Furin promotes epithelial-mesenchymal transition in pancreatic cancer cells via Hippo-YAP pathway

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Abstract. Furin, a well-characterized proprotein convertase, plays an important role in many diseases and links to tumor metastasis. However, the role of Furin in pancreatic cancer progression remains to be elucidated. In the present study, we found that Furin promotes the growth and the epithelial-mesenchymal transition (EMT) of pancreatic cancer cells. First, we found that Furin knockdown significantly inhibited proliferation, invasion and migration in BxPC3 and SW1990 cells, while Furin overexpression promoted the above behavior in PANC1 and PaTu8988 cells. Further evidence revealed that Furin knockdown resulted in the upregulation of E-cadherin (epithelial marker), and the downregulation of N-cadherin and Vimentin (mesenchymal markers) in BxPC3 and SW1990 cells, whereas Furin overexpression remarkably led to the opposite effects in PANC1 and PaTu8988 cells. Furthermore, our data showed that Furin knockdown, Furin inhibitor D6R or overexpression significantly affected YAP phosphoration level and total YAP protein level, indicating that Furin was involved in Hippo-YAP pathway. It is suggested that Furin promotes epithelial-mesenchymal transition in pancreatic cancer cells probably via Hippo-YAP pathway and may be a potential target for anti-pancreatic cancer.

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Introduction

Pancreatic cancer is one of the most serious diseases, which lacks specific symptoms and progresses rapidly. It ranks the fourth in all cancer mortality rates. Despite decades of effort, the 5-year survival rate remains at only ∼5% (1). Therefore, it is urgent to take a deep look of its biological characteristics, to insure that we can detect and diagnose pancreatic cancer in the early stage.

Epithelial-mesenchymal transition (EMT) is a developmental process where cells lose their epithelial features including loss of their sheet-like architecture, loss of polarity and develop a mesenchymal phenotype. As cells undergo EMT, they start expressing mesenchymal markers, such as N-cadherin, Vimentin and downregulating E-cadherin (2-5). EMT was identified to be regulated by YAP through modulation of TGFβ-Smad signaling (6). It is suggested that YAP/Smad pathway potentially plays an important role in EMT.

Furin, a member of proprotein convertases (PCs), which belongs to a family of serine proteases capable of cleaving carboxyl-terminal of specific basic amino acid motifs and activating various precursor proteins (7,8). These precursor proteins include growth factors and differentiation factors, receptors, adhesion molecules and enzymes like matrix metalloproteases (MMPs), which have been associated with different stages of tumor development, progression, vascularization and metastasis (9,10). Previously, it has been reported that Furin is highly linked to various human primary tumors (11,12) including skin tumor (13), colon tumor (14), head and neck (15) and breast (16) and ovarian cancer (17). Moreover, Furin can correctly cleave the TGF-β precursor, which has been identified as a key regulator of EMT (18), indicating that Furin may link to EMT of tumor cells. However, the role of Furin in pancreatic cancer cells remains to be clarified.

In the present study, we found that Furin was critical for the growth and EMT of pancreatic cancer cells. Also, Furin upregulated total YAP protein level and downregulated YAP phosphoration level, indicating that Furin was involved in the YAP activation. Therefore, we speculate that Furin promotes epithelial-mesenchymal transition in pancreatic cancer cells probably via Hippo-YAP pathway. All these findings prove that Furin promotes EMT and may play a crucial role in pancreatic cancer progression.

Materials and methods

Cell culture. The pancreatic cancer cells PaTu8988, BxPC3, PANC1 and SW1990 were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA) and the Cancer Cell Repository (Shanghai, China). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) at standard cell culture conditions (37˚C, 5% CO_2 in humidified incubator). DMEM, FBS and trypsin were purchased from Gibco (Carlsbad, CA, USA).

Plasmid construction. The construction of sh-Furin: the oligonucleotide sequences were inserted into the *Eco*RI and *Age*I sites of the pLKO.1-TRC plasmid and ligated into the vector (Sigma-Aldrich, St. Louis, MO, USA). The targeting sequences for Furin was searched from Sigma-Aldrich and produced by Sangon Biotech Co., Ltd. (Shanghai, China). The oligo sequences of Furin shRNA included: Furin shRNA (F), CCG GGT GGC AAA GCG ACG GAC TAA ACT CGA GTT TAG TCC GTC GCT TTG CCA CTT TTT G and Furin shRNA (R), AAT TCA AAA AGT GGC AAA GCG ACG GAC TAA ACT CGA GTT TAG TCC GTC GCT TTG CCA C.

The construction of Flag-Furin: the full-length complementary DNA (cDNA) for human Furin was obtained from a cDNA library via polymerase chain reaction (PCR) amplification using primers Furin-all-F (5'-CCCAAGCTTATGGAGCTG AGGCCCTGGTTGC-3') and Furin-all-R (5'-CCGGAATTC GAGGGCGCTCTGGTCTTTGATAAA-3') and cloned into the *Eco*RI/*Hin*dIII site of p3xFLAG-Myc-CMV-24. The sequence was confirmed by DNA sequencing.

