Inhibiting Effects of Down-regulating of Fascin 1 on Proliferation and Migration in Hepatoma Cells

Wanglu Gu
Medical School of Nantong University
Guilan Wang
Medical School of Nantong University
Xinyang Zhang
Medical School of Nantong University
Li Chen (bl1@ntu.edu.cn)
Medical School of Nantong University
Jiaming Zhou
Nantong University Xinglin College

Research Article

Keywords: fascin 1, siRNA, proliferation, migration, hepatoma cells

Posted Date: October 1st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-942724/v1

Abstract

Objective: To investigate the inhibiting effects of fascin 1 gene knock-down on the proliferation and migration of hepatoma cells by means of small interfering RNA (siRNA).

Methods: SiRNA targeting fascin 1 gene (si-fascin) and non-specific sequence siRNA (si-NC□were constructed and transfected into human hepatoma cell lines (HepG2 and Huh7) to down-regulate the expression of fascin 1. RT-qPCR, Western blotting, and Immunofluorescence technique were used to evaluate the efficiency of si-fascin. The proliferation and migration of cells were detected by MTT method and Transwell experiments, and the protein expression of genes related to proliferation and migration in cells were detected by Western blotting. The apoptosis and pseudopodia formation of cells were observed under scanning electron microscope (SEM).

Results: Compared with human normal liver cells (LO2), the expressions of fascin 1 mRNA and protein were significantly higher in HepG2 and Huh7 cells. The expression of fascin 1 was overall inhibited in HepG2 and Huh7 cells transfected by the constructed four si-fascins, among which, fascin_siR3 had the highest inhibitory efficiency, therefore was selected in this study. In HepG2 and Huh7 cells transfected by si-fascin significant knock-down target gene expression, while reducing cell proliferation, migration and the formation of pseudopods, and causes reduced protein expression associated with proliferation and migration.

Conclusion: This study further confirmed that fascin 1 gene has the function of promoting hepatoma cells proliferation and migration, suggesting that downregulating the expression of fascin 1 in hepatoma cells may be one of the strategies to intervene in liver cancer.

Intruduction

Hepatocellular carcinoma (HCC) ranks the sixth in the most common malignant tumor and the fourth in cancer mortality in the world [1]. Only 40% of early or local HCC patients are suitable for surgical resection, liver transplantation, and local radiofrequency ablation, and 20% are suitable for transcatheter arterial chemoembolization (TACE) and other treatments. Because of the lack of effective early diagnosis methods, about 80% of patients can only be diagnosed in the advanced stage. The prognosis of these advanced patients is poorer, the overall median survival is about 1 to 2 months [2], the main cause of death is the excessive proliferation, infiltration, and metastasis of the tumor, and the lack of effective intervention means. Therefore, it is of great significance for improving the prognosis of HCC patients by exploring the key genes that can regulate tumor proliferation and migration and developing them as intervention targets.

Fascin 1 belongs to the Fascin family. It is an actin-binding protein that can tightly bind to F -action, involved in tumor proliferation, growth, invasion and other malignant processes in a variety of human tumors, and is associated with poorer prognosis [3–6, 7–8]. By using small interfering RNA (siRNA) to knock down the gene [9–11] or by inhibiting certain signaling pathways, such as ERK, JNK, rpNK-lysin, PI3K/AKT and STAT3 signaling pathways [5, 12–15], the expression of fascin 1 was down-regulated, that reducing the invasion and metastasis ability of malignant tumor cells. Therefore, it is considered that fascin 1 is probable to become a therapeutic target for some malignant tumors (from epithelial and mesenchymal origin) [16–19]. In any case, there are still few studies on fascin 1 gene and protein in tumor tissue and cells, and its mechanism and regulatory factors still not very clear, and further research and verification are needed.

In this paper, small interfering RNA (siRNA) was used to down-regulate the expression of fascin 1 in human hepatoma cells (HepG2, Huh7), and to investigate the influence of fascin 1 on proliferation and migration of hepatocellular cancer cells and its meaning of the intervention.

1. Materials And Methods

1.1 Cells and reagents

Human hepatoma cells (HepG2, Huh7) and human normal liver (LO2) were purchased from the Liver Cancer Institute of Shanghai Zhongshan Hospital and stored in Biomics Biotechnologies (Nantong) Co., Ltd. DMEM medium and fetal bovine serum (FBS) were purchased from Gibco; Trizol, RNA reverse transcription kit and Lipofectamine^{TM2000} were purchased from Invitrogen; SYBR Green real-time quantification kit was purchased from Roche; rabbit anti- Fascin1 antibody, rabbit anti-hepatocyte growth factor (HGF), rabbit anti-fibronectin (FN), rabbit anti-matrix metalloproteinases (MMP7)antibody, rabbit anti-B-actin antibody, goat anti-rabbit IgG-HRP, and Goat anti- Rabbit IgG- TRITC all purchased from Proteintech. MTT staining solution was purchased from Nanjing Shengxing Biological Company; Transwell chamber was purchased from Corning Company; siRNA and Real-time PCR primers were synthesized by Biomics Biotechnologies Co., Ltd.

1.2 Methods

1.2.1 Construction and screening of siRNA targeting fascin1 gene

siRNAs targeting fascin 1 gene (si-fascin) including four si-fascin sequences (Fascin_siR1, Fascin_siR2, Fascin_siR3, and Fascin_siR4) and one non-specific sequence siRNA (si-NC) were designed according to the guideline proposed by Tuschl from GenBank. The sequences of above siRNAs were shown in Table 1. Among four si-fascin sequences, the most effective one in down-regulating target genes was screened out for subsequent experiments.

Table 1

siRNA sequence

siRNA ID		Sequence (5'-3')
Fascin_siR1	S	GCAAGUUUGUGACCUCCAAdTdT
	As	UUGGAGGUCACAAACUUGCdTdT
Fascin_siR2	S	GAUCGACCGCGACACCAAAdTdT
	As	UUUGGUGUCGCGGUCGAUCdTdT
Fascin_siR3	S	CGUUCGGGUUCAAGGUGAAdTdT
	As	UUCACCUUGAACCCGAACGdTdT
Fascin_siR4	S	AGUUCUGCGACUAUAACAAdTdT
	As	UUGUUAUAGUCGCAGAACUdTdT
Si-NC	S	UUCUCCGAACGUGUCACGUdTdT
	As	ACGUGACACGUUCGGAGAAdTdT

1.2.2 Cells transfection and grouping

The experimental cells were stored in Biomics Biotechnologies (Nantong) Co., Ltd. The cells were grown in DMEM medium containing 10% FBS and routinely cultured in a humidified incubator at 37°C with 5% CO2. The density of logarithmic growth cells as adjusted to 1×10^6 cells/mL, and the cells were seeded in a 24-well plate (1 ml per well). SiRNAs was respectively transfected into HepG2 and Huh7 cells according to the instructions of Lipofectamine^{TM2000} (In each well, appropriate amount of siRNA was add to 50 μL Opti-MEM, then mixed gently. The final concentration of siRNA is 100 nM).

