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FABP4 accelerates glioblastoma cell growth and metastasis through Wnt10b signalling

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Abstract. – OBJECTIVE: Glioblastomas are one of the most dangerous types of malignancies because of their metastatic capacity and challenge to treat with chemotherapy or radiotherapy. Hence, detailed research to explain the molecular mechanisms of glioblastoma metastasis is crucial to improve glioblastoma treatment.

PATIENTS AND METHODS: The expression level of fatty acid-binding protein 4 (FABP4) in glioblastoma cell lines and tissues was detected by Western blotting and quantitative Real-time polymerase chain reaction (qRT-PCR) assays. Proliferation assay, colon formation analysis and transwell/migration assay were performed to detect the relationship between FABP4 and malignant behaviors of glioblastoma cells *in vitro.* Subcutaneous xenograft and intravenous metastasis models were used to determine the role of FABP4 *in vitro.* Rescue assays were conducted to confirm the contribution of Wingless-Type MMTV Integration Site Family, Member 10B (Wnt10b) to the progression of glioblastoma cells regulated by FABP4.

RESULTS: Glioblastoma cells exhibited a higher level of FABP4 expression than control cells, and down-regulation of FABP4 suppressed tumor cell growth and metastasis *in vitro* and *in vivo.* Wnt10b, as a regulator gene of FABP4, restored the effects of FABP4 down-regulation in glioblastoma cells.

CONCLUSIONS: We provided substantive evidence that FABP4 is a growth and metastasis promoter *in vivo* and revealed that it functions in part through Wnt10b, which suggests that FABP4 might act as a probable target to block glioblastoma metastasis.

Key Words:
Glioblastoma, Neoplasm Metastasis, Wnt Signaling Pathway.

Introduction

Glioblastoma is one of the most common carcinomas and is the main cause of cancer-related death throughout the world[1]. Although, early diagnosis and increased awareness substantiality improves the survival rate for patients, long-term prognosis remains poor because of cancer cell metastasis[2]. Metastasis is a complex process by which tumor cells diffuse to distant locations, requiring individual cancer cells to locally invade and grow at metastatic sites[3]. More than a third of patients with glioblastoma develop metastatic disease; nevertheless, the basic mechanisms underlying glioblastoma metastasis remain poorly understood[4]. Sufficient investigations into the core molecules involved in glioblastoma metastasis to identify potential therapeutic targets could provide novel insights for combating glioblastoma. The fatty acid-binding protein (FABP) family, which consists of nine conserved cytosolic proteins, is abundantly expressed in a tissue-dependent manner[5]. FABPs bind to a panel of hydrophobic ligands, including leukotrienes, long-chain fatty acids, prostaglandins, and eicosanoids[6]. The biological functions of FABPs are not well understood, but they have been shown to be involved in various cellular processes, including regulation of cell survival and intracellular trafficking of fatty acids. Among the FABP family, FABP3 and FABP4 are the most important regulators of fatty acid metabolism and transport[7]. Previous investigations[8] have validated that metabolic processing of fatty acids participates in oncogenesis and tumor progression through control of tumor proliferation and tumor metastasis. Several investigations[9] have even suggested that FABPs exist in various cancer cells and are regarded as biomarkers of tumor progression. Researches[10] have identified the relationship between FABP4 and the poor prognosis of patients with cancer. FABP4 involvement in tumorigenesis has been explored in breast, ovarian, bladder, and oral squamous cell cancers[11]. FABP4 is well known as a prognostic factor of infiltrating or invasive

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bladder cancer, and FABP4 is a therapeutic target in metastatic prostate cancer[12]. However, whether FABP4 plays any essential roles in the metastasis of glioblastoma remains unclear. In the present investigation, we reported the function of FABP4 in glioblastoma cell growth and metastasis. Using glioblastoma cells with stable reduction of FABP4 expression, we demonstrated that loss of FABP4 decreased U-87MG cell migration and invasion *in vitro*. Furthermore, FABP4 knockdown suppressed tumor growth in a xenograft model and inhibited tumor metastasis to the lungs following tail vein injection. Further investigation demonstrated that Wnt10b was responsible in part for the FABP4-mediated effects and independently impaired cancer cell metastasis. Wnt10b was also overexpressed in human glioblastoma tissues. These data identify FABP4 as a regulator of tumor growth and metastasis, report a potential previously undescribed mechanistic role for Wnt10b in modulating FABP4, and suggest that FABP4 may be an important regulator of glioblastoma metastasis.

Patients and Methods

Cell Culture and Tissues

Glioblastoma cell lines (U251, KNS-81, U-87MG and TJ905) were bought from Cobioer Biotechnology Co., Ltd. (Nanjing, Jiangsu, China). The cell lines were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640), Eagle's Minimal Essential Medium (EMEM) or Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS) (Wisent, Quebec, Canada), streptomycin (100 µg/mL), and penicillin (100 Ul/mL). All cells were maintained in an incubator containing 5% CO_2 at 37°C. The samples of 24 glioma and 9 non-tumor tissues were both obtained from Xian Alenabio Biotechnology Co., Ltd (Xian, Shanxi, China). None of the patients had received previous chemotherapy or radiation treatments. In histological experiments, the resected glioma and non-tumor brain tissues were immobilized in formalin, embedded in paraffin, and cut into 5-µm-thick slices. For qRT-PCR analysis, the tissues were instantly frozen in liquid nitrogen and stored at -80°C until analysis.

