

MPZL1 promotes tumor cell proliferation and migration via activation of Src kinase in ovarian cancer

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Received March 10, 2018; Accepted May 28, 2019

DOI: 10.3892/or.2019.7199

Abstract. Tumor metastasis is the leading cause of mortality in patients with advanced ovarian cancer. Myelin protein zero like 1 (MPZL1) is a transmembrane glycoprotein that promotes migration of hepatocellular carcinoma cells and is involved in extracellular matrix-induced signal transduction. However, the functional role of MPZL1 in ovarian cancer has not been well elucidated. The present study conducted western blotting, phase-contrast imaging and immunohistochemistry to reveal the functions of MPZL1 in ovarian cancer. The present study demonstrated that the expression levels of MPZL1 were associated with malignant features of ovarian cancer. Furthermore, overexpression of *MPZL1* significantly promoted cell proliferation, migration and invasion of ovarian cancer cells. Conversely, *MPZL1* depletion by short hairpin RNA inhibited migration and invasion of ovarian cancer cells. In addition, this study demonstrated that phosphorylation of Src kinase was increased upon *MPZL1* overexpression. Additionally, phosphorylation and activation of pro-metastatic proteins p130 and cortactin were induced by phosphorylated Src kinase. Collectively, these findings indicated that *MPZL1* may be a novel pro-metastatic gene, which promotes tumor cell proliferation and migration through Src-mediated phosphorylation of p130 and cortactin in ovarian cancer.

Introduction

Ovarian cancer has the highest mortality rate among gynecological malignancies worldwide (1,2). Tumor metastasis is a complex biological process involving cell signaling, regulation of cell proliferation, motility and invasion; in

addition, metastasis is the primary cause of death in patients with advanced ovarian cancer and is closely associated with unfavorable outcomes and poor prognosis (3-7). Recently, the rat sarcoma-mitogen-activated protein kinase, phosphoinositide 3-kinase-protein kinase B and janus kinase-signal transducer and activator of transcription signaling pathways have been reported to be associated with tumor metastasis and invasion in ovarian cancer (8). Furthermore, the tumor microenvironment, which includes stromal cells, extracellular matrix components and exosomes, can establish a communication circuit that enhances cancer cell invasion and metastasis via reciprocal signaling (9). However, the molecular mechanisms underlying ovarian cancer metastasis are currently not well elucidated (10).

Myelin protein zero like 1 (MPZL1), also known as protein zero-related, is a hyperphosphorylated transmembrane glycoprotein involved in extracellular matrix-induced signal transduction (11-15). Previous studies have demonstrated that MPZL1 promotes hepatocellular carcinoma cell migration through the Src tyrosine kinase, and may be involved in adhesion-dependent signaling (11,14,16). Furthermore, MPZL1 forms a complex with the growth factor receptor-bound protein 2 adaptor and tyrosine-protein phosphatase non-receptor type 11 phosphatase, and is involved in cell adhesion in human epidermal growth factor receptor 2-positive breast cancer cells (12). Additionally, as a major receptor of concanavalin A, MPZL1 has an important role in cell signaling via c-Src (13). However, the functional role and clinical implications of MPZL1 in ovarian cancer are largely unknown.

The present study demonstrated that amplification of MPZL1 was associated with malignant features of ovarian cancer, and promoted tumor cell proliferation, migration and invasion. Furthermore, overexpression of *MPZL1* significantly promoted cell growth and metastasis of ovarian cancer. Conversely, knockdown of *MPZL1* via short hairpin RNA (shRNA) attenuated proliferation and migration of ovarian cancer cells. This study also demonstrated that *MPZL1* overexpression-induced activation of Src kinase mediated the phosphorylation and activation of cortactin and p130 in ovarian cancer. Taken together, these findings suggested that *MPZL1* may be considered a novel pro-metastatic gene that promotes tumor cell proliferation and migration via activation of Src kinase in ovarian cancer.

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Key words: MPZL1, proliferation, migration, Src kinase, ovarian cancer

Materials and methods

Collection of ovarian cancer specimens. The present study was approved in January 2017 by the Ethics Committee of the Tongren Hospital, Shanghai Jiao Tong University School of Medicine. The collection of ovarian cancer specimens was performed in conformity to ethical standards. All participants provided written informed consent. The tissues were obtained during surgery and were fixed with 4% paraformaldehyde (Beijing Solarbio Science & Technology Co., Ltd.) for 24 h at room temperature and embedded in paraffin. Specimens were obtained from 78 patients (age, 29-77 years, including 16 benign, 16 borderline and 46 malignant patients) with epithelial ovarian cancer who had not previously undergone treatment; the benign group consisted of patients with a benign ovarian tumor. No distant metastasis was detected in the selected patients prior to surgery. Detailed pathological data, including histological type, tumor size, tumor stage and lymph node metastasis, were obtained and summarized. The tumor stage was defined using the 8th edition of the Union for International Cancer Control-tumor node metastasis classification system (17).

Reagents and cell lines. The human ovarian cancer cell lines 293T, HO8910, SKOV3, HEY and TOV-21G were purchased from American Type Culture Collection and were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin/streptomycin (Gibco; Thermo Fisher Scientific, Inc.).