The experiment groups: si-fascin transfected group(si_fascin), si-NC transfected group (si_NC) and untreated group. The latter two groups served as experimental control groups.

1.2.3 Real-time quantitative PCR (RT-qPCR)

Transfected cells and untreated cells at logarithmic growth phase were collected by centrifugation. The total RNA was extracted separately with Trizol reagent and reversely transcribed into cDNA. RT-qPCR amplification was performed according to the instructions of the SYBR Green Real-Time Quantitative Kit. The cycle parameters of PCR amplification were 95℃ for 20sec, 55℃ for 30sec, 72℃ for 30sec, which repeated 45 cycles. The $2^{-\triangle\triangle Ct}$ value method [20] was used to calculate the relative expression of the target gene. Relative messenger RNA levels were normalized to housekeeping gene GAPDH (glyceraldehyde-3-phosphate dehydrogenase) from the same sample. The primer sequences were shown in Table 2. All samples were run in triplicate. RT-PCR primers were synthesized by Biomics Biotechnologies Co., Ltd.

Table 2

The primer sequences and length

Primer		Sequence (5'-3')	length（bp）
Fascin	F	CCTACAACATCAAAGACT	186 bp
	R	CAGAACTCGAAGAAGAAG	
GAPDH	F	GAAGGTGAAGGTCGGAGTC	226 bp
	R	GAAGATGGTGATGGGATTTC	

1.2.4 Western blotting

The cells were inoculated into 6-well plates and cultured in 37℃ and 5% CO2 incubator for 24 hours. When the fusion degree was 70% ~ 80%, the cells were harvested and lysed with SDS Lysis Buffer, extracted total protein and determined protein concentration by BCA method. After the protein lysate was electrophoretic with SDS-PAGE (5% laminating gel, 8% separating gel) and transferring for 2 h at 200 mA constant Flow using a wet transfer instrument, the proteins in the gels were transferred onto the PVDF membrane. After blocked for 2 hours at room temperature with TBS blocking solution containing 5% skimmed milk powder, the PVDF membrane was incubated at 37°C for 2 hours with primary antibody, including fascin 1 (1:2000), HGF (1:2000), FN (1:2000), and MMP7 (1:2000), then added the secondary antibody (1:1000) and incubated at 37°C for 2 h. Finally, the cleaned membrane was developed by electrochemiluminescence (ECL) method. Result analysis: the internal reference gene β-actin (1:1000) was used as an internal control, the gray value of the target band was analyzed by Image J software. The relative expression of the target gene is equal to the ratio of the gray value of the target band to the gray value of the internal reference of the same sample.

1.2.5 Immunofluorescence Staining

The growth phase cells were collected and plated for 24 hours. When the fusion degree reached 50% ~ 60%, the cells were fixed with 4% paraformaldehyde for 30 min, and treated with 0.5% Triton membrane for 15 min, blocked with 1% BSA solution for 30 min, and incubated with a primary fascin 1 antibody (1:50 diluted) at 4°C overnight, then added goat anti- rabbit IgG-TRITC antibody (1:50 diluted) for 30 min at room temperature. The cells were counter-stained with 1 μg/mL Hoechst in the dark, incubated at room temperature for 10 min, mounted with anti-fluorescence quenching mounting solution, observed under a fluorescent microscope, and captured image.

1.2.6 MTT detection

In each group, the cells at logarithmic growth phase were harvested by centrifugation, plated in 4 parallel wells of 96-well plate at 5×10^4 cells/mL (100 μl /well). Then, 10 μL MTT was added to each well and store at 37°C in the dark for 4 hours, followed by 150 μL/well of DMSO at 37°C for 10 min. After pipetting and mixing, 120 μL was taken out and transferred to another clean 96-well plate, and 120 μl DMSO was taken as a blank control to zero. Then the OD value was measured at 0 h, 24 h, 48 h, 72 h and 96 h after transfection on the microplate reader with a wavelength of 490 nm, and cell proliferation curve was drawn with time as the horizontal axis and OD value as the vertical axis.

1.2.7 Transwell experiment

The logarithmic growth phase cells were taken to inoculate in 24-well plates, 100μL and 600 μL DMEM complete medium were added to the upper and lower chambers of the Transwell chamber, respectively, then incubated overnight at 37°C with 5% CO2. After 48 h of transfection, the cells were resuspended in DMEM basal medium and the cell density was adjusted to 1×10^6/mL. After 24 h, the medium in the upper and lower chambers was aspirated and discarded. The cells in the upper chamber were gently wiped with a cotton swab. After washing with PBS, the cells were fixed with 10% methanol for 30 sec. The lower chamber was immersed in 0.2% crystal violet solution for 5 min. The membrane of the small chamber was cut off along the edge, and the cells that passed through the membrane and moved to the lower chamber were counted under an inverted microscope, and each membrane was counted with 5 different fields (×250) and photographed.

1.2.8 Preparation of electron microscope samples

The sterilized small round slides were placed in 24-well plates, and cells were respectively seeded on slides, and transfection was performed when the cell climbing density reaches about 60%, then added 2.5% glutaraldehyde and fixed at 4℃ for 24 h. The preparation of electron microscope samples was assisted by the Electron Microscope Room of Nantong University.

1.3 Statistical analysis

Each group of experiments should be repeated at least 3 times. All data are expressed as mean±SD, and GraphPad Prism 8 software was used for statistical analysis. The comparison between groups of samples used t test. Differences were considered statistically significant when $P<0.05$.

2. Results

2.1 Expression of fascin 1 in HepG2 and Huh7 cells

The expression of fascin 1 in HepG2 and Huh7 cells were detected by RT-qPCR, Western blot and immunofluorescence staining, respectively, and LO2 cells were used as a control (Fig. 1). Figure 1 showed that, compared with LO2, the expression of fascin 1 mRNA and protein in HepG2 and Huh7 cells were significantly up-regulated ($P< 0.01$). Immunofluorescence Staining results showed that fascin 1 protein was fine granules diffusely distributed in the cytoplasm of HepG2 and Huh7 cells, while coarse granules were scattered in the nucleus. But almost no expression was seen in LO2 cells (Fig. 1c). These results suggest that the gene and protein of fascin 1 were significantly overexpressed in HepG2 and Huh7 cells.

2.2 Si-fascins Selection

After four si-fascins were transfected into HepG2 and Huh7 cells, respectively, the effects of si-fascin on target gene knockdown were detected and screened by RT-qPCR (Fig. 2). As shown in Fig. 2, the mRNA level of fascin 1

was significantly down-regulated in si-fascin cells ($p < 0.05$), compared with the untreated cells and si-NC cells. Fascin_siR3 has the best inhibitory efficient in the four si-fascins. Therefore, Fascin_siR3 was used in subsequent experiments.

2.3 si-Fascin inhibited the proliferation of HepG2 and Huh7 cells

The proliferation levels of HepG2 and Huh7 cells transfected by si-Fascin were detected by MTT method at 0h, 24h, 48h, 72h, and 96h respectively (Fig. 3).

