A novel matrine derivative, WM130, inhibits activation and movement of human hepatic stellate LX-2 cells by targeting coflin 1

Yang Xu · Jicheng Duan · Weidan Ji · Chao Liu · Xiang Li · Qiuye Wu · Chunfang Gao · Changqing Su

Abstract Matrine, one of the active ingredients of *Sophora flavescens* Ait., has a protective effect in animal models on acute liver injury and liver fibrosis. However, since the protective effects are short-lived, a structural modification of matrine is needed to improve its anti-fibrotic effects. In the previous study we obtained a stable, highly active new matrine derivative, WM130, and explored its anti-fibrotic effects on the human hepatic stellate cell line, LX-2. CCK-8, wound healing, and transwell assays were used to investigate cell proliferation and migration, while 3D mimic study was used to determine the target of WM130. Western blots investigated the levels of α-SMA, coflin 1, p-cofilin 1, F-actin, PI3K, p-Akt, Akt, and PTEN in LX-2 cells treated with MW130. The results revealed that WM130 can significantly inhibit the proliferation of LX-2 cells at an IC\(_{50}\) of 60 μg/ml. At 30 μg/ml, matrine or WM130 significantly inhibited the migration of LX-2 cells. Moreover, WM130 significantly reduced the expression of α-SMA, coflin 1, F-actin, PI3K, and p-Akt, and increased PTEN levels. In conclusion, WM130 inhibits the proliferation, activation, and migration of human hepatic stellate LX-2 cells by targeting coflin 1.

Keywords Novel matrine derivative WM130 · Liver fibrosis · Hepatic stellate cells · LX-2 cell line · Cofilin 1

Abbreviations

- ECM: Extracellular matrix
- HSCs: Hepatic stellate cells
- MFB: Myofibroblast
- α-SMA: α-Smooth actin
- CCK-8: Cell counting kit-8
- SDS-PAGE: SDS–polyacrylamide gel electrophoresis
- IC\(_{50}\): Half-inhibitory concentration
Introduction

Liver fibrosis is the excessive accumulation of extracellular matrix (ECM) proteins in the liver and is caused by various liver diseases, such as nonalcoholic fatty liver disease, alcoholic liver disease, and viral hepatitis (Aydin and Akcali 2018; Estes et al. 2018; Younossi et al. 2018). Liver fibrosis is a reversible wound-healing response; even in the late stages of cholestasis and viral hepatitis, liver function can recover if risk factors are removed. However, when liver fibrosis has progressed to cirrhosis, the abnormally remodeled blood vessels and liver cells are replaced by scar tissue, which indicates that it is difficult to recover liver function (Okada et al. 2017; Kong et al. 2019). Hepatic fibrosis is a common pathological process in the development of chronic liver diseases that could progress to cirrhosis. Therefore, reversing hepatic fibrosis in chronic liver disease is an important strategy for preventing cirrhosis.

A previous study reported that hepatic stellate cells (HSCs) are important effector cells in liver fibrosis since they are the main source of ECM, which plays a key role in the development of liver fibrosis. HSCs are located in Disse's space, between hepatic sinusoidal endothelial cells and hepatic cells. Inactive HSCs play a primary role in the regulation of vitamin A metabolism and hepatic sinus blood flow in normal liver tissue (Higashi et al. 2017). However, HSCs become activated and transform into a proliferative myofibroblast (MFB) with increased contractility and up-regulated α-smooth actin (α-SMA) levels (Gressner 1996) when stimulated by certain physical factors, chemical factors, or microbial infections (Wallace et al. 2015; Koyama and Brenner 2017). Activated HSCs secrete collagen continuously and promote the abnormal accumulation of ECM, ultimately leading to liver fibrosis. In summary, activation of HSCs leads to increased cell proliferation and collagen synthesis, which is considered to be the major pathological process of liver fibrosis (Tsuchida and Friedman 2017). Therefore, inhibition of HSC activation is viewed as one of the main therapeutic strategies for blocking the progression of liver fibrosis.

