litwin M, Nowak D, Baczynska D, Wegrowski Y, Maquart FX and Malicka-Blaszkiewicz M. Overexpression of lumican affects the migration of human colon cancer cells through up-regulation of gelsolin and filamentous actin reorganization. Exp Cell Res 2012; 318: 2312-2323.
[24] Sadaria MR, Yu JA, Meng X, Fullerton DA, Reece TB and Weyant MJ. Secretory phospholipase A2 mediates human esophageal adenocarcinoma cell growth and proliferation via ERK 1/2 pathway. Anticancer Res 2013; 33: 1337-1342.
[25] Wang XF, Zhou QM, Du J, Zhang H, Lu YY and Su SB. Baicalin suppresses migration, invasion and metastasis of breast cancer via p38MAPK signaling pathway. Anticancer Agents Med Chem 2013; 13: 923-931.
[26] Drigotas M, Affolter A, Mann WJ and Brieger J. Reactive oxygen species activation of MAPK pathway results in VEGF upregulation as an undesired irradiation response. J Oral Pathol Med 2013; 42: 612-619.
[27] Mirzoeva OK, Das D, Heiser LM, Bhattacharya S, Siwak D, Gendelman R, Bayani N, Wang NJ, Neve RM, Guan Y, Hu Z, Knight Z, Feiler HS, Gascard P, Parvin B, Spellman PT, Shokat KM, Wyrobek AJ, Bissell MJ, McCormick F, Kuo WL, Mills GB, Gray JW and Korn WM. Basal subtype and MAPK/ERK kinase (MEK)-phosphoinositide 3-kinase feedback signaling determine susceptibility of breast cancer cells to MEK inhibition. Cancer Res 2009; 69: 565-572.