RNA Interference

FABP4 and Wnt10b short hairpin RNA (shRNA) samples were manufactured and produced by Biomics Biotechnologies Co. Ltd. (Biomics Biotechnologies, Shanghai, China). Glioblastoma cells were then transfected with FABP4 shRNA (sense, 5′-AAGGTGAAGAGCATCATAACC-3′ and anti-sense, 5′-TCACGCCTTTCATAACACATT-3′) or non-specific shRNA; Wnt10b shRNA (sense, 5′-GTGAGCGAGACCCCACTATGC-3′ and anti-sense, 5′-CACTCTGTAACCTTGCACTCA-3′) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Full-length human Wnt10b complementary DNA (cDNA) was produced by PCR and cloned into the pcDNA3.1 (+) expression vector before it was transfected into U-87MG cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

Colony Formation Assay

In brief, after transfection with plasmid or FABP4 shRNA for 48 h, 2×10^4 cells were plated in 60-mm culture dishes with Roswell Park Memorial Institute-1640 (RPMI-1640) medium. The plates were then incubated for 4 weeks and stained with 0.1% crystal violet. Colonies with more than 50 cells were quantified[13].

Wound Closure Analysis

For this analysis, 3×10^5 cells per well were plated in 6-well plates. After cells reached 80-90% monolayer convergence, a scratch was made in the cell layer. Cell debris was removed, and cells were then cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium for 24 h. The width of the wound was measured under the microscope, and the relative percept of wound closure was measured relative to that observed in the control cells[14].

Transwell Invasion Assay

In this assay, 200 µL containing 5×10^3 cells were seeded in the upper chamber of a transwell (Corning, NY, USA), and 600 µL of Roswell Park Memorial Institute-1640 (RPMI-1640) medium with 10% fetal bovine serum (FBS) was added to the lower chamber. After 6 h, the cells invaded the lower surface of the polycarbonate membrane and were fixed with 4% formaldehyde following by staining with 0.1% crystal violet. The number of invaded cells was calculated from five randomly selected fields in each group[15].

Western Blot Analysis

Cells were lysed in lysis buffer. Cell lysates (25 µg) were resolved on 8% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene difluoride

(PVDF) membranes. Membranes were incubated with monoclonal antibodies against FABP4 (1:1000, Signalway Antibody, College Park, MD, USA), Wnt10b (1:1000, Signalway Antibody, Nanjing, Jiangsu, China), and GAPDH (1:5000, Bioworld, Nanjing, Jiangsu, China). Then, PVDF membranes were incubated with goat anti-rabbit IgG (H&L) HRP (1:10000, Bioworld, Nanjing, Jiangsu, China). Antibody signals were determined with the enhanced chemiluminescence (ECL) Gel system (Millipore, Billerica, MA, USA) and imaged using the ChemiDoc XRS system (Bio-Rad, Hercules, CA, USA)[16].

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The cDNA was generated using 1 μg of total RNA with the PrimeScript Real-Time reagent kit (Takara Bio, Otsu, Shiga, Japan). qRT-PCR was conducted using IQTM SYBR Green Supermix and the iQ5 Real-time recognition system (Bio-Rad, Hercules, CA, USA). The $2^{-\Delta\Delta Ct}$ method was adopted for quantifying gene expression. Primers of the target genes were as follows: glyceraldehyde-3-phosphate dehydrogenase (GAPDH): 5′-TGTGGGCATCAATGGATTTGG-3′ (forward) and 5′-ACACCATGTATTCCGGGTCAAT-3′ (reverse); FABP4: 5′-TGGATTCGACTTAGACTTGACCT-3′ (forward) and 5′-GGTGGGTTATGGTCTTCAAAAGG-3′ (reverse). GAPDH RNA expression was adopted for normalizing the target gene quantities.

Microarray Analysis

1 μg of RNA extracted from parental U-87MG and FABP4-knockdown cells was marked with either Cy5 or Cy3. The 0.8 μg of Cy-labeled complementary RNA (cRNA) was cut into nucleotides by incubation with fragmentation buffer solution (Agilent Technologies, CA, USA) at a temperature of 60°C for 0.5 h. Equivalent Cy-labeled cRNA was placed under water and hybridized to SurePrint G3 Human Gene Expression 8×60K v2 arrays at a temperature of 65°C for 17 h. Microarrays were scanned using an Agilent microarray scanner (Agilent Technologies, Santa Clara, CA, USA) at a wavelength of 535 nm for Cy3 and 625 nm for Cy5. Differentially expressed genes were identified by the signal intensities and p-value log ratios[17].

Xenograft Tumor Assay

Female BALB/c nu/nu mice were obtained from Shanghai SLAC Laboratory Animal Co., LTD (Shanghai, China). Animal experimental protocols were approved by the Ethics Committee for the Care and Use of Laboratory Animals of Dezhou People's Hospital. 0.1 mL (1×10^5 cells/μL) of cells was subcutaneously injected into the mice (n = 6 for each group). Tumor volume was determined every 3 days and calculated as ($W^2 \times L$)/2. Ki67 and caspase-3 immunohistochemistry (IHC) staining was conducted on tumor tissue sections using the Super Sensitive IHC Detection Systems (Beijing, China).

Statistical Analysis

Multi-group comparisons of the means were carried out by the one-way analysis of variance (ANOVA) test with post hoc contrasts by the two-tailed Student's t-test. The data were shown as the Mean ± Standard Deviation (SD). The differences with $p < 0.05$ were considered to be statistically significant.