Virus production and infection. 293T cells (60% confluent) in a 10 cm dish were co-transfected with 5 μ g lentiviral constructs (pLenti 7.3; Invitrogen; Thermo Fisher Scientific, Inc.), 5 μ g plasmid Δ 8.9 (Invitrogen; Thermo Fisher Scientific, Inc.) and 3 μ g plasmid vesicular stomatitis virus G (Invitrogen; Thermo Fisher Scientific, Inc.) using Lipofectamine[®] 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). Cells were incubated at 37°C and the medium was replaced after 12 h. Virus-containing medium was collected 48 h post-transfection and supplemented with 8 μ g/ml polybrene to infect target cells in 6-well dishes (60% confluent) at 37°C. Infected cells were selected with 3 μ g/ml puromycin at 37°C for \geq 1 week post-infection. The lentiviral shRNA vectors targeting *MPZL1* and scrambled control shRNA were purchased from Open Biosystems; Dharmacon Inc. For *MPZL1* knockdown, the shRNA sequences were: *MPZL1*-shRNA-1, 5'-TGACATCAC AGATATAGGT-3'; *MPZL1*-shRNA-2, 5'-TCAAGTGGCATA GCCAATG-3'; and shRNA-negative control (NC), 5'-ACC TCCACCCTCACTCTGCCAT-3'. For *MPZL1* overexpression, the coding sequence of *MPZL1* was inserted into the lentiviral vector pLenti 7.3.

For *Src* knockdown, *Src*-small interfering RNA (siRNA) was used at a final concentration of 25 nM and was transfected into cells (60% confluent) using Lipofectamine[®] RNAiMAX reagent (Invitrogen; Thermo Fisher Scientific, Inc.) for 72 h at 37°C, according to the manufacturer's protocol. For *Src* knockdown, the siRNA sequences (Shanghai GenePharma Co., Ltd.) were: *Src*-siRNA, 5'-CAGGCUGAGGAGUGGUAUUT-3'; and siRNA-NC, 5'-TTCTCCGAACGTGTCACGT-3'.

Western blotting. Cells were lysed in RIPA buffer [Tris (pH 7.4), 50 mM; NaCl, 150 mM; 1% NP-40; 0.1% SDS; EDTA, 2 μ M] containing proteinase inhibitors (Roche Molecular Diagnostics) and phosphatase inhibitors (Roche Molecular Diagnostics), and the protein concentration was determined using the bicinchoninic acid assay. The cell lysates (20 μ g total protein) were subjected to 8-10% SDS-PAGE and immunoblotting. Subsequently, proteins were transferred to nitrocellulose membranes, which were blocked for 1 h with 5% nonfat milk at room temperature, and were then incubated with primary antibodies (1:1,000) at 4°C overnight. The membranes were then incubated for 1 h with horseradish peroxidase-linked anti-rabbit IgG antibody (1:1,000; cat. no. 7074; Cell Signaling Technology, Inc) at room temperature. Blots were visualized with ECL western blotting reagents (Thermo Fisher Scientific, Inc.) using ChemiDoc XRS+ (Bio-Rad Laboratories, Inc.). Semi-quantification was conducted using ImageLab 2.0 software (Bio-Rad Laboratories, Inc.). Antibodies against the following proteins were used: p130 (cat. no. 13846), phosphorylated (p)-p130 (cat. no. 4015), cortactin (cat. no. 3502), p-cortactin (cat. no. 4569), Src (cat. no. 2108), p-Src (cat. no. 12432), *MPZL1* (cat. no. 9893) and GAPDH (cat. no. 5174) (all Cell Signaling Technology, Inc). Notably, since *MPZL1* has three alternatively spliced isoforms, at least three bands can be seen in *MPZL1* blots, and all bands were measured when semi-quantifying the blots.

Cell proliferation assay. Cell proliferation was assessed using the Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc.). Cells (5,000 cells/well) were seeded in triplicate in 96-well plates. After 24, 48, 72, 96 or 120 h at 37°C, CCK-8 reagent (1/20, volume/volume) was added to the cells for 2 h at 37°C. The absorbance of each well was measured at 450 nm, according to the manufacturer's protocol.

Cell migration and invasion assays. Transwell chambers with 8 μ m pore membranes (Corning, Inc.) were placed in 24-well culture plates and were incubated with serum-free RPMI-1640 medium at 37°C for 1 h. A 200 μ l suspension of 0.5-1x10⁵ cells was seeded into the upper compartment of the Transwell chambers in FBS-free medium, whereas 600-800 μ l RPMI-1640 medium supplemented with 10% FBS was added into the bottom wells; cells were incubated at 37°C for 12-24 h. The migrating cells that were attached to the lower membranes of the Transwell chambers were stained with crystal violet (0.1%) at room temperature for 2 h and images were captured at x200 magnification under a light microscope. Images of three random fields from three replicate wells were obtained, and the migrated cells were counted. The cell invasion assay was the same as the migration assay; however, the chambers were coated with Matrigel.

Colony formation assay. Cells were seeded in triplicate in 6-well plates. After 12 days, the cells were washed with PBS, fixed with methanol for 1 h and stained with 0.1% crystal violet for 1 h at room temperature. Subsequently, images of the colonies were captured and counted.

Immunohistochemistry. Ovarian cancer specimens were collected from the 78 patients during surgery at Tongren

Table I. Association between clinicopathological characteristics and MPZL-1 expression.

Variable	Cases (n)	MPZL-1 expression score				P-value
		Low level		High level		
		-	+	++	+++	
Age (years)						
<55	28	1	8	9	10	0.69
≥55	18	0	4	4	10	
Clinical stage						
I-II	9	1	5	2	1	0.01 ^a
III	25	0	5	8	12	
IV	12	0	2	3	7	
CA125 (U/ml)						
≤200	33	1	12	10	10	0.01
>200	13	0	0	3	10	

^aStage III vs. stage I-II; ^bstage IV vs. stage III. Data were analyzed by Fisher's exact test. CA125 cancer antigen 125; MPZL1, myelin protein zero like 1.