As shown in Fig. 3, MTT method detection showed a significant decrease in OD values for si-fascin treating HepG2 cells at 48 h, 72 h, and 96 h over against si-NC and untreated cells(all $p < 0.05$), for si-fascin treating Huh7 at 48 h, 72 h and 96 h over against si-NC and untreated cells ($p < 0.01$, $p < 0.05$), respectively. These results suggested that downregulating fascin 1 can suppresses cell proliferation.

2.4 si-Fascin inhibited the migration of HepG2 and Huh7 cells

The results of Transwell migration experiment showed that number of migration cells in si-Fascin treating HepG2 (115 ± 7.23) and Huh7 (125 ± 10.02) were significantly less than the si-NC cells (273 ± 10.02) and the untreated cells (247 ± 19.30) (all $p < 0.001$) (Fig. 4). The results suggested that down-regulating fascin 1 can suppresses the migration of hepatoma cells.

2.5 Down-regulation of fascin 1 affected the expression of related genes

Western blot examined the expressions of target gene proteins and related gene proteins in si-fascin treated cells (Fig. 5).

As shown in Fig. 5, comparison with untreated cells and si-NC treated cells, not only significantly decreased fascin 1 expression, but also significantly decreased HGF, FN, and MMP7 protein expression in si-fascin treated cells, suggesting that si-fascin downregulated expression of fascin 1 protein and simultaneously inhibited expression of HGF, FN, and MMP7 in target cells, which may be one of the mechanisms for cancer suppression by si-fascin.

2.6 Morphological changes related to cell growth and migration were observed by scanning electron microscope (SEM)

The surface morphology related to cell growth and migration of HepG2 and Huh7 cells transfected by si-fascin was observed by SEM (Fig. 6). As shown in the Fig. 6, the HepG2 and Huh7 transfected by si-fascin were shrinkage, size decreased, and the connection with neighboring cells reduces, showing apoptotic changes, while the cell surface pseudopodia branches decrease and the shorter, loss of some inter-cell connection. On the contrary, si-NC treated cells and untreated cells were normal size and had no obvious tendency of apoptosis; the pseudopodia branches of the cells were more and rich, and abundant inter-cellular connections exist.

3. Discussion

Fascin was found in the cytoplasm of sea urchin oocytes by Bryan et al. in the 1970s [21]. It was named as fascin because it could bind tightly to F-action and stabilized into bundles. Fascin have 3 forms in humans, named as fascin 1 (also known as fascin), fascin 2 and fascin 3, locating on chromosomes 7p22, 17q25, and 7q31, respectively. The human fascin 1 gene encodes a cytoskeletal protein with a molecular weight of 55 kDa, which is located in cytoplasmic tension fibers and filaments, lamellar pseudopods, and core actin bundles of microspikes at the edges of cell membrane folds [22]. In normal tissues and organs, fascin-1 is expressed in mesenchymal tissues and nervous system, mainly in endothelial cells, dendritic cells and nerve cells, and plays an important role in cell migration, cell adhesion, and communication between cells [23]. However, there is almost no expression in most normal epithelial tissues. But in human tumors, such as dephosphorylation at fascin S39 [24], activated HIF1α [25], leucine aminopeptidase 3 (LAP3) [26], epidermal growth factor [5] and TGFβ [27], etc, these factors can cause the activation of NF-K, JAK-STAT, ERK-JNK and other signal pathways [12–13, 28], which further induced the up-regulation of fascin expression in cancer, and promote the proliferation and invasion of cancer tissues. Especially in metastatic and recurrent lesions, fascin has a higher expression [8, 29–30]. So fascin is even considered as a crucial protein that promotes tumor cells to participate in the invasion function [6]. Similar to the literature report [31], the results of RT-qPCR, Western blot and immunofluorescence staining in HepG2 and Huh7 cells, showed that the expression levels of fascin 1 mRNA and protein were significantly up-regulated compared with normal liver LO2 cells. And the four siRNAs specifically targeting fascin 1 constructed in this article all down-regulated the mRNA expression of fascin 1 gene in HepG2 and Huh7 cells, among them, Fascin_siR3 had the best inhibitory efficiency and was used in subsequent experiments.

Lin et al [32] found that the proliferation of Lewis lung cancer cells lacking fascin 1 was significantly slower. Maria et al [13] found that down-regulation of fascin 1 inhibited the migration and invasion of more aggressive glioblastoma cells. Similar to reports in the literature, MTT assay showed that compared with the transfected si-NC cells and the untreated cells, the cell proliferation ability of the si-fascin cells decreased. Similarly, the results of the Transwell experiment showed that number of migration cells in si-Fascin treating HepG2 and Huh7 were significantly less than the si-NC cells and the untreated cells. These suggested that the proliferation and migration ability of hepatoma cells was inhibited after knockdown of fascin 1. This is because fascin participates in the regulation of focal adhesion transition and nuclear translocation in migrating cells [33, 34], plays an actin-bundling independent role [35]. Scanning electron microscopy showed that after HepG2 and Huh7 cells transfected with si-fascin, the cellular size was reduced, the surface was wrinkled, and there was a tendency of apoptosis, the cell surface pseudopodia branches decrease and the shorter, loss of some inter-cell connection. These morphological changes are manifested by si-fascin inhibiting cell proliferation and migration. These just indicate that fascin 1, as an actin-binding protein, is involved in the formation of filopodia and pseudopodia in hepatoma cells. The decrease in pseudopodia density may be due to the down-regulation of fascin 1 expression affecting the assembly of cell filaments by orchestrating membrane protrusion and cell adhesion [36], thereby weakening the ability of cell movement, at the same time, the direction of cell movement was changed. The above electron microscopy results explain the inhibitory effect of down-regulating the expression of fascin 1 on the migration of HepG2 and Huh7 cells from the ultrastructural level. However, Lin et al. concluded that fascin can promote metastatic expansion independent of its role in cell motility[32]. Similarly, Western blot detection showed that not only significantly decreased Fascin 1 expression, but also significantly decreased proliferation-related genes HGF, migration and invasion related genes FN and MMP7 protein expression in si-fascin treated

cells, which may be one of the mechanisms for cancer suppression by si-fascin. The results of this paper are further indicating that the down-regulation of fascin 1 gene may also inhibit the proliferation and migration of hepatoma cells by reducing the expression of proliferation-related genes and migration-related genes. Based on this speculation, the highly expressed fascin 1 may increase the protein expression of FN, MMP7 and HGF, which is beneficial to the metastasis and spread of cancer cells.

In summary, in hepatoma cells, constructed si-fascin (especially by Fascin_siR3) effectively down-regulated the mRNA and protein levels of fascin 1, and inhibited the proliferation and migration, increased cell apoptosis, and reduced the formation of pseudopodia of hepatoma cells. Its inhibitory mechanism on proliferation and migration may partly be due to the low expression of fascin 1 which weakens the protein expression of gene related to proliferation and migration. The research of this project will be a deeper understanding of the invasion and metastasis mechanism of HCC, which will help guide the clinic to find more effective new targeted treatment sites, block the malignant process of HCC, and thereby improve the clinical efficacy and prognosis of HCC patients. But in any case, the detailed inhibitory mechanism remains to be further studied.