Results

FABP4 Knockdown Suppresses Glioblastoma Cell Growth in vitro

To investigate the level of endogenous FABP4 in glioblastoma cells, we analyzed a panel of human glioblastoma cell lines by Western blot for FABP4 expression. The levels of FABP4 expression were remarkably increased in U251 and U-87MG cells compared to expression in two other glioblastoma cell lines, KNS-81 and TJ905 (Figure 1A). Considering that U-87MG had higher FABP4 expression levels than to the other glioblastoma cell lines, the U-87MG cell line was chosen to generate stable FABP4 knockdown cells using shRNA specifically targeting FABP4 (indicated as shFABP4). Western blot (Figure 1B) analysis demonstrated that transfection with shFABP4 significantly decreased the expression of FABP4 compared with transfection with non-targeting scrambled control shRNA transfection (indicated as shCtrl). To examine whether loss of FABP4 affected U-87MG cell proliferation, we subjected shCtrl and shFABP4 U-87MG cells to cell proliferation assays. As shown in Figure 1C, shFABP4 markedly inhibited cell proliferation over a 3-day period. To determine whether FABP4 knockdown affected cell growth *in vitro*, we seeded cells in 25-mm^3 dishes. For shFABP4 U-87MG cells, several tumor colonies were identified in the control cells, while no significant colony formation was observed in the shFABP4 U-87MG cells (Figure

1D). Thus, loss of FABP4 in glioblastoma cells resulted in decreased cell viability *in vitro*.

FABP4 Modulates Tumor Growth in vivo

We next researched the role of FABP4 in glioblastoma tumor growth *in vivo*. The shCtrl and shFABP4 U-87MG cells were subcutaneously injected in athymic nude mouse flanks. As shown in Figure 2A, the tumor weight in the FABP4-shRNA group was dramatically smaller than that in the control-shRNA group. The tumor volumes were significantly inhibited through the end of the study. The final average tumor volumes were 306.4 mm^3 for U-87MG shCtrl cells and 671.8 mm^3 for U-87MG shFABP4 cells (Figure 2B). Tumor sections were subjected to Ki67 staining to assess glioblastoma cell proliferation *in vivo*. The immunohistochemical staining of Ki67 further revealed that knockdown of FABP4 inhibited glioblastoma cell proliferation *in vivo* (Figure 2C). To determine whether apoptosis was regulated *in vivo*, we performed cleaved caspase-3 staining. As shown in Figure 2D, no significant changes were observed in cleaved caspase-3-positive cells. Collectively, these data suggest that FABP4 plays an important role in glioblastoma cell growth *in vivo*.

FABP4 Knockdown Impairs Cancer Cell Metastasis

To investigate if functional loss of FABP4 affects glioblastoma cell invasion *in vitro*, we next evaluated the effects of FABP4 knockdown on invasion through matrigel using coated transwell filters. Representative images are shown in Figure 3A, and the quantity of invaded glioblastoma cells was measured. The mean number of invaded cells was decreased more than 4-fold in U-87MG cells following FABP4 knockdown *vs.* that in control cells. Furthermore, shFABP4-transfected U-87MG cells were then subjected to a wound-healing assay. Consistently, disruption of FABP4 expression significantly reduced U251 cell migration compared to transfection with scrambled shRNA (Figure 3B). We next investigated whether FABP4 regulates metastatic implantation of cancer cells *in vivo* using a tail vein model. The shCtrl and shFABP4 cells were injected into athymic nude mice via the tail vein. In the control group, mice injected with shCtrl cells had obvious lung metastatic lesions at the end of the study, whereas the mice injected with shFABP4 cells exhibited few lung metastasis loci (Figure 3C). Finally, the mice were dissected, and the lungs were fixed and stained with Hematoxylin and Eo-

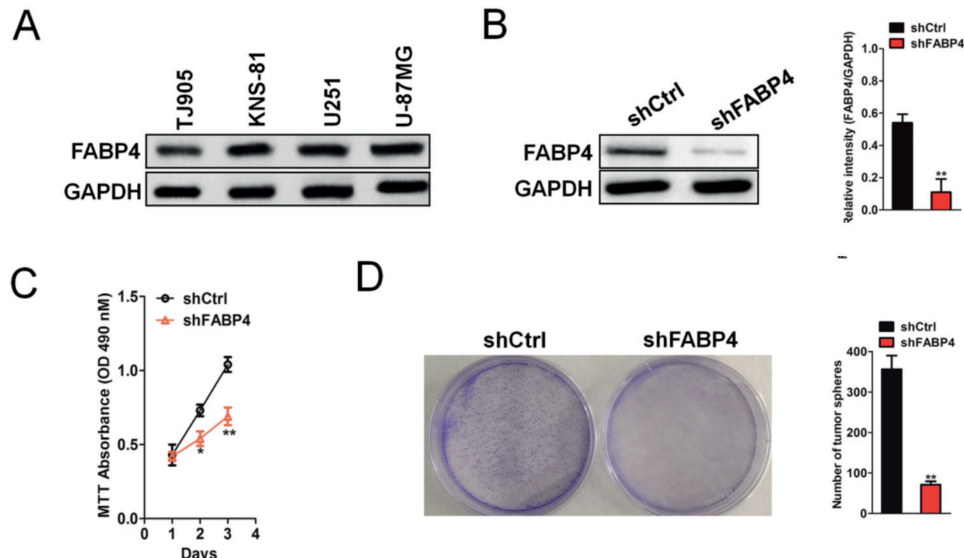

Figure 1. FABP4 knockdown inhibits glioblastoma cells growth *in vitro*. ***A***, Endogenous FABP4 expression was studied by western blot essay in four established glioblastoma cell lines. ***B***, FABP4 knockdown efficiency of shFABP4 stable cells was confirmed using western blot analysis. ***C***, Cells were hatched into 96-well plates, and cell viability was examined at 24, 48, and 72 h after seeding. Each data point represents as the mean ± SD of three independent experiments. ***D***, Representative micro-graphs of the colonies produced by indicated U-87MG cells (left panel). Histograms described the mean ± SD of the colonies formed by indicated cells (right panel). ** $p < 0.01$, ** $p < 0.01$ as compared to shCtrl.