The active ingredients of *Sophora flavescens* Ait. include matrine and oxymatrine. An increasing number of studies have revealed the pharmacological effects of matrine, including anti-inflammatory, immunosuppressive, anti-tumor, and anti-fibrotic effects. It has been used clinically to treat certain acute and chronic liver diseases (Zhang et al. 2001a, 2011; Wang et al. 2012; Liu et al. 2014). Oxymatrine has been demonstrated to have protective effects in an acute liver injury model in mice and a liver fibrosis model in rats (Zhang et al. 2001a, b). However, the protective effect was weak, which may be due to a short half-life ($t_{1/2} = 0.5$ h) (Yang et al. 2014). In this study, we modified the structure of matrine to obtain a novel matrine derivative, WM130 (Fig. 1A). Our previous results have shown that WM130 is structurally stable and has high pharmacological activity, which includes inhibiting the activity of HSC-T6 cells, thereby inhibiting the progression of liver fibrosis in rats (Xu et al. 2015). This study aimed to further explore the anti-fibrotic effects of WM130 on a human HSC cell line, LX-2, and determining its target and mechanism of action. This study may provide a theoretical basis for further work using WM130.

Materials and methods

Cell lines and cell culture

The human stellate cell line, LX-2, was obtained from Shanghai University of Traditional Chinese Medicine. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; GIBCO, New York, USA) supplemented with 10% fetal bovine serum (FBS; GIBCO, New York, USA), 100 μg/ml streptomycin, and 100 U/ml penicillin. They were grown in a humidified incubator at 37 °C with 5% CO₂. LX-2 cells have been known to retain virtually all features of activated HSCs.

Cell counting kit-8 (CCK-8) assay and IC₅₀ detection

LX-2 cells ($5 \times 10^3$) were plated into 96-well plates overnight and treated with different concentrations of WM130 (0–100 μg/ml) for 24 h. After adding 10 μl CCK-8 (Beyotime Institute of Biotechnology, Hainen, China), cells were incubated for 2 h and OD was measured at 450 nm. The IC₅₀ was determined using the trimmed Spearman-Karber method (Hamilton et al. 1977). Each concentration was repeated five times.
Wound healing assay

LX-2 cells ($2 \times 10^5$) were plated into 6-well plates. When 80% confluency was reached, the plates were scratched with a tip, washed using PBS, and incubated overnight with 30 μg/ml matrine or WM130, which is one-half of the IC$_{50}$. An untreated group was set up as a control. All groups were photographed at 0 and 24 h. Migration distance was determined using Adobe Photoshop ver. 3.0.

Transwell assay

LX-2 cells at $1 \times 10^6$ cells/ml were seeded (200 μl) into the upper chambers of 24-well transwell plates (8.0 μm pore size; Corning, NY, USA) and the cells were treated with 30 μg/ml matrine or WM130. An untreated control group was also set up. The bottom chamber contained 500 μl media with 20% FBS and the cells were incubated for 24 h to allow for migration. The cells in the bottom chamber were fixed with 1% paraformaldehyde and stained by crystal violet for 15 min, washed with PBS, and the number of migrated cells was determined using a microscope.

3D mimic study for the binding of WM130 to cofilin

WM130 resin was synthesized (SupFig. 1). The total protein from the LX-2 cells was used as the mobile phase and the protein adsorbed on the WM130 resin was collected. The proteins that were bound to WM130 were separated by SDS–polyacrylamide gel electrophoresis (SDS-PAGE). The amino acid sequence of the WM130 target protein from the LX-2 cells was identified using the MALDI-TOF/TOF mass spectrometry method. All the molecular modeling calculations were performed using SYBYL 6.9 version (Tripos International, St. Louis, MO, USA). And the structures of the compounds were assigned with Gasteiger–Hückle partial atomic charges. Energy minimization was performed using the Tripos force field, Powell optimization method, and MAXIMIN2 mini-mizer with a convergence criterion 0.001 kcal/mol Å. Simulated annealing was then performed.
The system was heated to 1000 K for 1.0 ps and then annealed to 250 K for 1.5 ps. The annealing function was exponential; 50 such cycles of annealing were run and the resulting 50 conformers were optimized using methods described above. The lowest energy conformation was selected. All the other parameters were default value. The structure of WM130 was drawn by Chemdraw software, and the protein structure of cofilin 1 was found in the protein data bank. The binding of cofilin 1 protein to compound WM130 was simulated with software Discovery Studio 3.5. The binding region and possible binding sites were determined by the size of binding free energy.