Transfection of the cell line. psPAx2 and pMD2.G were co-transfected with sh-EGFP or sh-Furin into HEK293T cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). After 48 h the supernatants were collected and concentrated. BxPC3 and SW1990 cells were stably transfected with either sh-EGFP or sh-Furin plasmid. Cells were infected with $1x10^6$ recombinant lentivirus transduction units in the presence of 8 mg/ml polybrene (Sigma-Aldrich). Cells were then cultured with puromycin (1:10,000 dilution) and the cells in blank group all became unviable.

PANC1 and PaTu8988 cells at 60-80% confluence were transfected with Lipofectamine 2000 reagent according to the manufacturer's instructions. The amount of vector and Flag-Furin DNA used for transfection was 2 μg/well in a 6-well plate, after cultured for 48-72 h, the cells transfected were washed and harvested for further study.

D6R treatment. The pancreatic cancer cells BxPC3 were seeded at a final density of 100,000 cells/well on a 6-well culture plates and the cells were plated at 30-50% confluence after 8 h. Then, we added different doses of Furin inhibitor D6R. After treatment with D6R for 48 h, cells were collected for different assays. The total cellular proteins were collected after treated with D6R for 72 h.

Cell proliferation assay. To analyze the cell proliferation and the viability, we performed Cell Counting kit-8 (CCK-8). The pancreatic cancer cells were collected by trypsinization, and incubated in a 96-well plate at a final density of $2x10^3$ cells/well for counting. A CCK-8 kit was added to assess the cells viability at 24, 48, 72, 96 and 120 h and the absorbance was finally determined at 490 nm.

Colony formation assay. The colony formation assay was used to detect the anchorage-independent growth of the pancreatic cancer cells. The cells were plated at a final density of 500 cells/well. Each transfection group was seeded on 6-well culture plates. After the cells were incubated for 10-14 days, the cell colonies with >50 cells were counted then fixed with 4% paraformaldehyde and stained with crystal violet. Following the colony count a graph was prepared.

Scratch wound healing assay. To detect the ability of migration we incubated the cells at the density of $1x10^5$ cells/well in a 24-well culture plate, and disrupted the confluent monolayer with a 10-μl pipette tip and then washed with phosphate-buffered saline (PBS) three times. The wounded monolayer was photographed over the following 24 h. The migration ability of the cells was calculated by the ratio of the healing width at 24 h to the wound width at 0 h.

Cell invasion and migration assay. The cells were incubated in the Transwell chambers which were coated with 4 μl/well Matrigel (for an invasion assay; BD Biosciences) or without Matrigel (for a migration assay) in a serum-free DMEM according to the manufacturer's instruction and in the lower chambers 10% FBS WAS added. At 24 h, the cells that remained on the top of the filter were wiped off and the invasive cells on the lower chamber were stained and counted.

Real-time PCR. Total RNA was extracted using RNAiso Plus (Takara). Reverse transcription was performed using RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific) according to the manufacturer's specification. Real-time PCR was performed in triplicate in 20 μl reactions with iQ SYBR® Premix Ex Taq™ Perfect Real-Time (Bio-Rad Laboratories, Hercules, CA, USA), 50 ng first strand cDNA and 0.2 μg each primer. The primer pair used for the amplification of the human Furin gene was as follows: forward primer, 5'-CCAAAGACATCGGGAAACG-3' and reverse primer, 5'-TTAAACCCATCTGCGGAGTAG-3'; and GAPDH primer: forward, 5'-GGTGAAGGTCGGTGTGAACG-3' and reverse, 5'-CTCGCTCCTGGAAGATGGTG-3'. Samples were cycled once at 95˚C for 2 min, subjected to 35 cycles of 95˚C, 56˚C and 72˚C for 30 sec each. The relative mRNA content was calculated using the $2^{-\Delta\Delta CT}$ method with GAPDH as an endogenous control.