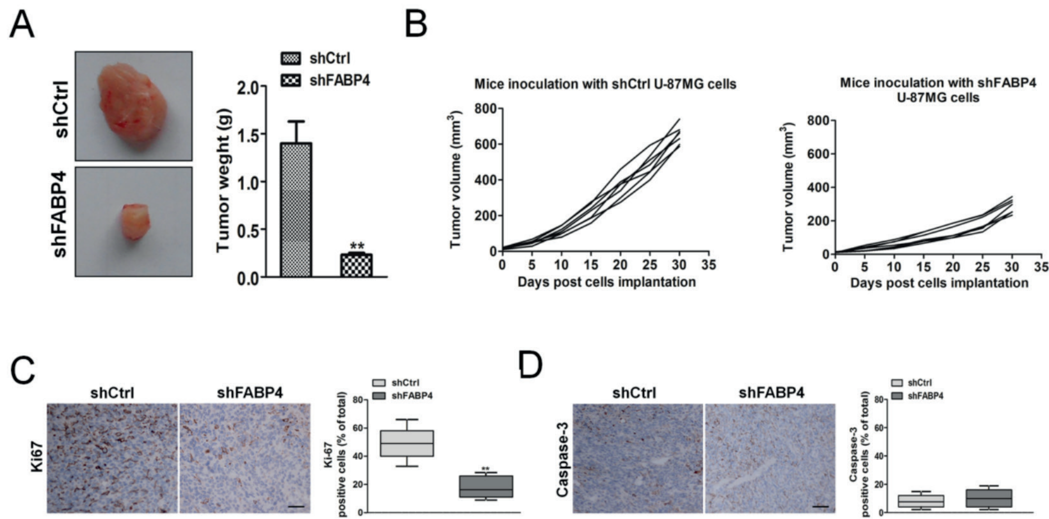

Figure 2. FABP4 knockdown suppresses tumor growth *in vivo*. ***A***, 100 µl 1×10^6 shCtrl and shFABP4 U-87MG cells were implanted subcutaneously into athymic nude mouse flanks. After 30 days, the xenografts were harvested. Mean tumor mass of xenografts in different groups. ***B***, Mean tumor volume of xenografts in different groups. ***C***, Immunohistochemistry analysis of Ki67 in xenografts of the indicated groups (left panel). Quantification by immune scoring as shown by percentage of positive staining for Ki67 (right panel). ***D***, Immunohistochemistry analysis of caspase-3 in xenografts of the indicated groups (left panel). Box plots of caspase-3 staining for the tumors (right panel). Five random fields were quantified and averaged for each animal. ** $p < 0.01$ as compared to shCtrl.

sin (H&E). Histology examination confirmed the lower frequency of lung metastases in shFABP4 cells than in shCtrl cells (Figure 3D). In total, these results indicate that down-regulation of FABP4 inhibits the aggressiveness of glioblastoma cells *in vitro* and *in vivo*.

Wnt10b Has a Functional Role in FABP4-Regulated Invasion

The database STRING (http://string-db.org/) is a pre-computed global resource for the exploration and analysis of functional links between proteins. Based on the information in the STRING database, FABP4 was highly correlated with several molecules, such as cluster of differentiation 36 (CD36), peroxisome proliferative activated receptor, gamma (PPARG) and Wnt10b (Figure 4A). To determine the genes responsible for FABP4 knockdown glioblastoma metastasis, we studied gene expression profiles by microarray analysis using the parental and shFABP4 U-87MG cells. Decreased levels of EMT-associated genes, such as matrix metallopeptidase 2 (MMP-2), Wnt10b, MMP-9, transforming growth factor beta receptor 1 (TGFBR1), neuropilin 1 (NRP1), and bone morphogenetic protein 2 (BMP2); metastasis-associated cytokines, such as interleukin 8 (IL-8), IL-6, IL-1β and s6 kinase 1 (S6K1); and other metastasis-related genes, including vascular endothelial growth factor A (VEGF-A), epidermal growth factor (EGF), CD24, and CD74, were observed in the FABP4 knockdown U-87MG cells (Figure 4B). We focused on the genes that were remarkably downregulated in shFABP4 U-87MG cells compared with the expression in control cells, and Wnt10b signaling was identified. Altered expression of Wnt10b was confirmed by immunofluorescence analysis and qRT-PCR assay (Figure 4C). To explore the clinical relevance of Wnt10b in glioblastoma, we assessed the expression of Wnt10b in clinical glioblastoma specimens from the human protein atlas (www.proteinatlas.org). We discovered strong expression of Wnt10b in glioblastoma and weak expression in normal tissues (Figure 4D). We next examined Wnt10b mRNA levels by qRT-PCR in a human glioma tissue array containing 24 duplicated samples of human glioblastoma tissues and 9 cases of normal tissues. As shown in Figure 4E, the expression of FABP4 was increased in glioblastoma tissues in comparison with the paired normal tissues.