Immunofluorescent colocalization assay

WM130-Biotin was constructed (SupFig. 3). Then, $5 \times 10^4$ LX-2 cells were seeded on a cover slip and after an overnight growth. Treated with WM130-Biotin, the cells were fixed with 4% paraformaldehyde for 10 min at temperature and permeabilized with PBS-Triton X-100 for 20 min. Wash the cells three times with PBS+0.1% Triton X-100 (PBST) and block in PBST containing 10% goat serum and 5% BSA for 1 h at 37 °C. After three washes, incubate cells for 1 h at 37 °C with anti-cofilin 1 antibody (Cell Signaling Technology, Danvers, MA) diluted in blocking solution containing 2% Triton X-100 (1:200). After washing with PBST, maintain the cells in dark for 1 h with Alexa Fluor 594 conjugate Goat anti-rabbit IgG (Cell Signaling Technology, Danvers, MA). Wash samples in PBST and stain in DAPI (4′,6-diamidino-2-phenyindole, Sigma) for 5 min for nucleus labelling. After washing with PBST, add ProLong1 Gold anti-fade reagent (Thermo Fisher) and observe cells using an Olympus BX41 laser scanning confocal microscope.

RT-PCR assay

LX-2 cells were seeded in six-well plate at a density of $2 \times 10^5$ cell/well. Cells were incubated with 30 μg/ml matrine or WM130 (PBS was used as control). Total RNA was extracted with Trizol (Life Technologies Corporation, New York, USA). Reverse transcription was performed with PrimeScript RT Reagent Kit (TaKaRa, Dalian, China). The cDNA was then amplified by polymerase chain reaction (PCR). The primers include: GAPDH: Forward, 5'-TGT GGT CAT CAA TGG ATT TGG-3'; reverse, 5'-ACA CCA TGT ATT CCG GGT CAA T-3'. Cofilin 1: Forward, 5'-TTC AAC GAC ATG AAG GTG CGT-3'; reverse, 5'-TCC TCC AGG ATG ATG TTC TTC T-3'. p-Cofilin 1: Forward, 5'-TGG CCC TCG TAG CCT TGA GGA C-3'; reverse, 5'-CCA GTG CTG CAG GGT CCG AGG T-3'. F-actin: Forward, 5'-CTC TAC TTC GCA GTG GAA GAA AC-3'; reverse, 5'-ACG CAG GTC GTC GAT CTC ACC A-3'.

Western blot analysis

LX-2 cells were treated with 30 μg/ml matrine or WM130 and then collected. RIPA cell lysis buffer (Beyotime Institute of Biotechnology, Haimen, China) supplemented with phosphatase and protease inhibitors was used to extract proteins from 3x10^6 cells. Proteins were separated by SDS-PAGE and transferred onto a PVDF membrane. The primary antibodies used included anti-cofilin 1, anti-p-cofilin 1, anti-F-actin, anti-PI3K, anti-p-Akt, anti-Akt, anti-PTEN (Cell Signaling Technology, Danvers, MA), and anti-α-SMA (Abcam Inc. Cambridge, MA). The membranes were incubated with primary antibodies overnight at 4 °C, washed with TBST, and incubated with secondary antibodies (Abcam Inc. Cambridge, MA). The bands were scanned for densitometry analysis relative to the loading control using ImageJ software.

Statistical analysis

SPSS software version 17.0 was used to analyze data, which was expressed as mean values ± standard errors. A one-way ANOVA was used to compare the data between groups. Differences were considered significant at a p-value < 0.05.

Results

WM130 inhibits the proliferation and activation of LX-2 cells

WM130 strongly inhibited the proliferation of LX-2 cells in a concentration-dependent manner (Fig. 1B),
with an IC$_{50}$ of 60 μg/ml. WM130 at 30 μg/ml was used as a working concentration in further experiments. Activated LX-2 cells can express a large amount of α-SMA and ECM, leading to excessive accumulation of ECM. However, 30 μg/ml of matrine or WM130 can reduce α-SMA levels in LX-2 cells (Fig. 1C), with WM130 being more effective. These results indicated that WM130 significantly inhibits the activation of LX-2 cells.