Hospital, Shanghai Jiao Tong University. The tissue specimens were fixed in 4% paraformaldehyde (Beijing Solarbio Science & Technology Co., Ltd.) for 24 h at room temperature and embedded in paraffin, before being cut into 5 μ m sections. The tissue sections were deparaffinized, treated with 3% H₂O₂ for 10 min at room temperature, autoclaved in 10 mM citric sodium (pH 6.0) for 30 min to unmask antigens and rinsed in PBS. Subsequently, sections were incubated with primary antibodies against MPZL1 (1:200; cat. no. GTX46451; GeneTex, Inc.) at 4°C overnight, followed by incubation with a biotinylated secondary antibody (1:1,000; cat. no. ab6844; Abcam) for 1 h at room temperature. Signal amplification and detection was performed using the DAB system (Dako) according to the manufacturer's instructions.

Immunohistochemistry scoring. All cases were analyzed independently with the help of two expert pathologists and were assigned a score according to the following criteria: Strongly positive, 3+; positive, 2+; weakly positive, 1+; and negative, 0.

Statistical analysis. Statistical analysis was performed using GraphPad Prism 6 software (GraphPad Software, Inc.). Genomic analysis of *MPZL1* in ovarian cancer (489 high-grade serous ovarian cancer specimens were surgically resected prior to systemic treatment; all patients received a platinum agent and 94% received a taxane) was performed with The Cancer Genome Atlas (TCGA) copy number portal (www.broadinstitute.org/tcga). In all experiments, comparisons between two groups were conducted using two-sided Student's t-test, and one-way analysis of variance followed by Tukey's multiple comparisons test was used to test for differences among more groups. Fisher's exact test was used to determine differences between stages, as presented in Table I. P<0.05 was considered to indicate a statistically significant difference.

Results

Overexpression of *MPZL1* is associated with malignant features of ovarian cancer. Copy number analysis of TCGA ovarian serous adenocarcinoma samples in the cBioportal database revealed that *MPZL1*, located at chromosome 1q24 (magnified section in Fig. 1A), was genomically amplified in a considerable proportion of cases (Fig. 1A). Notably, the expression levels of *MPZL1* were associated with its amplification status (Fig. 1B), thus indicating that the high expression of *MPZL1* in tumor tissues may be regulated by copy number amplification of the *MPZL1* gene. To further determine the association of *MPZL1* expression with the malignant features of ovarian cancer, *MPZL1* protein expression was assessed by immunohistochemistry among benign, borderline and malignant epithelial ovarian cancer patient tissues (Fig. 1C; Table I). The results revealed that *MPZL1* expression was almost undetectable in patients with benign ovarian cancer, whereas it was expressed at significantly higher levels in malignant cancer patient tissues compared with in benign/borderline cancer patient tissues (Fig. 1D). In addition, the expression profile of the *MPZL1* protein was determined using The Human Protein Atlas (18). *MPZL1* was ubiquitously expressed in different human tissues as well as various cancer types. In normal ovarian tissues, *MPZL1* was lowly expressed, whereas in some ovarian cancer tissues, *MPZL1* was overexpressed (data not shown). Taken together, these data indicated that *MPZL1* was overexpressed in a subset of patients with ovarian cancer, suggesting that *MPZL1* may serve a pivotal role in ovarian cancer.

Overexpression of *MPZL1* promotes ovarian cancer cell proliferation and migration. To study the biological function of *MPZL1*, the protein expression levels of *MPZL1*