In conclusion, fascin 1 gene can regulate the proliferation and migration of hepatoma cells, fascin 1 can be used as an promising target for intervention in hepatoma.

Declarations

Funding

This study is supported by the Innovation and Entrepreneurship Training Program for College Students in Jiangsu Province (No.201913993018Y), and the Natural Science Project of Medical School, Nantong University (TDYXY2019012).

Conflicts of interest

There is no conflict of interest in this research work.

Ethics approval

Not applicable

References

1. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Pineros M, Znaor A, Bray F (2019) Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer 144(8):1941–1953. https://doi.org/10.1002/ijc.31937
2. Ren Z, Chen X, Hong L, Zhao X, Cui G, Li A, Liu Y, Zhou LN, Sun RR, Shen S, Li J, Lou JM, Zhou HQ, Wang JM, Xu GW, Yu ZJ, Song YJ, Chen XH (2020) Nanoparticle conjugation of ginsenoside Rg3 inhibits hepatocellular carcinoma development and metastasis. Small 16(2):e1905233. https://doi.org/10.1002/smll.201905233

3. Barnawi R, Al-Khaldi S, Colak D, Tulbah A, Al-Tweigeri T, Fallatah M, Monies D, Ghebeh H, Al-Alwan M (2019) β1 Integrin is essential for fascin-mediated breast cancer stem cell function and disease progression. Int J Cancer 145(3):830–841. https://doi.org/10.1002/ijc.32183
4. Misiura M, Zińczuk J, Zarba K,KamińskaD, Pryczynicz A (2020) Actin-Bundling Proteins (Actinin-4 and Fascin-1) are Involved in the Development of Pancreatic Intraepithelial Neoplasia (PanIN)[J]. AMERICAN JOURNAL OF THE MEDICAL SCIENCES 359(3):147–155. https://doi.org/10.1016/j.amjms.2019.11.013
5. Wang CQ, Li Y, Huang BF,Zhao YM, Yuan H, Guo DSu CM,Hu GN, Wang Q, Long T (2017) EGFR conjunct FSCN1 as a Novel Therapeutic Strategy in Triple-Negative Breast Cancer. Sci Rep 7(1):15654. https://doi.org/10.1038/s41598-017-15939-9
6. Poli G, Ruggiero C, Cantini G, Canu L, Baroni G, Armignacco R, Jouinot A, Santi R, Ercolino T, Ragazzon B, Assie G, Mannelli M, Nesi G, Lalli E, Luconi M (2019) Fascin-1 Is a Novel Prognostic Biomarker Associated With Tumor Invasiveness in Adrenocortical Carcinoma. J Clin Endocrinol Metab 104(5):1712–1724. https://doi.org/10.1210/jc.2018-01717
7. Wang CQ, Wang Y, Huang BF, Tang CH, Du Z, Zeng Y, Wang Q, Shao JK, Jin LL (2020) High Expression of Both Resistin and Fascin-1 Predicts a Poor Prognosis in Patients with Colorectal Cancer. BioMed Research International Volume **2020** Article ID 8753175:7 pages. https://doi.org/10.1155/2020/8753175
8. Gunal A, Onguru O, Safali M, Beyzadeoglu M (2008) Fascin expression in glial tumors and its prognostic significance in glioblastomas. Neuropathology 28:382–386. https://doi.org/10.1111/j.1440-1789.2008.00889
9. Huang FK, Han S, Xing B, Huang J, Liu B, Bordeleau F, Reinhart-King CA, Zhang JJ, Huang XY (2015) Targeted inhibition of fascin function blocks tumour invasion and metastatic colonization. Nature communications **6**:7465. DOI: 10.1038/ncomms8465 | www.nature.com/naturecommunications
10. Feng Y, Zhu J, Ou C, Deng Z, Chen M, Huang W, Li L (2014) MicroRNA-145 inhibits tumour growth and metastasis in colorectal cancer by targeting fascin-1. Br J Cancer 110:2300–2309. https://doi.org/10.1038/bjc.2014.122
11. Liu R, Liao J, Yang M, Sheng JY, Yang H, Wang Y, Pan EC, Guo W, Pu YP, Kim SJ, Yin LH (2012) The cluster of miR- 143 and miR-145 affects the risk for esophageal squamous cell carcinoma through co-regulating fascin homolog 1. PLoS One 7:e33987. https://doi.org/10.1371/journal.pone.0033987
12. Khan A, Fan KH, Sun N, Yin W, Sun YG, Sun PP, Jahejo AR, Li HQ (2019) Recombinant porcine NK-lysin inhibits the invasion of hepatocellular carcinoma cells in vitro. Int J Biol Macromol 40:249–1259. https://doi.org/10.1155/2019/5318729
13. Maria L, Apostolos Z, Triantafyllos S, Vasiliki G (2020) ILK silencing inhibits migration and invasion of more invasive glioblastoma cells by downregulating ROCK1 and Fascin1. Mol Cell Biochem 471:143–153. https://doi.org/10.1007/s11010-020-03774-y
14. Barnawi R, Al-Khaldi S, Bakheet T, Fallatah M, Alaiya A, Ghebeh H, Al-Alwan M (2020) Fascin Activates β-Catenin Signaling and Promotes Breast Cancer Stem Cell Function Mainly Through Focal Adhesion Kinase (FAK): Relation With Disease Progression Front. Oncol 10:440. doi:10.3389/fonc.2020.00440
15. Greten FR, Grivennikov SI (2019) Inflammation and Cancer: Triggers, Mechanisms, and Consequences. Immunity 51:27–41. https://doi.org/10.1016/j.immuni.2019.06.025
16. Francis S, Croft D, Schüttelkopf AW, Parry C, Pugliese A, Cameron K, Claydon S, Drysdale M, Gardner C, Gohlke A, Goodwin G, Gray CH, Konczal J, McDonald L, Mezna M, Pannifer A, Paul NR, Machesky L,

McKinnon H, Bower J (2019) Structure-based design, synthesis and biological evaluation of a novel series of isoquinolone and pyrazolo[4,3-c]pyridine inhibitors of fascin 1 as potential anti-metastatic agents. Bioorg Med Chem Lett 29:1023–1029. https://doi.org/10.1016/j.bmcl.2019.01.035