Wnt10b Knockdown Reduces the Migration and Invasion of U-87MG Cells in vitro

To confirm that the gene identified was responsible for metastasis in glioblastoma cells, we researched whether the mobility of U-87MG cel-

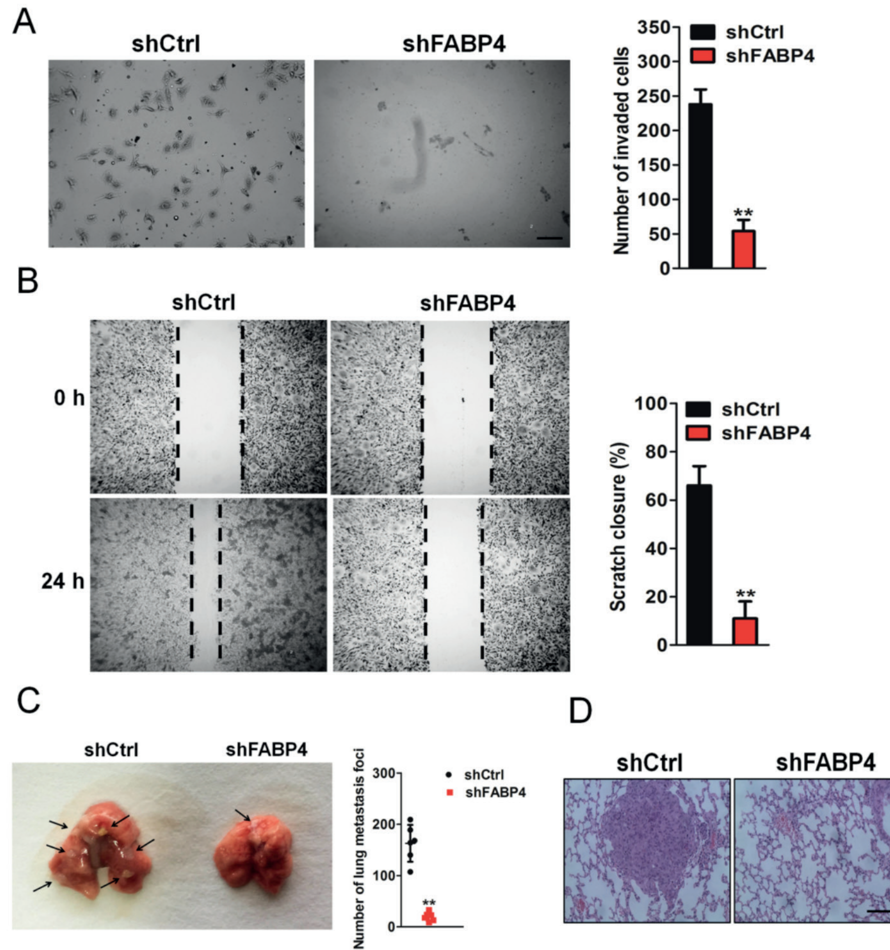

Figure 3. Knock-down of FABP4 decreases the metastasis of U-87MG cells. ***A,*** U-87MG cells transfected with shCtrl or shFABP4 were placed into the upper chamber of Transwell. After 6 h, the U-87MG cells invaded through the membrane were stained using 0.1% crystal violet and counted. Scale bar: 100 μM. ***B,*** shCtrl or shFABP4 transfected U-87MG cells were wounded and the percent rate of wound closure was measured at 24 h after scratch relative to that at 0 h. $^{**}p<0.01$ in comparison with the shCtrl cells. Scale bar: 100 μm. ***C,*** Representative images of pathological section of lung tissues for the existence of microscopic lesions two weeks after injected with U-87MG cells via tail vein (left panel). Quantification test of lung metastasis foci in the lungs of each group was presented (right panel). $^{**}p<0.01$ in comparison with the shCtrl cells. ***D,*** Representative results of H&E-stained lungs with metastases. Scale bar: 100 μm.

ls was inhibited by silencing Wnt10b in U-87MG cells with a shRNA plasmid targeting Wnt10b (shWnt10b). As shown in Figure 5A, the levels of Wnt10b in cells transfected with shWnt10b were diminished in comparison to the levels in cells transfected with shCtrl. After transfection, cells were subjected to wound closure and Transwell analysis. U-87MG cells transfected with shCtrl closed the wound within 24 h, whereas the wound in cells transfected with shWnt10b were not closed within 24 h (Figure 5B). The transwell invasion experiment consistently revealed that fewer shWnt10b cells than shCtrl cells invaded the membrane (Figure 5C). Together, the results demonstrate that Wnt10b knockdown drastically reduces metastasis of U-87MG glioblastoma cells.