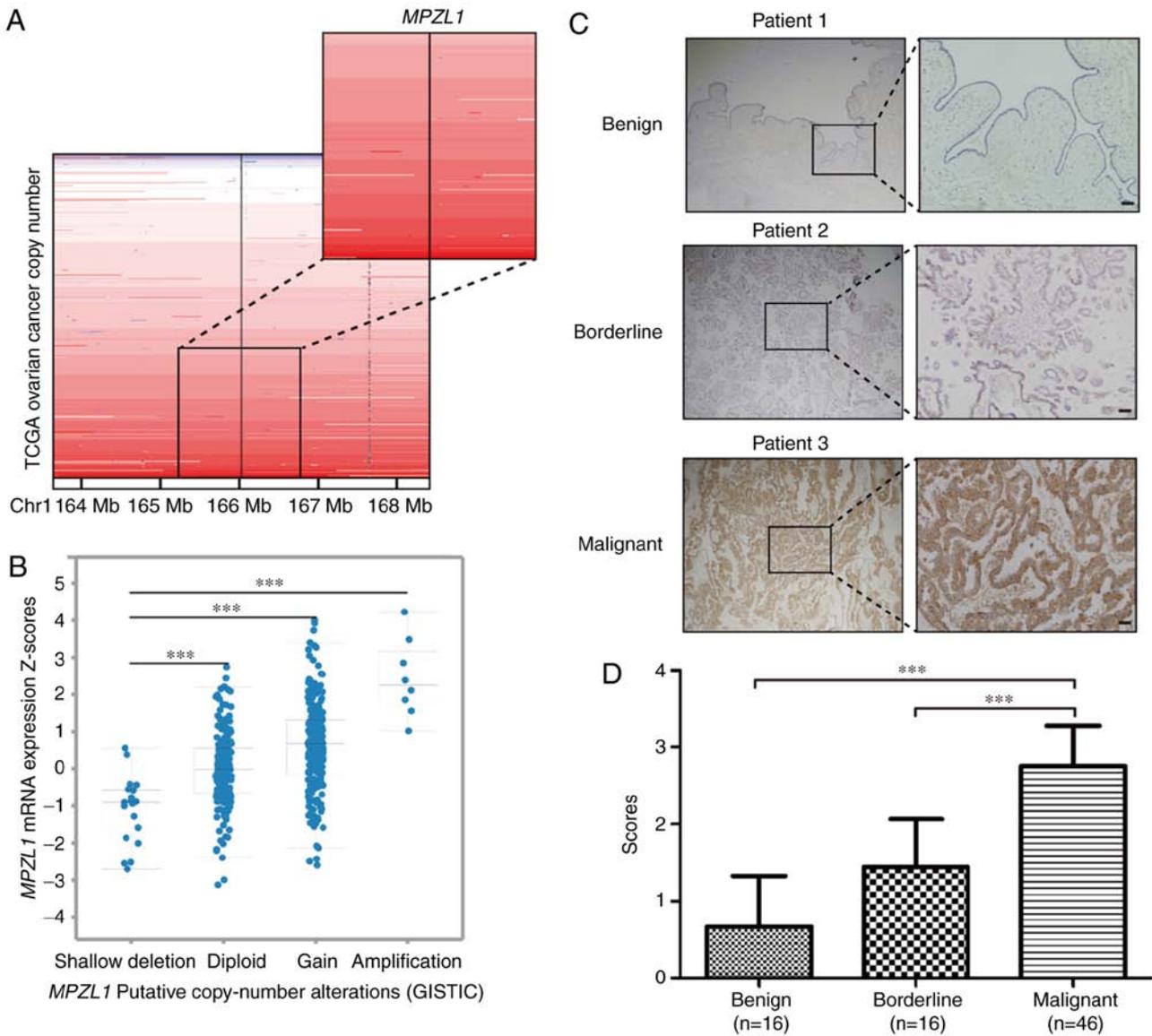


Figure 1. Overexpression of *MPZL1* is associated with ovarian cancer. (A) Copy number analysis of *MPZL1* in TCGA ovarian cancer samples. Color scale: Red, amplification; blue, deletion. (B) *MPZL1* gene expression in ovarian cancer with different *MPZL1* copy number alterations. (C) Representative images of immunohistochemical staining of the *MPZL1* protein in human ovarian cancer samples (scale bar, 50 μ m). (D) Histogram of the relative immunohistochemistry scores of *MPZL1* in the three ovarian cancer groups (benign, n=16; borderline, n=16; malignant, n=46). Data are presented as the mean \pm SEM. ***P<0.0001. GISTIC, Genomic Identification of Significant Targets in Cancer; *MPZL1*, myelin protein zero like 1; TCGA, The Cancer Genome Atlas.

were detected in four ovarian cancer cell lines (Fig. 2A). Subsequently, the *MPZL1* gene was overexpressed via lentiviral infection in SKOV3 and TOV-21G cells (Fig. 2B); these two cell lines were selected as they exhibited relatively lower endogenous *MPZL1* expression. Subsequently, the effects of *MPZL1* overexpression on the proliferation of these ovarian cancer cells were determined using the CCK-8 assay. Notably, the results demonstrated that exogenous overexpression of the *MPZL1* gene promoted ovarian cancer cell proliferation (Fig. 2C). Similarly, the results of the colony formation assay demonstrated that the number of colonies was significantly increased in *MPZL1*-overexpressed SKOV3 and TOV-21G cells (Fig. 2D). Furthermore, the effects of *MPZL1* overexpression were examined on the migratory and invasive abilities of ovarian cancer cells by Transwell assays; ectopic expression of *MPZL1* significantly enhanced the *in vitro* migration and invasion of ovarian

cancer cells (Fig. 2E). These results indicated that *MPZL1* may serve an important role in promoting cell migration and metastasis of ovarian cancer.

Targeted downregulation of MPZL1 attenuates ovarian cancer cell proliferation and migration. To further verify the role of *MPZL1* in ovarian cancer cell migration, HO8910 and HEY cells were selected as cellular models for loss-of-function studies. Firstly, the *MPZL1* gene was stably knocked down in HO8910 and HEY cells using a lentiviral shRNA specifically targeting *MPZL1* (Fig. 3A). Consequently, cell proliferation was significantly inhibited under normal growth conditions (Fig. 3B). Consistently, the colony formation assay revealed that the number of colonies was evidently decreased following knockdown of *MPZL1* in HO8910 and HEY cells (Fig. 3C). Furthermore, Transwell assays demonstrated that the *in vitro* migration and invasion of ovarian cancer cells