17. Alburquerque-González B, Bernabé-García M, Montoro-García S, Bernabé-García Á, Rodrigues PC, Ruiz Sanz J, López-Calderón FF, Luque I, Nicolas FJ, Cayuela ML, Salo T, Pérez-Sánchez H, Conesa-Zamora P (2020) New role of the antidepressant imipramine as a Fascin1 inhibitor in colorectal cancer cells. Exp Mol Med 52:281–229. https://doi.org/10.1038/s12276-020-0389-x
18. Montoro-García S, Alburquerque-González B, Bernabé-García Á, Bernabé-García M, Rodrigues PC, denHaan H, Luque I, Nicolás FJ, Pérez-Sánchez H, Cayuela ML, Salo T, Conesa-Zamora P (2019) Novel anti-invasive properties of a Fascin1 inhibitor on colorectal cancer cells. J Mol Med 98:383–394. https://doi.org/10.1007/s00109-020-01877-z
19. Chen Y, Tian T, Li ZY, Wang CY, Deng R, Deng WY, Yang Ak, Chen YF, Li H (2019) FSCN1 is an effective marker of poor prognosis and a potential therapeutic target in human tongue squamous cell carcinoma. Cell Death Dis 10:356. https://doi.org/10.1038/s41419-019-1574-5
20. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods **25**: 402–408. doi: 10.1006/meth.2001.1262. PMID: 11846609
21. Bryan J, Kane RE (1978) Separation interaction of the major components of sea urchin actin gel. J Mol Biol 125(2):207–224. https://doi.org/10.1016/0022-2836(78)90345-5
22. Gigek CO, Chen ES, Calcagno DQ, Wisnieski F, Burbano RR, Smith MA (2012) Epigenetic mechanisms in gastric cancer. Epigenomics 4(3):279–294. https://doi.org/10.2217/epi.12.22
23. Yamashiro S, Yamakita Y, Ono S, Matsumura F (1998) Fascin, an Actin-bundling Protein, Induces Membrane Protrusions and Increases Cell Motility of Epithelial Cells. Mol Biol Cell 9(5):993–1006. https://doi.org/10.1091/mbc.9.5.993
24. Zanet J, Jayo A, Plaza S, Millard T, Parsons M, Stramer B (2012) Fascin promotes filopodia formation independent of its role in actin bundling. J Cell Biol 197:477–486. https://doi.org/10.1083/jcb.201110135
25. Bu M, Liu X, Liu X, Xu W (2019) Upregulation of fascin-1 is involved in HIF-1α-dependent invasion and migration of hypopharyngeal squamous cell carcinoma. Journal of Oncology 55(2):488–498. DOI:10.3892/ijo.2019.4827
26. Fang C, Zhang J, Yang H, Peng L, Wang K, Wang Y, Zhao X, Liu H, Dou C, Shi L, Zhao C, Liang S, Li D, Wang X (2019) Leucine aminopeptidase 3 promotes migration and invasion of breast cancer cells through upregulation of Fascin and matrix metalloproteinases-2/9 expression. J Cell Biochem 120(3):;3611–3620. https://doi.org/10.1002/jcb.27638
27. Yang J, Zhang N, Gao R, Zhu Y, Zhang Z, Xu X, Wang J, Li Z, Liu X, Li Z, Li J, Bi J, Kong C (2018) TGF-β1 induced fascin1 expression facilitates the migration and invasion of kidney carcinoma cells through ERK and JNK signaling pathways. Biochem Biophys Res Commun 501:913–919. https://doi.org/10.1016/j.bbrc.2018.05.081
28. Snyder M, Huang XY, Zhang JJ (2011) Signal transducers and activators of transcription 3 (STAT3) directly regulates cytokine-induced fascin expression and is required for breast cancer cell migration. J Biol Chem 286:38886–38893. https://doi.org/10.1074/jbc.M111.286245

29. Bi J, Chen X, Zhang Y, Li B, Sun J, Shen H, Kong C (2012) Fascin is a predictor for invasiveness and recurrence of urothelial carcinoma of bladder[. Urol Oncol 30(5):688–694. https://doi.org/10.1016/j.urolonc.2010.08.001
30. Ma Y, Faller WJ, Sansom OJ, Brown ER, Doig TN, Melton DW, Machesky LM (2015) Fascin expression is increased in metastatic lesions but does not correlate with progression nor outcome in melanoma. Melanoma Res 25:169–172. doi:10.1097/CMR.0000000000000135
31. Wang G, Zhu S, Gu Y, Chen Q, Liu X, Fu H (2015) MicroRNA-145 and MicroRNA-133a Inhibited Proliferation, Migration, and Invasion, While Promoted Apoptosis in Hepatocellular Carcinoma Cells Via Targeting FSCN1. Dig Dis Sci 60(10):3044–3052. https://doi.org/10.1007/s10620-015-3706-9
32. Lin S, Huang C, Gunda V, Sun J, Chellappan SP, Li Z, Izumi V, Fang B, Koomen J, Singh PK, Hao J, Yang S (2019) Fascin Controls Metastatic Colonization and Mitochondrial Oxidative Phosphorylation by Remodeling Mitochondrial Actin Filaments. Cell Reports 28:2824–2836.e8. https://doi.org/10.1016/j.celrep.2019.08.011
33. Elkhatib N, Neu MB, Zensen C, Schmoller KM, Louvard D, Bausch AR, Betz T, Vignjevic DM (2014) Fascin plays a role in stress fiber organization and focal adhesion disassembly. Curr Biol 24:1492–1499. https://elkssl0a75e822c6f3334851117f8769a30e1clib.v.ntu.edu.cn:4443/10.1016/j.cub.2014.05.023
34. Jansen S, Collins A, Yang C, Rebowski G, Svitkina T, Dominguez R (2011) Mechanism of actin filament bundling by fascin. J Biol Chem 286:30087–30096.https://elkssl0a75e822c6f3334851117f8769a30e1clib.v.ntu.edu.cn:4443/10.1074/jbc.M111.251439
35. Jayo A, Malboubi M, Antoku S, Chang W, Ortiz-Zapater E, Groen C, Pfisterer K, Tootle T, Charras G, Gundersen GG, Parsons M (2016) Fascin Regulates Nuclear Movement and Deformation in Migrating Cells. Dev Cell 38:371–383. https://elkssl0a75e822c6f3334851117f8769a30e1clib.v.ntu.edu.cn:4443/10.1016/j.devcel.2016.07.021
36. Lin S, Taylor MD, Singh PK, Yang S (2021) How does fascin promote cancer metastasis? The FEBS Journal 288:1434–1446. https://doi.org/10.1111/febs.15484