Wnt10b is Involved in FABP4-mediated U251 Cell Migration and Invasion

To further define the potential role of Wnt10b in FABP4-mediated glioblastoma cell metastasis, we expressed constitutively active Wnt10b in FABP4-silenced cells and determined the level of Wnt10b by immunoblotting (Figure 6A). As expected, constitutively active Wnt10b rescued the impaired migration and invasion in FABP4 knockdown cells (Figure 6B and 6C). In addition,

Figure 4. FABP4 regulates Wnt10b signaling in glioblastoma cell. ***A,*** Modules from the protein-protein interaction network of FABP4. Edges represent protein-protein associations. ***B,*** In microarray analysis, a heat-map of differential expression of tumor metastasis associated genes was displayed. ***C,*** Immunofluorescence (left panel) and qRT-PCR analysis (right panel) of the FABP4 expression level in the control and shFABP4 U-87MG cells. **$p<0.01$ in comparison with the shCtrl cells. ***D,*** Wnt10b expressing quantity in normal tissue and cancer specimens. Pictures were photographed from the Human Protein Atlas (http://www.proteinatlas.org) database. ***E,*** The level of Wnt10b in 9 cases of normal tissues and 24 cases of glioblastoma tissues was calculated by qRT-PCR. ** $p<0.01$ as compared to the normal.

cells were treated with shWnt10b to dissect the role of Wnt10b in FABP4-mediated glioblastoma cell migration and invasion. No significant changes were observed in cells co-transfected with shFABP4 and shWnt10b compared to cells that were transfected with shFAPB4 alone (Figure 6D and 6E). In conclusion, the results show that Wnt10b is involved in FABP4-mediated metastasis of glioblastoma cells.

Overexpression of FABP4 Correlates with Glioblastoma Progression and Poor Prognosis

Finally, we discovered that FABP4 is up-regulated in glioblastoma, as determined by immunohistochemical staining with the FABP4 antibody and qRT-PCR assays ($p < 0.05$, Figure 7A-7B), and is positively associated with Wnt10b expression (Figure 7C) in clinical glioblastoma tissues.

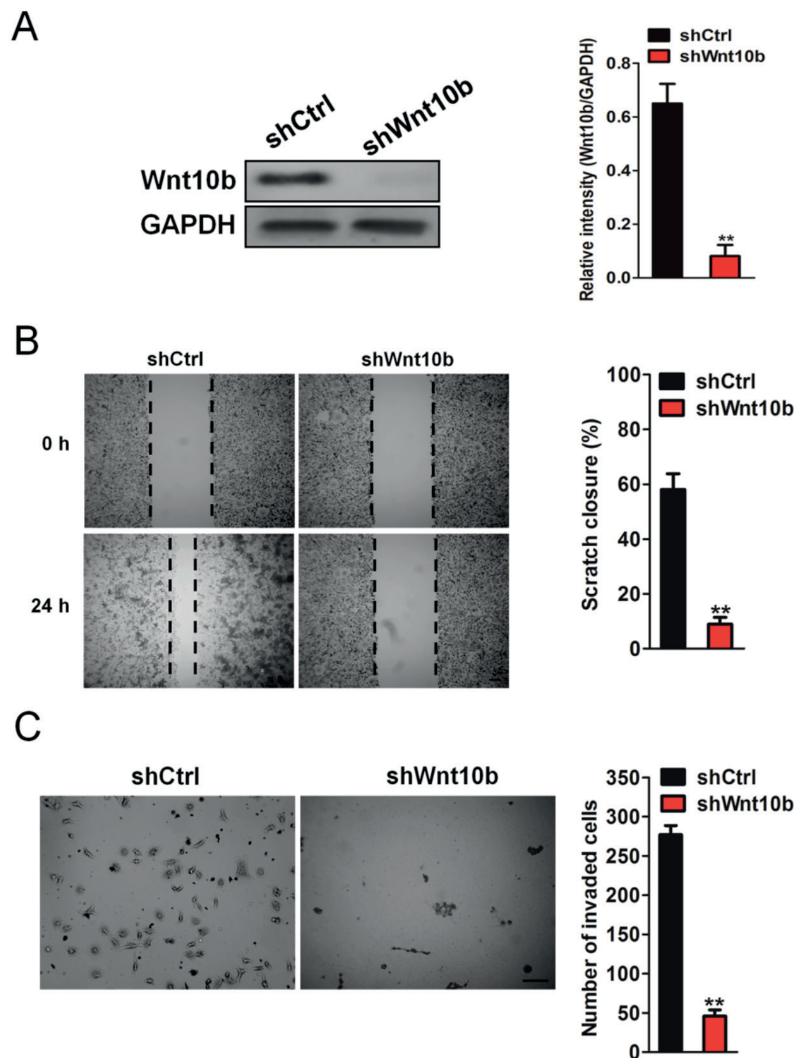

Figure 5. Wnt10b knockdown suppresses the mobility and invasion of U-87MG cells. ***A***, Wnt10b knockdown efficiency of shWnt10b stable cells was confirmed using western blot analysis. GAPDH was used as control. ***B***, The cell motility was determined by wound closure analysis and the percentage of wound healing was computed. Scale bars: 1000 µm. ***C***, The invasion of U-87MG cells was evaluated by Transwell assay. Scale bars: 1000 µm. Data were from three tests and were average ± SD. values. ** $p < 0.01$, in comparison with cell transfected with shCtrl.

Importantly, patients with high levels of FABP4 expression exhibited shorter survival times, and patients with lower levels of FABP4 expression had longer survival times (Figure 7D). These results suggest that FABP4 is over-expressed in glioblastoma and positively associates with disease progression.