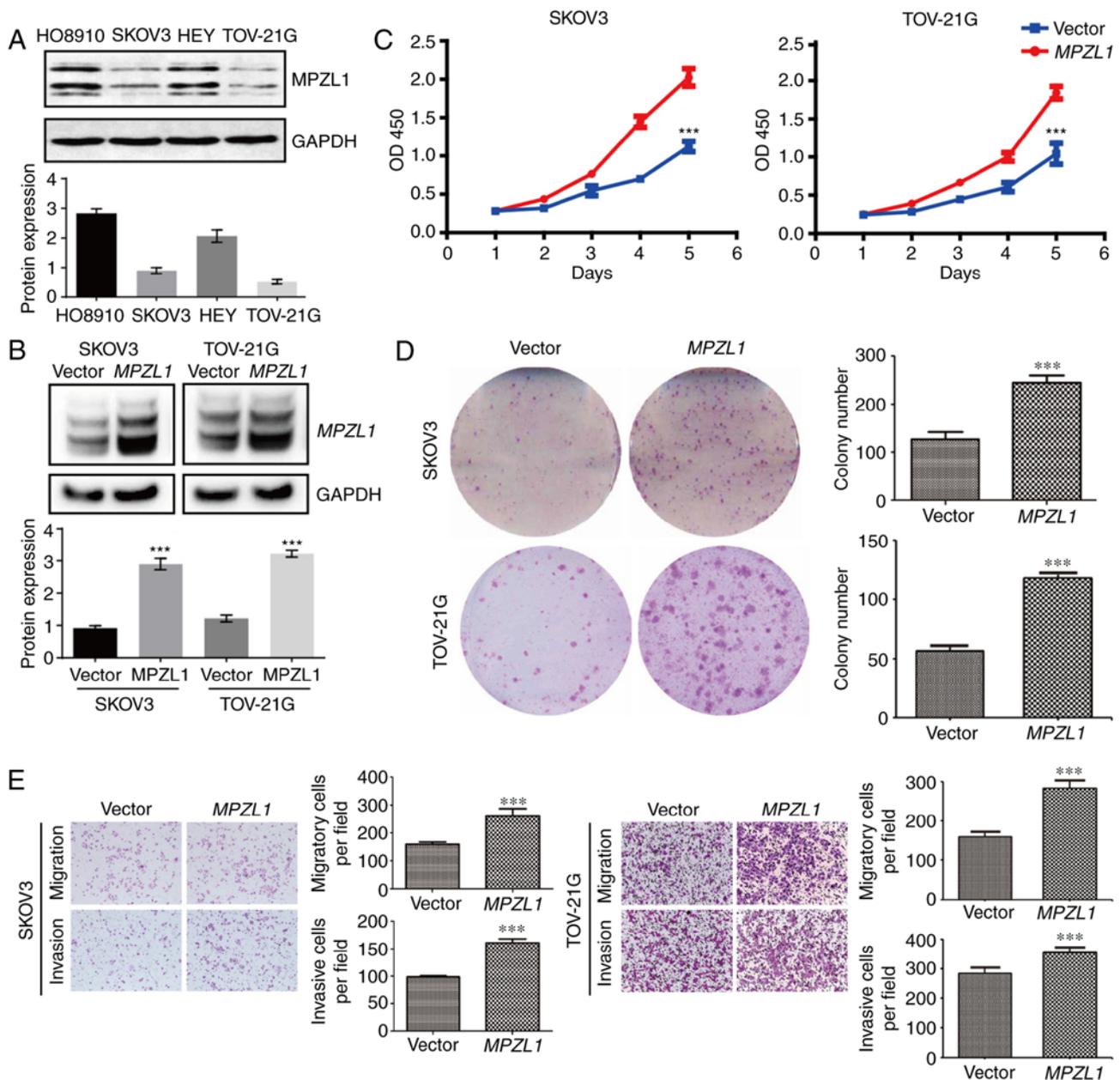


Figure 2. Overexpression of *MPZL1* promotes ovarian cancer cell proliferation and migration. (A) Detection of *MPZL1* protein expression in four ovarian cancer cell lines by western blotting. (B) Detection of lentivirus-mediated overexpression of *MPZL1* in SKOV3 and TOV-21G cells by western blotting. (C) *MPZL1* was overexpressed in SKOV3 and TOV-21G cells. Cell proliferation was analyzed by Cell Counting Kit-8. Error bars indicate standard deviation. (D) Colony formation assays in SKOV3 and TOV-21G cells (magnification, x200). (E) Representative results of the Transwell assays to determine the effects of *MPZL1* overexpression on the *in vitro* migratory and invasive abilities of SKOV3 and TOV-21G cells (magnification, x200). Each group consisted of three biological replicates, and data are presented as the mean \pm SEM. *** $P < 0.001$ vs. vector. *MPZL1*, myelin protein zero like 1; OD, optical density.

were inhibited by *MPZL1* depletion (Fig. 3D). These findings indicated that *MPZL1* knockdown attenuated proliferation, migration and invasion of ovarian cancer cells.

MPZL1 regulates phosphorylation levels of numerous pro-metastatic proteins. Recently, many studies have reported that pro-metastatic proteins, including p130, Src and cortactin, are important for cell migration and tumor metastasis (19-21). Furthermore, p130 and cortactin were originally identified as substrate proteins of the Src family kinases, and a previous report demonstrated that the *MPZL1*/Src/cortactin signaling cascade functions in the process of hepatocellular carcinoma cell migration (22).

However, to the best of our knowledge, the potential role of *MPZL1* in the phosphorylation of pro-metastatic proteins has not been determined in ovarian cancer. To investigate whether the *MPZL1*/Src/cortactin signaling cascade exists in ovarian cancer, the expression levels of pro-metastatic proteins were detected by western blotting. The results indicated that stable overexpression of *MPZL1* led to increased phosphorylation of p130, Src and cortactin in SKOV3 cells (Fig. 4A). Furthermore, targeted knockdown of the *MPZL1* gene by shRNA reduced phosphorylation of these three proteins in HO8910 cells (Fig. 4B). These findings indicated that *MPZL1* overexpression reprogrammed the pro-metastatic signaling network in ovarian cancer.