WM130 inhibits the migration of LX-2 cells

To examine the effects of WM130 on the migration of LX-2 cells, we used a scratch test and found that the migration distance of LX-2 was significantly reduced after pretreatment with matrine or WM130 (Fig. 2A), with WM130 having a stronger effect. These results indicated that WM130 could inhibit the migration of LX-2. A transwell assay confirmed the results; the number of migrated cells after WM130 treatment was significantly lower (Fig. 2C). Our adhesion assay data revealed that WM130 could significantly reduce the adhesion of LX-2 cells to collagen I, fibronectin, and laminin which are the major components of the ECM (SupFig. 3).

WM130 acts on LX-2 cells via coflin 1

The whole protein in the cells acts as a mobile phase and binds to the WM130 resin (The synthesized WM130 resin is shown in SupFig. 1), during the interaction between WM130 and LX-2 cells. WM130 resin, which was bound by proteins, and empty resin were collected and subjected to SDS-PAGE.
A protein was identified in the WM130-bound sample and the amino acid sequence was analyzed by MALDI-TOF/TOF mass spectrometry. However, the protein was identified as cofilin 1 (SupTab. 1) and this identification was confirmed by the cofilin 1 (19kd) monoclonal antibody (Fig. 3A). Immunofluorescent colocalization in LX-2 cells revealed that WM130-Biotin (SupFig. 2) colocalized with cofilin 1 (Fig. 3B), and the above experiment confirmed that the target of WM130 in LX-2 cells is cofilin 1.

Next, we are working on using simulation techniques to identify therapeutic target protein. The protein of cofilin 1 that combine with WM130 tightly can be screened from the proteins’ library. We noted the binding site was disappeared after Ser3 mutation when using computer to simulate the free energy of the mutant binding to WM130, which suggested WM130 bind with cofilin 1 in the cells, and the mutation of ser3 has a great influence on the whole binding region (Fig. 3C).

**Fig. 3** The target of WM130 in LX-2 cells is cofilin 1. A SDS-PAGE to detect proteins absorbed by the resin and western blot analysis confirmed that the protein is cofilin 1; B Immunofluorescent colocalization confirmed that cofilin 1 is the target of WM130 in LX-2 cells; C The binding of cofilin 1 to WM130 was simulated with software Discovery Studio 3.5. However, the binding site between WM130 and cofilin 1 was Ser3.
WM130 down-regulates cofilin 1 and filamentous actin (F-actin) protein, and inhibits the PI3K/Akt signaling pathway.

Research shows that block the PI3K and Akt signals involved in hepatic stellate cells proliferation (Riaz et al. 2021). After treatment of LX-2 cells with WM130, cofilin 1, p-cofilin 1, F-actin, PI3K, p-Akt, Akt, and PTEN were detected. The expression of cofilin 1, F-actin, PI3K and p-Akt were significantly down-regulated, while the levels of PTEN protein were significantly increased (Fig. 4). This suggests that the effects of WM130 on LX-2 cell activity may be achieved by down-regulating the expression of cofilin 1 and F-actin proteins.

Fig. 4 WM130 down-regulates cofilin 1 and F-actin proteins in LX-2 cells and inhibits the PI3K/Akt signaling pathway. A: the mRNA levels of cofilin 1 and F-actin in LX-2 cells; B: WM130 down-regulates the mRNA levels of cofilin 1 and F-actin in LX-2 cells; C: Cofilin 1 and F-actin proteins in LX-2 cells; D: WM130 down-regulates cofilin 1 and F-actin in LX-2 cells; E: PI3K, p-Akt, and PTEN protein levels after WM130 treatment of LX-2 cells; F: WM130 inhibits the PI3K/Akt signaling pathway in LX-2 cells: *p < 0.05, **p < 0.01 vs control group; #p < 0.01 vs matrine group.
cofilin 1 and F-actin, as well as through inhibition of the PI3K/Akt signaling pathway.