Figures

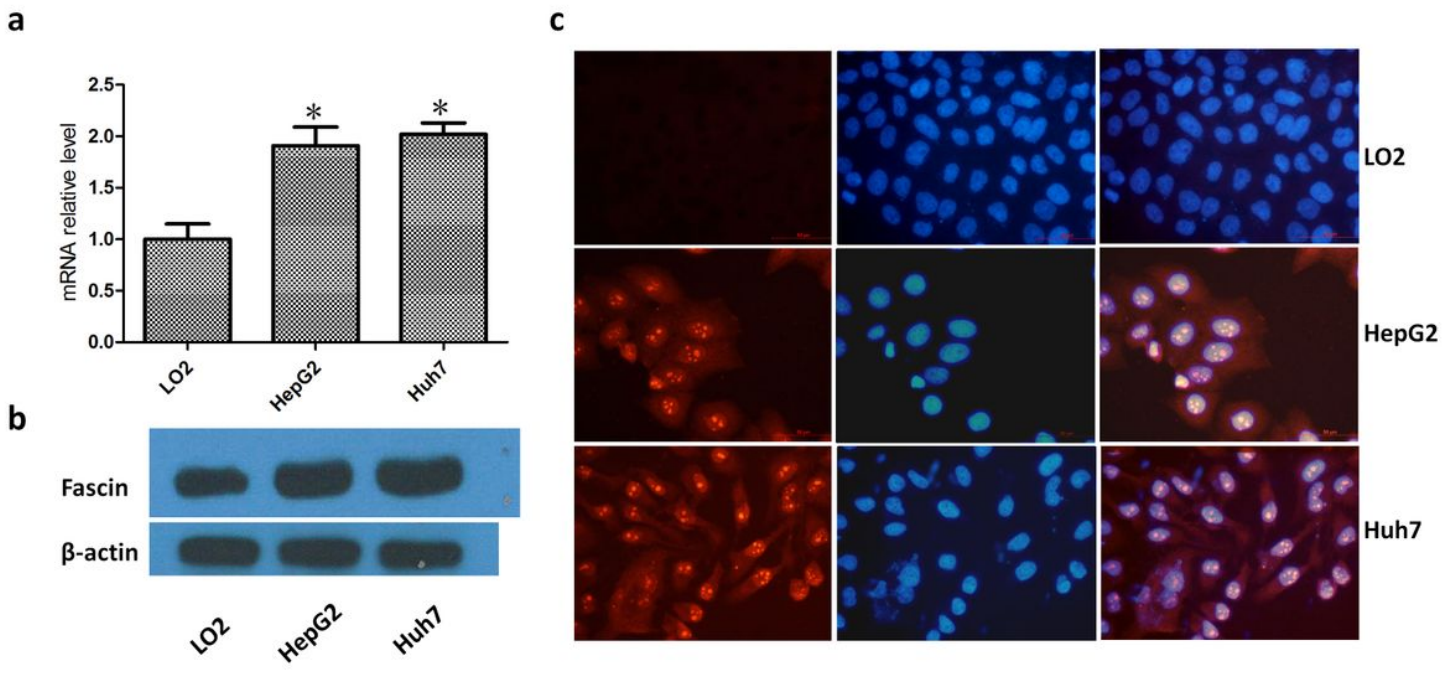

Figure 1

Expression of fascin 1 was upregulated in HepG2, Huh7 cell lines a. Histogram showing relative level of fascin 1 mRNA detected by RT-qPCR. *vs LC cell, p<0.01. b. Western blotting image showing fascin 1 protein expression; c. Immunofluorescence staining image showing fascin 1 protein expression (bar = 50 μm).

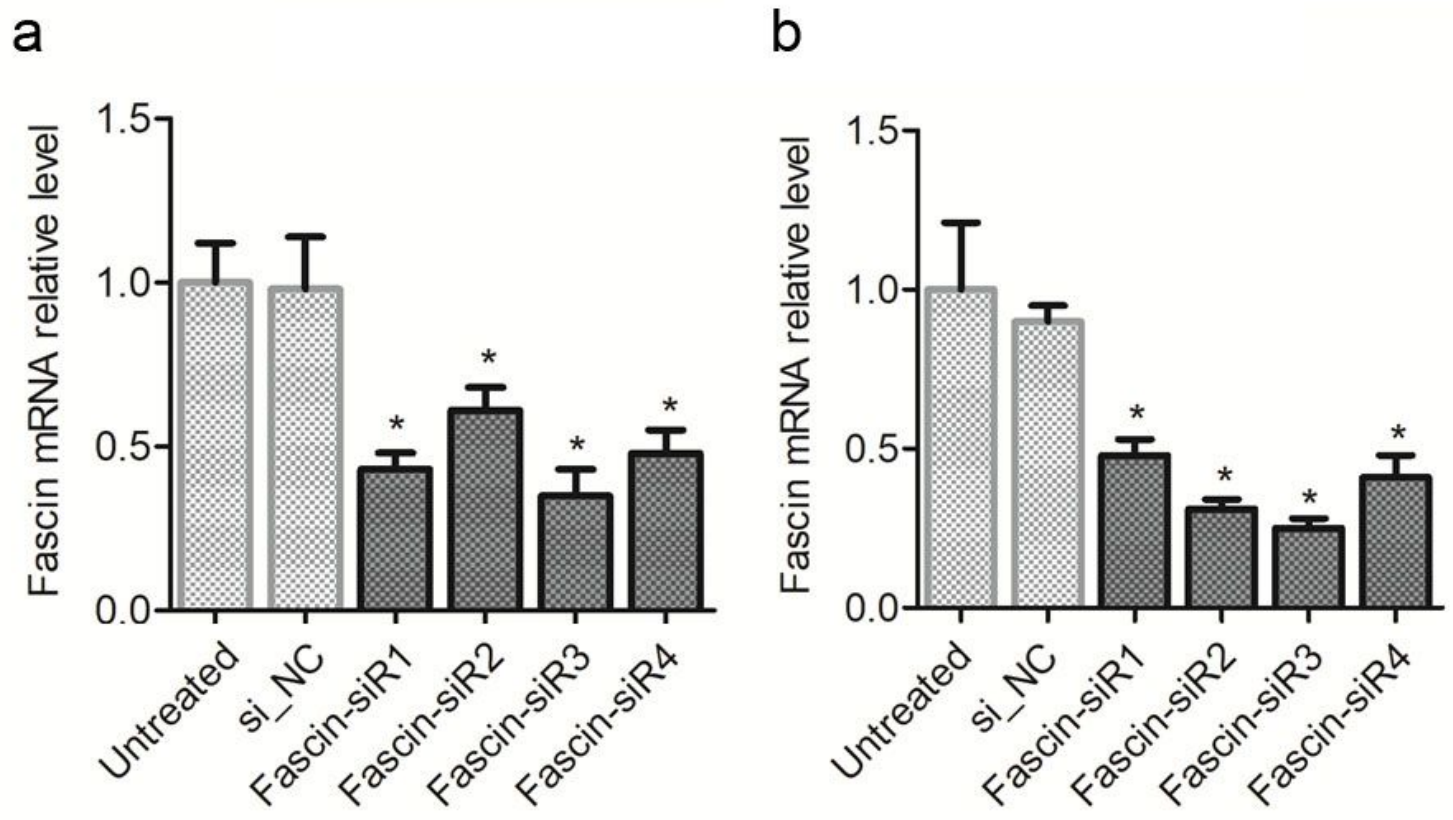

Figure 2

si-Fascin significantly downregulated the expression of target genes while showing inhibitory efficiency of different si-Fascin in HepG2 and Huh7 cells The histogram shows the relative expression levels of fascin mRNA in HepG2 and Huh7 cells transfected with different siRNA, respectively. *vs si-NC cells or vs untreated cells, p<0.05. a: HepG2 cells; b: Huh7 cells.