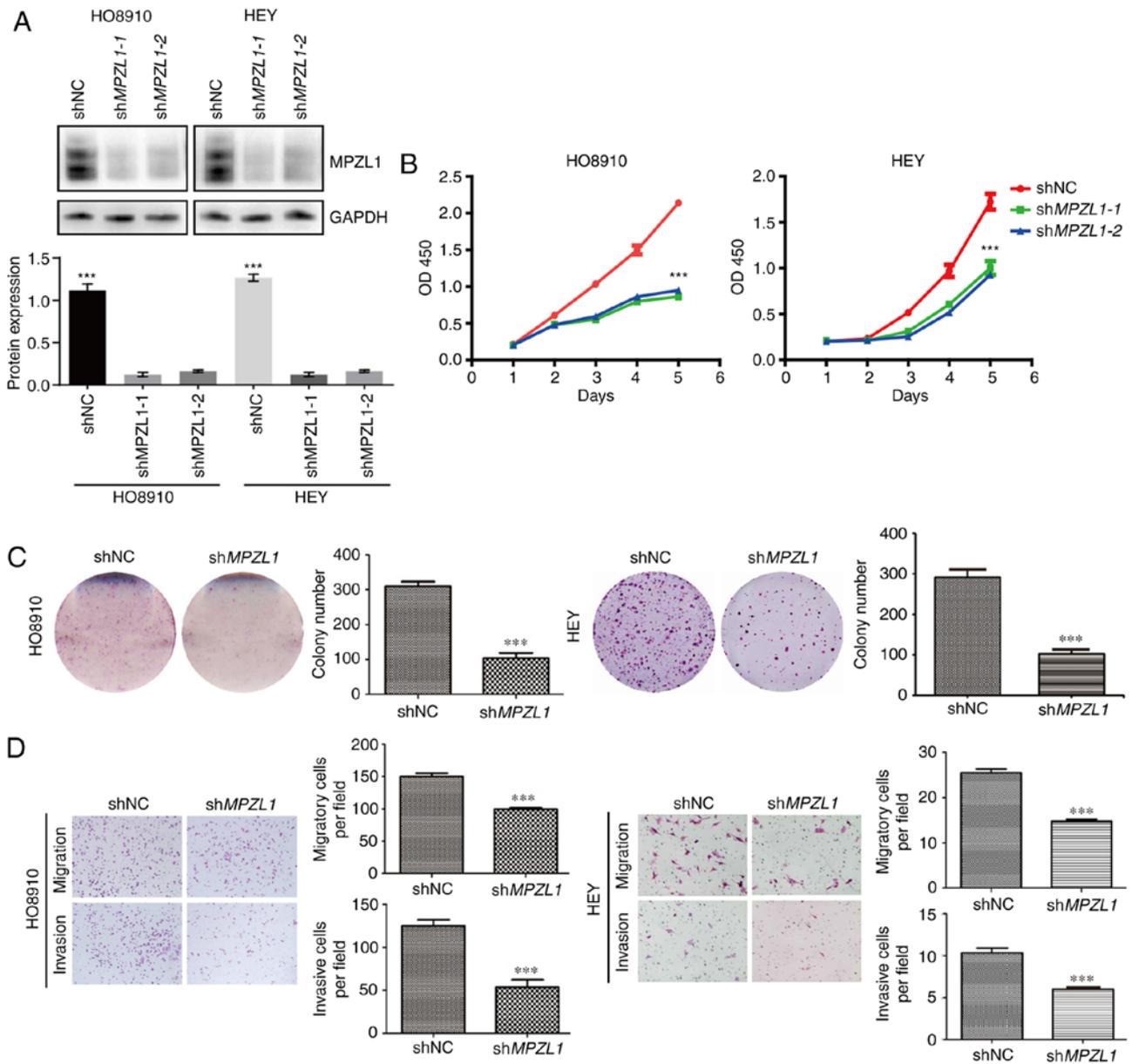


Figure 3. Targeted downregulation of *MPZL1* attenuates ovarian cancer cell proliferation and migration. (A) Detection of knockdown of *MPZL1* by shRNA in HO8910 and HEY cells by western blotting. (B) *MPZL1* was knocked down by shRNA in HO8910 and HEY cells, and cell proliferation was analyzed by Cell Counting Kit-8. Error bars indicate standard deviation. Data are presented as the mean \pm SEM. *** P <0.001 vs. shNC. (C) Colony formation assays in HO8910 and HEY cells (magnification, x200). (D) Representative results of the Transwell assays to determine the effects of *MPZL1* knockdown on the *in vitro* migratory and invasive abilities of HO8910 and HEY cells (magnification, x200). Each group consisted of three biological replicates. Data are presented as the mean \pm SEM. *** P <0.001 vs. shNC. *MPZL1*, myelin protein zero like 1; NC, negative control; OD, optical density; sh/shRNA, short hairpin RNA.

Transient transfection of Src siRNA suppresses migration and invasion in SKOV3-MPZL1 cells. According to previous studies, Src tyrosine kinases serve critical roles in several cellular signal transduction pathways that regulate cell proliferation, adhesion, migration and invasion (22–25). Additionally, as substrate proteins of Src kinase, p-p130 and p-cortactin participate in cell migration (26). Furthermore, amplification of *MPZL1* promotes tumor cell migration via Src-mediated cortactin phosphorylation in hepatocellular carcinoma cells (27). To ascertain whether Src kinase activity is essential for *MPZL1*-induced phosphorylation of p130 and cortactin in ovarian cancer cells, *MPZL1* was overexpressed in SKOV3 cells, and the *Src* gene was then knocked down by siRNA. Notably, stable overexpression of *MPZL1* significantly

increased phosphorylation of p130, cortactin and Src in SKOV3-*MPZL1* cells, and enhanced the migratory and invasive abilities of SKOV3-*MPZL1* cells (Fig. 5A-C). Conversely, targeted knockdown of *Src* via siRNA resulted in reduced phosphorylation of p130 and cortactin, and suppressed the migration and invasion of SKOV3-*MPZL1* cells (Fig. 5A-C). Taken together, these findings indicated that Src kinase activity may be essential for *MPZL1*-mediated migration and invasion of ovarian cancer cells.