**Discussion**

Matrine is one of the active ingredients of the traditional Chinese medicine *S. flavescens*. Numerous studies have identified its regulatory actions, which include anti-inflammatory, immune regulation, anti-tumor, and anti-fibrotic effects (Liu et al. 2014, 2015; Yu et al. 2014; Zhang et al. 2015). Although the pharmacological action of matrine is quite extensive, it has low activity levels. Therefore, an effective method was developed to structurally modify matrine to obtain a highly active, nontoxic derivative. We used a similar structure to modify matrine, with sophoridine as the parent nucleus. Studies have confirmed that the carbonyl group is the pharmacophore for matrine, and a series of thio-matrine compounds have been synthesized by addition of a thio group and a side-chain using the Michael addition method. In this process, WM130 and its maleate are obtained, producing a stable, highly active new matrine derivative. Our previous study (Xu et al. 2015) have found that HSCs are very sensitive to WM130, and their ability to proliferate is greatly impaired. The IC50 of the experimental cells is 68 μM. At concentrations of 34 μM and 68 μM, WM130 can significantly reduce α-SMA protein levels and induce apoptosis in HSC-T6 cells. In vivo experiments show that WM130 can effectively reduce the expression levels of TGF-β1 and α-SMA in fibrotic rat liver tissue. WM130 can also reduce collagen fiber formation and inhibit the progression of liver fibrosis in rats. However, the role of WM130 in human hepatic stellate cells is still unknown. The aim of the present study was to identify new targets and treatments for liver fibrosis.

The CCK-8 assay was used to detect the effects of WM130 on the proliferation of LX-2 cells. It was found that WM130 inhibits the proliferation of LX-2 cells, and the half-inhibitory concentration (IC50) of WM130 on LX-2 cells was calculated as 60 μg/ml. Therefore, we used half of the IC50 (30 μg/ml) of WM130 as the working concentration of WM130 or matrine on LX-2 cells. And the toxicity assay of WM130 was determined on normal liver cells WRL-68 in our previous study, IC50 of WM130 was 116.4 μM (about 68 μg/ml), which was much higher than the doses of 30 μg/ml used in vitro. So we think it is non-toxic at the concentration of 30 μg/ml of WM130 in vitro. Studies on liver fibrosis mechanisms suggest that HSCs activation is the core process for liver fibrosis. After activation, HSCs are transformed into myofibroblasts, which begin to produce α-SMA and secrete a large amount of ECM, forming scar tissue, and promoting the progression of liver fibrosis. Our experiments showed that WM130 significantly reduced α-SMA levels in LX-2 cells, indicating that WM130 can inhibit LX-2 cell activation. Scratch and transwell migration assays showed that WM130 inhibited LX-2 cell migration to a higher degree than matrine at the same concentration.