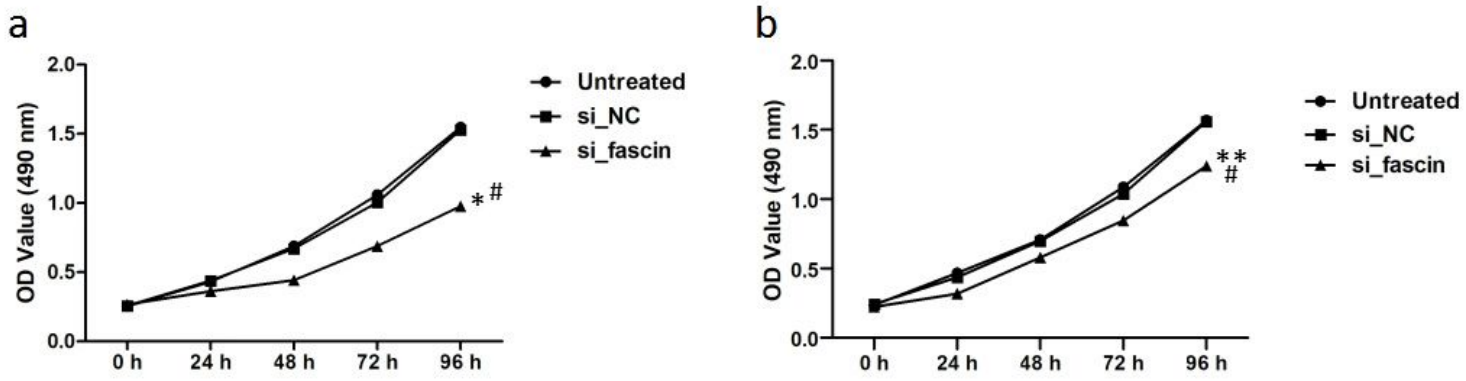

Figure 3

si-fascin impairs the proliferation ability of HepG2 and Huh7 cells The image of MTT assay showed the decreasing trend of proliferation ability of HepG2 and Huh7 cells transfected with si-fascin at 24 h, 48 h, 72 h and 96 h. *vs si-NC cells, p<0.05; ** vs si-NC cells, p<0.01; # vs untreated cells, p<0.05. a: HepG2 cells; b: Huh7 cells.

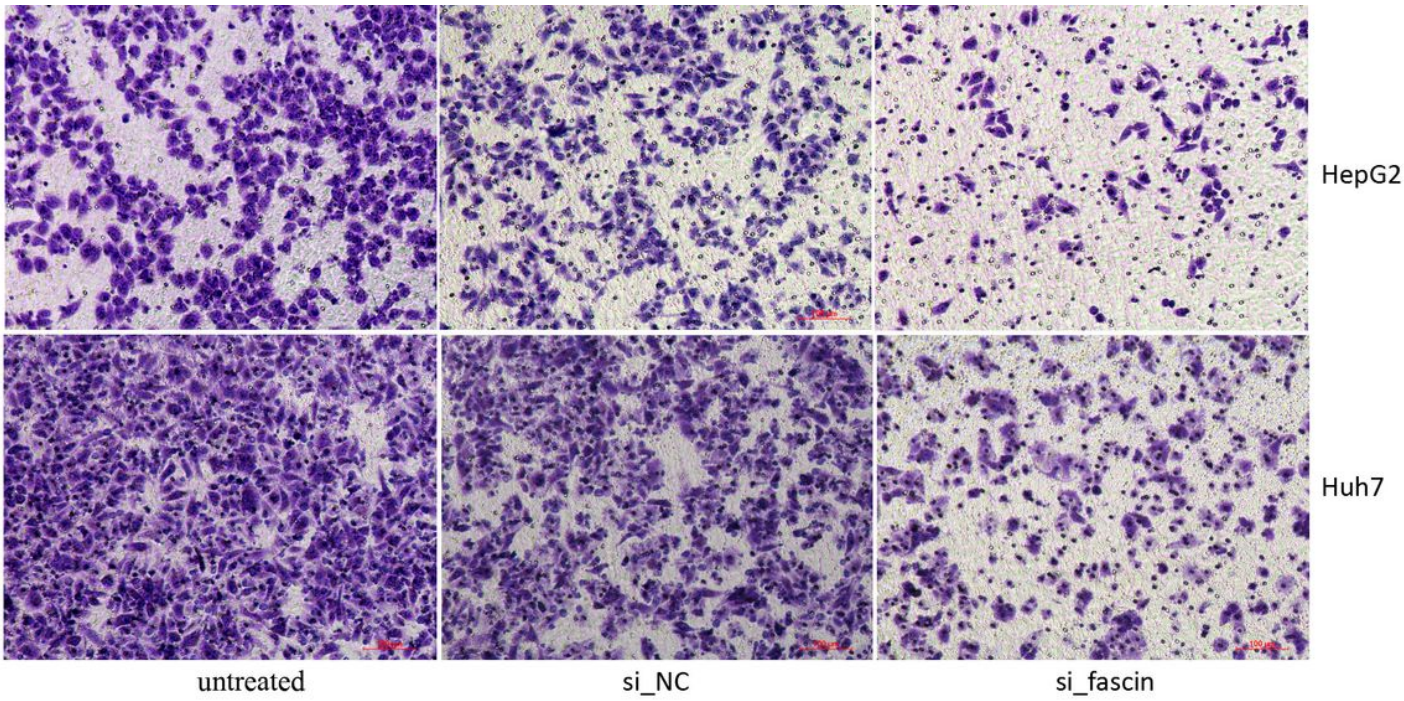

Figure 4

si-fascin impairs the migration of HepG2 and Huh7 cells Transwell results revealed fewer migration cells in si-fascin treating HepG2 and Huh7 cell group compared to si-NC cell group and untreated cell group (bar=100μm).