Discussion

In the present study, amplified *MPZL1* was associated with the malignant features of ovarian cancer. Subsequently,

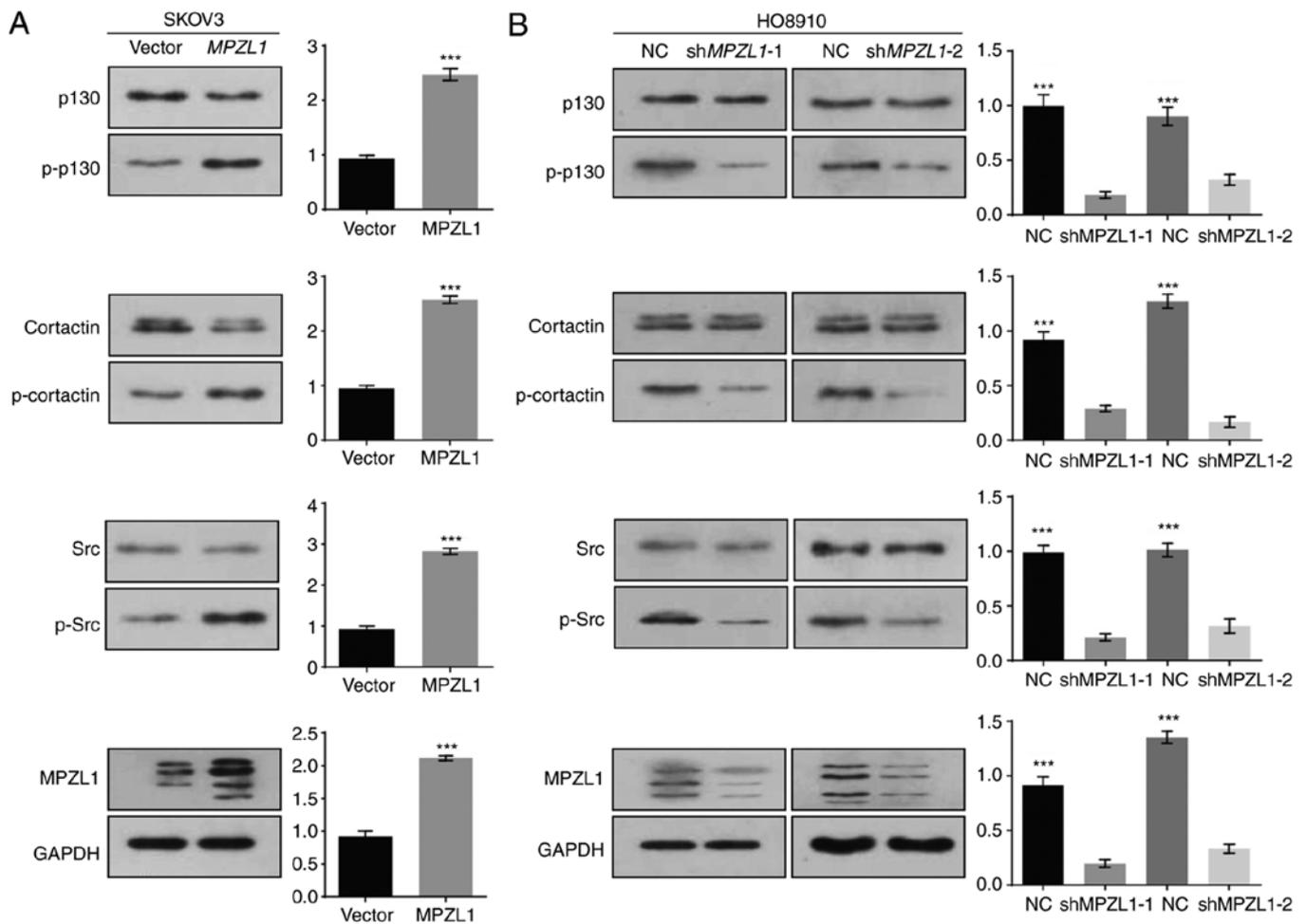


Figure 4. MPZL1 regulates the phosphorylation of numerous pro-metastatic proteins. (A) Overexpression of *MPZL1* in SKOV3 cells increased the phosphorylation of p130, cortactin and Src. *** $P < 0.001$ vs. vector. (B) Knockdown of *MPZL1* by shRNA in HO8910 cells decreased the phosphorylation of p130, cortactin and Src. Phosphorylated proteins were normalized to both total proteins and GAPDH. Each group consisted of three biological replicates and representative images are shown. Data are presented as the mean \pm SEM. *** $P < 0.001$ vs. NC. MPZL1, myelin protein zero like 1; NC, negative control; p-, phosphorylated; sh/shRNA, short hairpin RNA.

overexpression and knockdown of *MPZL1* indicated that *MPZL1* served a prominent role in the promotion of ovarian cancer cell proliferation, migration and invasion. In addition, amplification of *MPZL1* promoted tumor cell migration through Src-mediated phosphorylation of p130 and cortactin. Therefore, these data suggested that *MPZL1* may operate as a novel oncoprotein in ovarian cancer.

Previous studies have reported that the *MPZL1* protein is involved in cell signaling, proliferation, differentiation and transformation (15,28). Furthermore, amplification of *MPZL1* promotes tumor cell migration through Src-mediated phosphorylation of cortactin in hepatocellular carcinoma (27,29). However, the biological functions and clinical implications of *MPZL1* in other types of human cancer remain unclear. This study identified a positive association between the protein expression levels of *MPZL1* and cell proliferation, migration and invasion of ovarian cancer cells.

Src family protein tyrosine kinases participate in numerous signaling pathways that control cellular responses, including proliferation, survival, adhesion and migration in normal and cancer cells (30-33). As substrate proteins of Src kinase, p130 and cortactin become tyrosine-phosphorylated during integrin-mediated cell adhesion to the extracellular matrix, cell

attachment and cell invasion (24,34-36). Furthermore, it has been demonstrated that tyrosine-phosphorylated cortactin via Src kinase, which is activated upon *MPZL1* overexpression, increases the migratory potential of hepatocellular carcinoma cells (14,27). In this study, it was demonstrated that overexpression of *MPZL1* resulted in increased phosphorylation of Src, p130 and cortactin. Additionally, the migration and proliferation of ovarian cancer cells were promoted. These findings indicated that *MPZL1* may serve an important role in ovarian cancer cell metastasis. However, the physiological relevance of these data requires further mechanistic investigations and *in vivo* studies.