The human cofilin 1 gene (*CFL1*), which is located at 11q13, encodes a non-muscle tissue protein and is widely expressed in non-muscle tissues, especially in the brain and liver. Cofilin 1 binds actin and plays an important regulatory role in the depolymerization and polymerization of actin during cytoskeletal reorganization (Gressin et al. 2015; Wang et al. 2015). Cofilin 1 can sever F-actin, which is an important regulator of actin kinetics during cell migration. Cofilin 1 regulates actin recombination in normal cells, but under stressful conditions it binds to F-actin and utilizes F-actin’s instability to promote F-actin depolymerization (Schlau et al. 2018). Phosphorylation of Ser-3, a unique phosphorylation site in cofilin 1, causes cofilin 1 to lose the ability to bind F-actin, thereby enhancing the stability of F-actin (Tanaka et al. 2018). At present, most research on cofilin 1 has focused on its role in tumors. The activation of cofilin 1 was considered to be related to the invasion and metastasis of tumor cells (Virtanen et al. 2018). In addition, there is a dynamic balance between cofilin 1 and p-cofilin 1 in tumor cells, which plays an important role in the precise movement of cells, the regulation of cell and exogenous matrix adhesion, the nuclear division of cells, and the speed and depth of cell invasion. Local activation of cofilin 1 induces the formation of lamellipodia, which are rich in actin, the contractile force of which help move the cell forward (Han et al. 2007; Tahtamouni et al. 2013; Mousavi et al. 2018). Our study identified cofilin 1 as the target of WM130, and the binding site between WM130 and cofilin 1 was Ser3. Cofilin 1 and F-actin protein levels were significantly lower with WM130 treatment, indicating that the effects in LX-2 cells may be achieved by down-regulating these proteins. We suspect that the binding
of WM130 to cofilin 1 may affect the phosphorylation of cofilin 1 and the interaction of cofilin 1 with F-actin. The results of adhesion assay suggested that WM130 could affect cytoskeleton reorganization of LX-2 cells, which collate with the interaction between WM130 and cofilin 1/F-actin. The latest research confirms that hypoxia-induced cofilin 1 increases the proliferation, migration, invasion, and EMT in hepatocellular carcinoma by regulating the AKT pathway (Yao et al. 2021). BMP9 counteracts the tumorigenic and pro-angiogenic potential of glioblastoma by inhibiting the PI3K/AKT/MAPK and RhoA/Cofilin pathway (Porcù et al. 2018). These indicated that there are crosstalk between AKT pathway and cofilin 1. Then, WM130 downregulates the expression of cofilin 1 and F-actin not only at the protein but also at the mRNA level, possibly related to WM130 regulating AKT signaling pathway. However, we observed that the expression of cofilin 1 was difference in the stage of liver fibrosis. These might suggest that cofilin 1 played an important role on the progression from liver fibrosis to hepatocellular carcinoma.

Phosphatidylinositol-3-OH-kinase (PI3K) is an esterase that can be activated by cytokines and growth factors, which can bind the substrate 3,4-diphospholipid on the cell membrane. Phosphatidylinositol-3,4-bisphosphate (PIP2) is converted to 3,4,5-bisphosphoinositol (Phosphatidylinositol-3,4,5-bisphosphate, PIP3), which is the main mediator of PI3K’s biological effects, leading to activation of the downstream Akt signaling pathway (Ma et al. 2017). The PI3K/Akt pathway is considered to be the main signaling pathway for protein synthesis and plays an important role in regulating cell growth, proliferation, differentiation, and metastasis. The PTEN gene was originally thought to be a tumor suppressor gene with a dual-specificity phosphatase function. Based on the deeper understanding gained from recent research, PTEN is currently considered to be a negative regulator of protein kinase B (Akt) (Li et al. 2018). PTEN mainly inhibits Akt activity by dephosphorylation of the PI3P molecule to form PI2P, which blocks the PI3K/Akt signaling pathway (Zhang et al. 2018). Our study found that WM130 down-regulated PI3K and p-Akt protein levels in LX-2 cells while up-regulating the protein level of PTEN. This result suggests that the inhibitory effects of WM130 on LX-2 cells may be related to the activity of the PI3K/Akt signaling pathway.

In summary, the novel matrine derivative WM130 significantly inhibits the proliferation, activation, and migration of LX-2 cells. WM130 may down-regulate cofilin 1 and F-actin protein levels by targeting cofilin 1, which causes inhibition of LX-2 cell migration. Moreover, WM130 may regulate the proliferation and activation of LX-2 cells by inhibiting the PI3K/Akt signaling pathway.

Acknowledgements Not applicable.

Author contributions Yang Xu and Jicheng Duan wrote the main manuscript text. All authors reviewed the manuscript.

Funding This work was supported by The National Key Research and Development Program of China (No. 2018YFA0900902).

Declarations

Competing interests The authors declare no competing interests.

Conflict of interest None.