Discussion

Glioblastoma is one of the most malignant cancers, with the topmost risk of cancer-associated death because of metastasis[18]. The morbidity and mortality rates of glioblastoma have increased recently[19], but there are no effective therapeutic options for metastatic glioblastoma because of the complex mechanisms involved in brain metastasis[20]. Cancer metastasis, which is one of the ten cancer hallmarks, is the primary reason that the glioblastoma-associated survival-rate is low[21]. Many patients with glioblastoma develop metastatic disease, though the detailed molecular mechanisms underlying glioblastoma metastasis have not been well defined[22]. An improved understanding of the core molecules involved in the metastasis processes may bring about novel insights for the development of effective anticancer

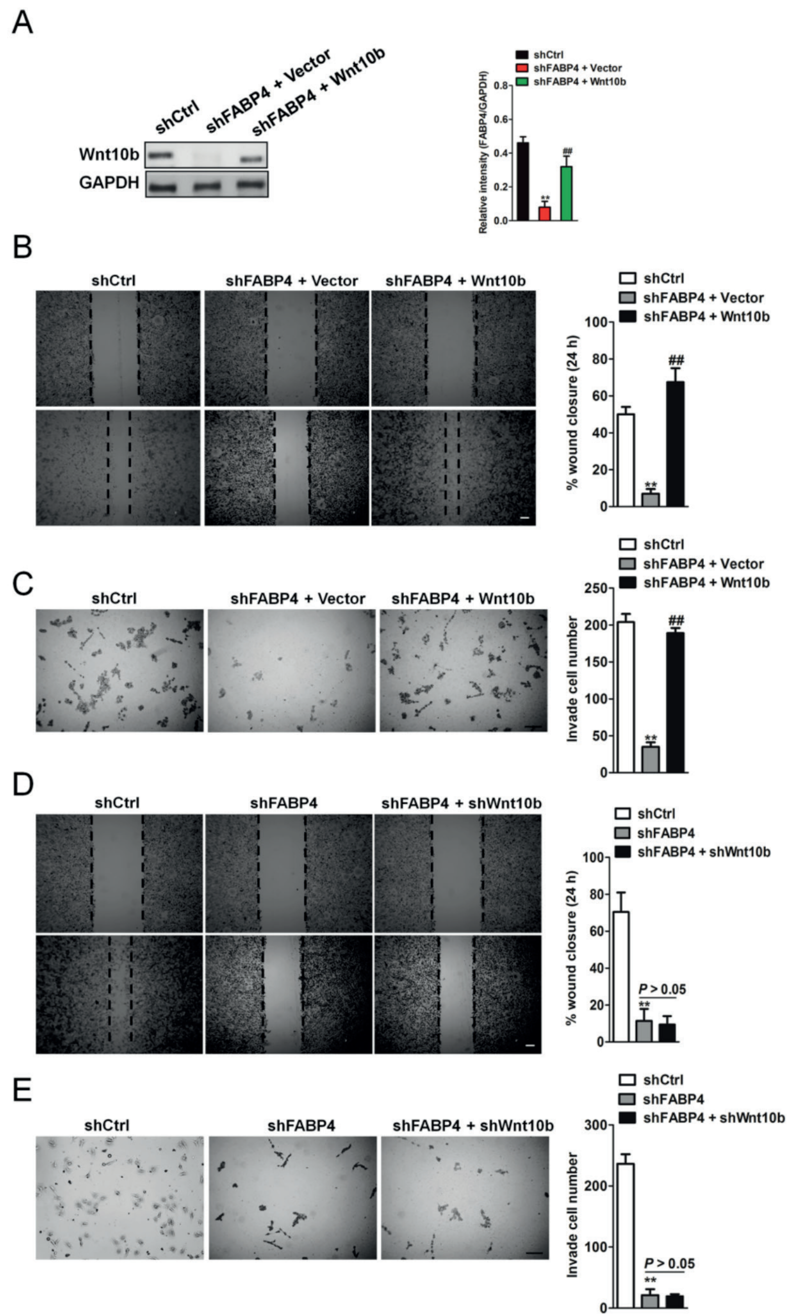

Figure 6. FABP4 facilities the metastasis of U-87MG cells via Wnt10b. ***A***, The expressing levels of Wnt10b in shCtrl transfected cells, cells transfected with shFABP4 and vector, or cells transfected with shFABP4 and Wnt10b were detected by immunoblotting analysis. GAPDH was used as control. ***B***, Wound-healing assays were conducted to assess the migration of U-87MG cells co-transfected with shFABP4 and Wnt10b plasmid. ***C***, Transwell invasion analysis was subjected to examine the assault of cells co-transfected with shFABP4 and Wnt10b plasmid. Columns were data collected from three experiments and were average ± SD. ** $p < 0.01$, in comparison with shCtrl cells. ## $p < 0.01$, compared with cells co-transfected with shFABP4 and vector. ***D***, Wound closure tests were adopted to assess the mobility of U-87MG cells co-transfected with shFABP4 and shWnt10b. ***E***, Transwell invasion assays were performed to assess the cell invasiveness after both shFABP4 and shWnt10b transfection. The typical photos were taken with crystal violet staining. Columns were data gathered from three independent experimentations and are average ± SD. values. ** $p < 0.01$, compared with shCtrl cells.