In conclusion, the present results suggested that amplification of *MPZL1* in ovarian cancer may be associated with the malignant features of ovarian cancer. *MPZL1* was involved in Src-mediated phosphorylation of p130 and cortactin, thereby promoting ovarian cancer cell proliferation, migration and invasion. Furthermore, these data identifies *MPZL1* as a novel pro-metastatic gene in ovarian cancer. Together with a report implicating *MPZL1* in other cancer types (27), the present study expands our understanding of human ovarian cancer metastasis and indicates potential therapeutic avenues for the treatment of ovarian cancer.

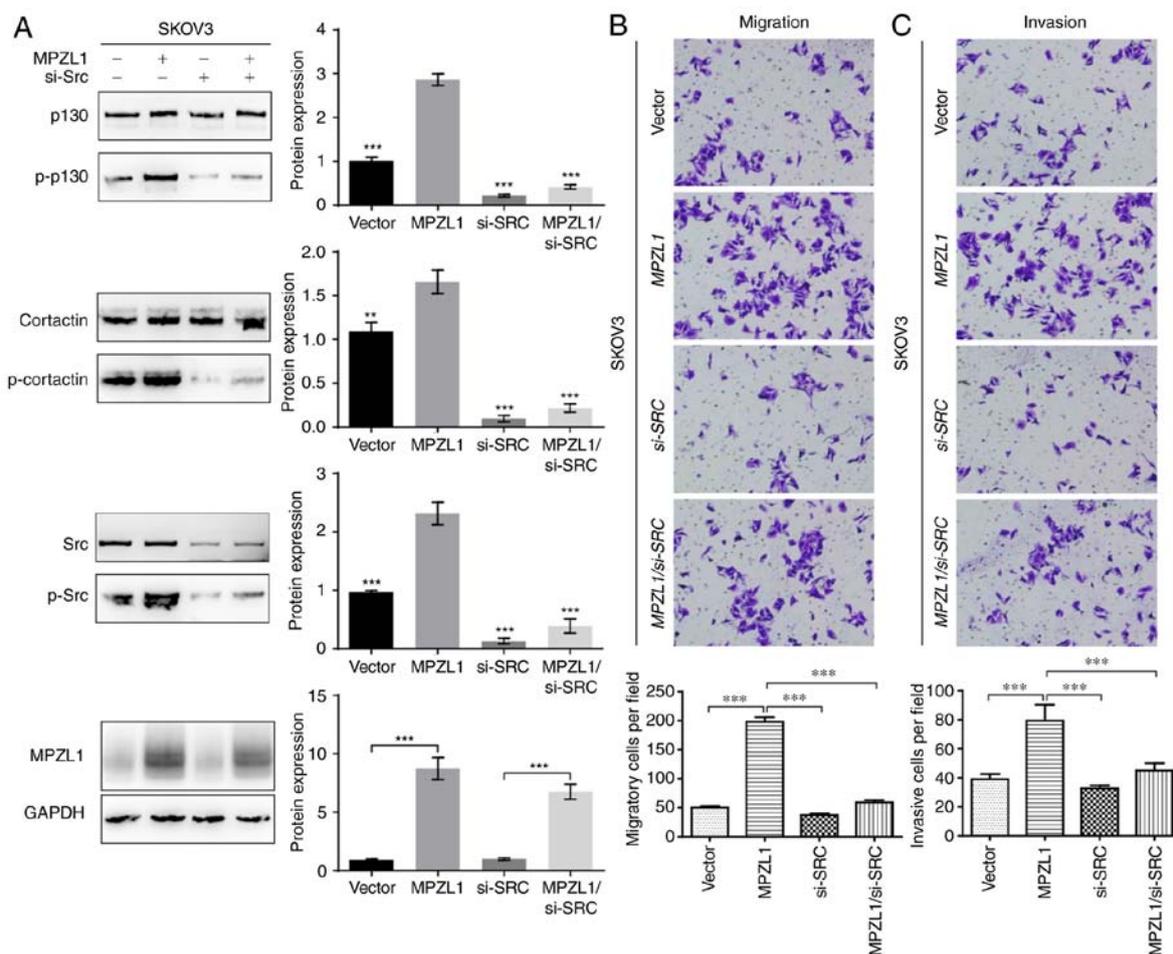


Figure 5. Transient transfection of *Src* siRNA decreases cell migration and invasion in SKOV3-*MPZL1* cells. (A) Western blot analysis following transient transfection of *Src* siRNA in SKOV3-*MPZL1* cells; control cells were transfected with NC siRNA. Phosphorylated proteins were normalized to both total proteins and GAPDH. (B) Transwell migration assays of SKOV3 cells (magnification, x200). (C) Transwell invasion assays of SKOV3 cells (magnification, x200). Each group consisted of three biological replicates. Data are presented as the mean \pm SEM. ** $P < 0.01$, *** $P < 0.001$ vs. MPZL1 or as indicated. MPZL1, myelin protein zero like 1; p-, phosphorylated; siRNA, small interfering RNA.

Acknowledgements

The authors would like to thank Dr Shengzhe Zhang and Ms. Zhenfeng Zhang (Shanghai Jiao Tong University School of Medicine) for their technical assistance.

Funding

This work was supported by a grant from the Science and Technology Commission of Changning District, Shanghai (grant no. CNKW2017Y08 to X.W.).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XW and DC designed and conceived the experiments. LC analyzed the data. XW, DC and LC drafted the manuscript and wrote the manuscript. XW supervised the study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All experimental protocols were approved by the Ethics Committee of the Tongren Hospital, Shanghai Jiao Tong University School of Medicine. All patients provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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