References

Aydin MM, Akcali KC (2018) Liver fibrosis. Turkish J Gastroenterol 29:14–21

Estes C, Razavi H, Loomba R, Younossi Z, Sanyal AJ (2018) Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. Hepatology 67:123–133

Gressin L, Guillotin A, Guerin C, Blanchoin L, Michelot A (2015) Architecture dependence of actin filament network disassembly. Curr Biol 25:1437–1447

Gressner AM (1996) Transdifferentiation of hepatic stellate cells (Ito cells) to myofibroblasts: a key event in hepatic fibrogenesis. Kidney Int Suppl 54:S39-45

Hamilton MA, Russo RC, Thurston RV (1977) Trimmed spearman-karber method for estimating median lethal concentrations in toxicity bioassays. Environ Sci Technol 11:714–719

Han L, Stope MB, de Jesús ML, Oude Weernink PA, Urban M, Wieland T, Rosskopf D, Mizuno K, Jakobs KH, Schmidt M (2007) Direct stimulation of receptor-controlled phospholipase D1 by phospho-cofilin. EMBO J 26:4189–4202

Higashi T, Friedman SL, Hoshida Y (2017) Hepatic stellate cells as key target in liver fibrosis. Adv Drug Deliv Rev 121:27–42

Kong Y, Sun Y, Zhou J, Wu X, Chen Y, Piao H, Lu L, Ding H, Nan Y, Jiang W, Xu Y, Xie W, Li H, Feng B, Shi G, Chen G, Li H, Zheng H, Cheng J, Wang T, Liu H, Lv F, Shao C, Mao Y, Sun J, Chen T, Han T, Han Y, Wang L, Ou X, Zhang H, Jia J, You H (2019) Early steep decline of liver stiffness predicts histological reversal of fibrosis in chronic liver disease. Hepatology 69:1865–1877
hepatitis B patients treated with entecavir. J Viral Hepatitis 26:576–585

Koyama Y, Brenner DA (2017) Liver inflammation and fibrosis. J Clin Invest 127:55–64

Li L, Zhu X, Shou T, Yang L, Cheng X, Wang J, Deng L, Zheng Y (2018) MicroRNA-28 promotes cell proliferation and invasion in gastric cancer via the PTEN/PI3K/AKT signaling pathway. Mol Med Rep 17:4003–4010

Liu Y, Xu Y, Ji W, Li X, Sun B, Gao Q, Su C (2014) Anti-tumor activities of matrine and oxymatrine: literature review. Tumour Biol 35:5111–5119

Liu ZW, Wang JK, Qiu C, Guan GC, Liu XH, Li SJ, Deng ZR (2015) Matrine pretreatment improves cardiac function in rats with diabetic cardiomyopathy via suppressing ROS/TLR-4 signaling pathway. Acta Pharmacol Sin 36:323–333

Ma Y, Chen F, Yang S, Duan Y, Sun Z, Shi J (2017) Silencing of TRB3 ameliorates diabetic tubule interstitial nephropathy via PI3K/AKT signaling in rats. Med Sci Monit 23:2816–2824

Mousavi S, Safaralizadeh R, Hosseinpour-Feizi M, Azimzadeh-Isfanjani A, Hashemzadeh S (2018) Study of cofilin 1 gene expression in colorectal cancer. J Gastrointest Oncol 9:791–796

Okada M, Enomoto M, Kawada N, Nguyen MH (2017) Effects of antiviral therapy in patients with chronic hepatitis B and cirrhosis. Expert Rev Gastroenterol Hepatol 11:1095–1104

Porch É et al (2018) BMP9 counteracts the tumorigenic and proangiogenic potential of glioblastoma. Cell Death Differ. https://doi.org/10.1038/s41418-018-0149-9

Riaz F, Chen Q, Lu K, Osoro EK, Wu L, Feng L, Zhao R, Yang L, Zhou Y, He Y, Zhu L, Du X, Sadiq M, Yang X, Li D (2021) Inhibition of miR-188-5p alleviates hepatic fibrosis by significantly reducing the activation and proliferation of HSCs through PTEN/PI3K/AKT pathway. J Cell Mol Med 25:4073–4087

Schlau M, Terheyden-Keighley D, Theis V, Mannherz HG, Theiss C (2018) VEGF triggers the activation of cofilin and the Arp2/3 complex within the growth cone. Int J Mol Sci 19:384

Tahtamouni LH, Shaw AE, Hasan MH, Yasir SR, Bamburg JR (2013) Non-overlapping activities of ADF and cofilin-1 during the migration of metastatic breast tumor cells. BMC Cell Biol 14:45

Tanaka K, Takeda S, Mitsuoka K, Oda T, Kimura-Sakiyama C, Maeda Y, Narita A (2018) Structural basis for cofilin binding and actin filament disassembly. Nat Commun 9:1860