therapies. In the current study, we identified novel roles for FABP4 in the metastasis of glioblastoma cells. We identified FABP4 as a potentially important metastasis-inducing protein based on *in vitro* and *in vivo* results that have identified it as a critical functional signaling molecule that is upstream of Wnt10b. Our results might provide a new target for intervention in glioblastoma metastasis and improve glioblastoma treatments. FABP is one of the most well characterized intracellular lipid transport proteins. FABP4 is implicated in several crucial cellular processes, such as uptake and storage of fatty acids and regulation of gene expression, cell growth, and survival[23]. In adipocytes, macrophages and endothelial cells, FABP4 is transcriptionally activated by agonists of the peroxisome proliferator associated receptor γ (PPARγ), fatty acids, and insulin[24]. Although PPARγ targets genes whose expression levels are in association with PPARγ activation, FABP4 acts as a PPARγ regulator by transporting nuclear ligands of FABP4 and controlling signal transduction in a positive feedback loop. Based on these findings, PPARγ and FABP4 seemingly are included in adipocyte-induced metabolic interchanges in the prostate microenvironment that might steer tumorigenesis[25]. As of yet, no in-depth investigations have been conducted to determine the involvement of FABP4 in glioblastoma. In the present study, using shFABP4, we were able to reduce FABP4 expression in the U-87MG glioblastoma cell line. We revealed that the metastasis of glioblastoma cells was considerably restrained when FABP4 was downregulated by shRNA. Additional examination of the effects of FABP4 knockdown revealed that the metastasis of glioblastoma cells was considerably suppressed as determined by wound closure analysis and invasion experiments. Meanwhile, there were no significant effects on cell apoptosis in the xenograft experiments after mice were injected with shFABP4 U-87MG cells. Metastatic progression is a highly coordinated process with a series of steps that involve complex interactions between cancer cells and the microenvironment[26]. The tail vein metasta-

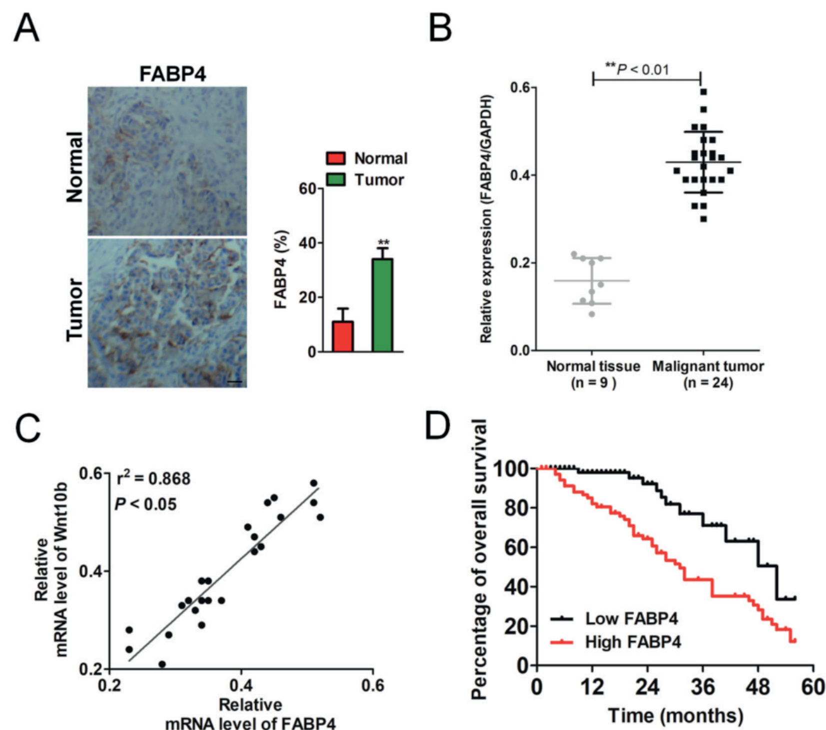

Figure 7. Overexpression of FABP4 correlates with glioblastoma progression and poor prognosis. ***A***, Representative images of immunohistochemical staining of FABP4 in glioblastoma tissues and corresponding normal tissues. ***B***, qRT-PCR was adopted to compute the level of FABP4 in glioblastoma tissues and corresponding normal tissues. ** $p < 0.01$, compared with normal. ***C***, The negative correlation analysis of Wnt10b and FABP4 in glioblastoma. ***D***, The Kaplan-Meier survival curves compare glioblastoma patients with low and high FABP4 expressing levels.

sis model provides evidence that FABP4 is a regulator in glioblastoma cell metastasis *in vivo*. These results have revealed that FABP4 participates in glioblastoma metastasis. The molecular mechanism of FABP4-mediated glioblastoma metastasis was found to be associated with Wnt10b. Our results demonstrated that down-regulation of FABP4 in glioblastoma U-87MG cells resulted in deregulation of Wnt10b expression. Furthermore, restoring Wnt10b activity maintained the lessened metastasis capacity of U-87MG cells induced by FABP4 knockdown. The Wnt pathway is known to be triggered in basal-like tumors. Wnt/β-catenin signaling is triggered by the interaction between Wnt ligands and receptors, subsequently resulting in β-catenin stabilization[27]. Stabilized β-catenin translocates to the nucleus and induces transcription of genes influencing cellular processes such as cellular growth, tumor cell metastasis, cell differentiation, neoplasia and stem cell maintenance[28]. Wnt10b also plays fundamental roles in mammary gland development, as it is the initial discernible ectodermal occasion that defines mammary gland development[29]. Over-expression of Wnt10b causes mammary tumorigenesis in mice and occurs in human breast carcinoma cell lines.

Conclusions

We provide evidence that FABP4 in glioblastoma cells regulates tumor growth and metastasis. Because of the crucial role of FABP4 in the metastasis of glioblastoma cells, FABP4 might function as a potential target for inhibition of glioblastoma metastasis. In future research, it will be meaningful to clarify the precise function of FABP4 in regulating Wnt10b and thus in mediating the metastasis of glioblastoma cells.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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