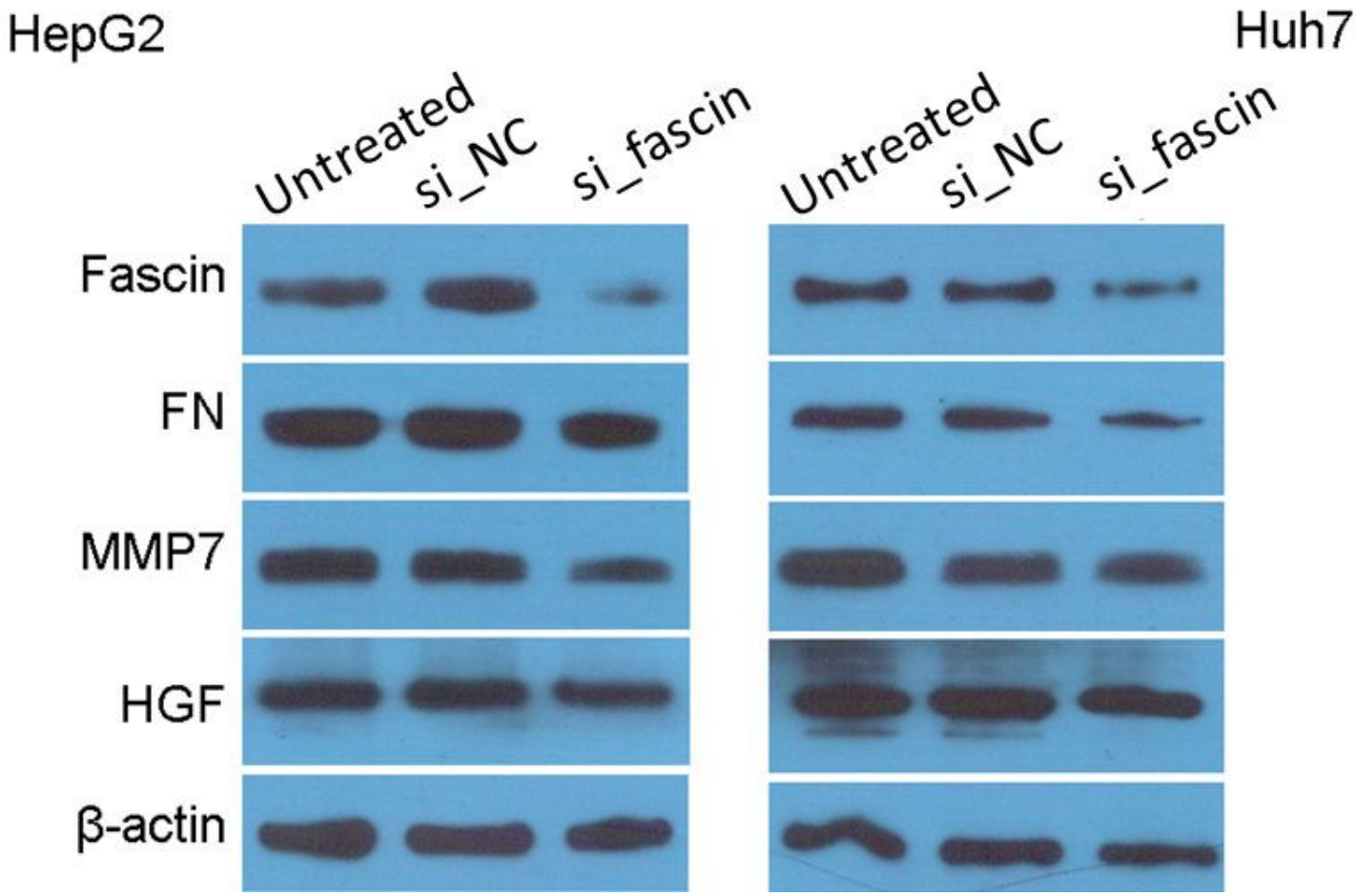

Figure 5

Si-fascin down-regulated the expressions of fascin 1, FN, MMP7 and HGF in HepG2 and Huh7 cells The Western blot images showed that, compared with si-NC cells and untreated cells, the protein expression of fascin 1, FN, MMP7 and HGF was significantly reduced in HepG2 and Huh7 cells transfected with si-fascin.

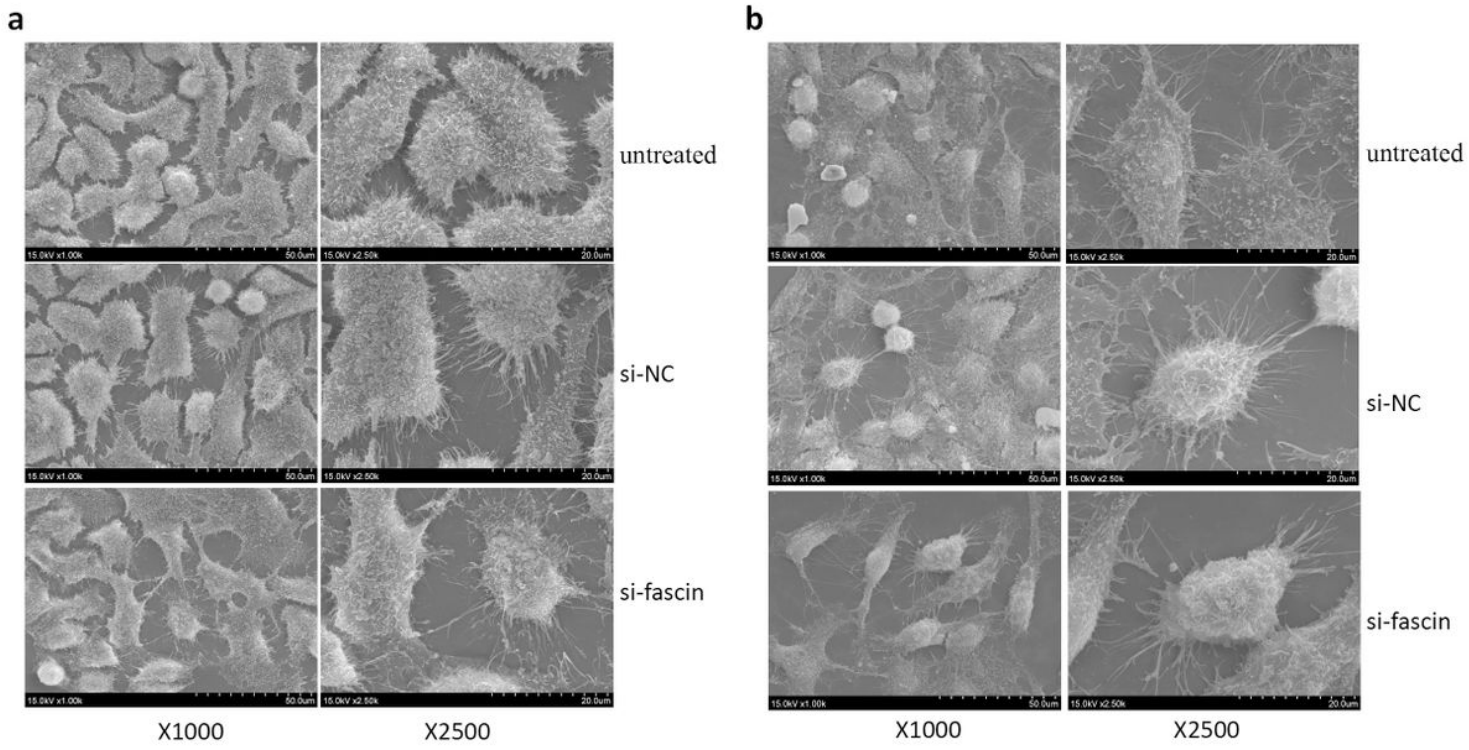

Figure 6

Surface morphology of HepG2 and Huh7 cells transfected with si-fascin The images of scanning electron microscope showed that the HepG2 and Huh7 cells transfected with si-fascin were shrinkage, appeared apoptotic changes, and accompanied by the reduction and shortening of pseudopodia branches. a:HepG2 cells; b: Huh7 cells; ×1000 and ×2500 are magnifications.