Tsuchida T, Friedman SL (2017) Mechanisms of hepatic stellate cell activation. Nat Rev Gastroenterol Hepatol 14:397–411

Virtanen SS, Ishizu T, Sandholm JA, Løyttyniemi E, Vaananen HK, Tuomela JM, Harkonen PL (2018) Ablendonate-induced disruption of actin cytoskeleton and inhibition of migration/invasion are associated with cofilin downregulation in PC-3 prostate cancer cells. Oncotarget 9:32593–32608

Wallace MC, Friedman SL, Mann DA (2015) Emerging and disease-specific mechanisms of hepatic stellate cell activation. Semin Liver Dis 35:107–118

Wang Y, Yuan J, Yuan X, Wang W, Pei X, Zhao Q, Cao H, Xu M, Liu Z (2012) Observation of antinociceptive effects of oxymatrine and its effect on delayed rectifier K(+) currents (Ik) in PC12 cells. Neurochem Res 37:2143–2149

Wang Y, Kuramitsu Y, Kitagawa T, Baron B, Yoshino S, Maehara S, Maehara Y, Oka M, Nakamura K (2015) Cofilin-phosphatase slingshot-1L (SSH1L) is over-expressed in pancreatic cancer (PC) and contributes to tumor cell migration. Cancer Lett 360:171–176

Xu Y, Peng Z, Ji W, Li X, Lin X, Qian L, Li X, Chai X, Wu Q, Gao Q, Su C (2015) A novel matrine derivative WM130 inhibits activation of hepatic stellate cells and attenuates dimethylnitrosamine-induced liver fibrosis in rats. Biomed Res Int 2015:203978

Yang J, Hou Y, Ji G, Song Z, Liu Y, Dai G, Zhang Y, Chen J (2014) Targeted delivery of the RGD-labeled biodegradable polymersomes loaded with the hydrophilic drug oxymatrine on cultured hepatic stellate cells and liver fibrosis in rats. Eur J Pharm Sci 52:180–190

Yao B, Li Y, Chen T, Niu Y, Wang Y, Yang Y, Wei X, Liu Q, Tu K (2021) Hypoxia-induced cofilin 1 promotes hepatocellular carcinoma progression by regulating the PLD1/AKT pathway. Clin Transl Med 11:e566

Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, George J, Bugianesi E (2018) Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol 15:11–20

Yu JL, Li JH, Chengz RG, Ma YM, Wang XJ, Liu JC (2014) Effect of matrine on transforming growth factor β1 and hepatocyte growth factor in rat liver fibrosis model. Asian Pac J Trop Med 7:390–393

Zhang JP, Zhang M, Zhou JP, Liu FT, Zhou B, Xie WF, Guo C (2001a) Antifibrotic effects of matrine on in vitro and in vivo models of liver fibrosis. Acta Pharmacol Sin 22:183–186

Zhang JP, Zhang M, Jin C, Zhou B, Xie WF, Guo C, Zhang C, Qian DH (2001b) Matrine inhibits production and actions of fibrogenic cytokines released by mouse peritoneal macrophages. Acta Pharmacol Sin 22:765–768

Zhang B, Liu ZY, Li YY, Luo Y, Liu ML, Dong HY, Wang YX, Liu Y, Zhao PT, Jin FG, Li ZC (2011) Antiinflammatory effects of matrine in LPS-induced acute lung injury in mice. Euro J Pharm Sci 44:573–579

Zhang L, Zhang H, Zhu Z, Jiang L, Lu X, Zhou M, Sun X, He L, Bai Y, Ma L (2015) Matrine regulates immune functions to inhibit the proliferation of leukemic cells. Int J Clin Exp Med 8:5591–5600

Zhang J, Li L, Peng Y, Chen Y, Lv X, Li S, Qin X, Yang H, Wu C, Liu Y (2018) Surface chemistry induces mitochondria-mediated apoptosis of breast cancer cells via PTEN/PI3K/AKT signaling pathway, biochimica et biophysica acta. Mol Cell Res 1865:172–185

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.

 Springer