Western blot analysis. Cells were washed with PBS three times and lysed in 2X loading buffer on ice, then collected. Total cellular protein were boiling for 5 min to denature the fractions, and then separated equally on 10% SDS-PAGE gels, transferred onto PVDF membranes. The membranes were blocked with 5% skimmed milk for 1 h at room temperature. The membranes were incubated in the primary antibodies overnight at 4˚C, and the secondary horseradish peroxidase-conjugated antibodies for 1 h at room temperature. The bands were detected by enhanced chemiluminescence. The antibodies were rabbit anti-Furin (18413-1-AP; Proteintech,
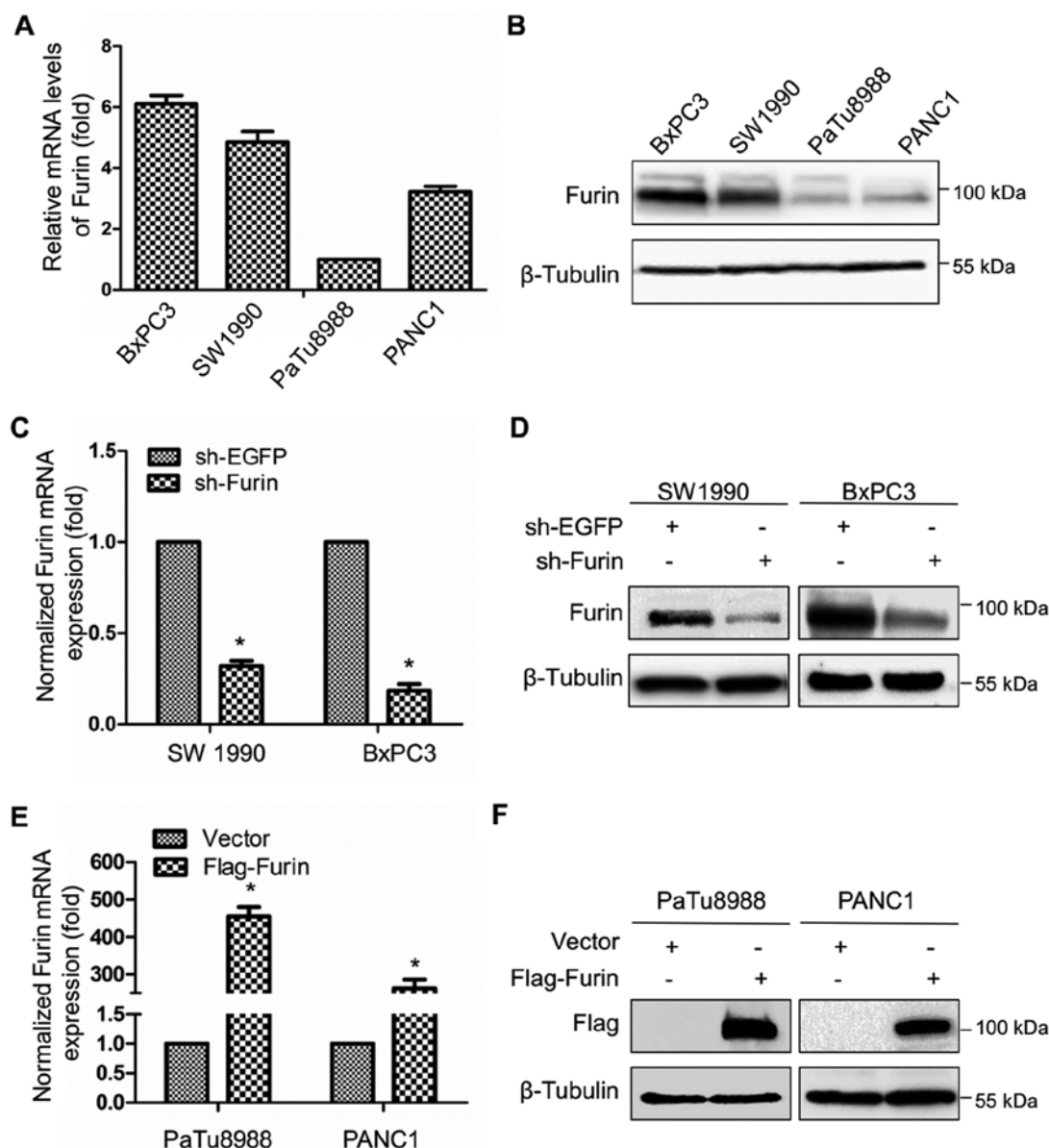
Figure 1. Detection of Furin expression in pancreatic cancer cells. Relative expression levels of (A) Furin mRNA and (B) protein were assessed in SW1990, BxPC3, PANC1 and PaTu8988 cells. Identification of shRNA-mediated Furin knockdown in SW1990 and BxPC3 cells by real-time-PCR and western blot analysis (C and D). GAPDH was used for normalization, the data are presented as the mean ± SD. (Student's t-test: *P<0.05 vs. sh-EGFP). The expression of Furin was verified in PANC1 and PaTu8988 cells, when transfected with vector or Flag-Furin plasmid (E and F). GAPDH was used for normalization, the data are presented as the mean ± SD. (Student's t-test: *P<0.05 vs. vector). All data were obtained from at least three independent experiments.

Rosemont, IL, USA), mouse anti-β-Tubulin (cat. no. 6181; Cell Signaling Technology, Danvers, MA, USA), mouse anti-Flag (cat. no. F1804; Sigma-Aldrich), rabbit anti-N-cadherin (cat. no. 13116; Cell Signaling Technology), rabbit anti-E-cadherin (cat. no. 3195; Cell Signaling Technology), rabbit anti-Vimentin (cat. no. 5741; Cell Signaling Technology), rabbit anti-YAP (cat. no. 8418; Cell Signaling Technology), rabbit anti-p-YAP (cat. no. 13619; Cell Signaling Technology), rabbit anti-Mob1 (cat. no. 13730; Cell Signaling Technology), rabbit anti-p-Mob1 (cat. no. 8699; Cell Signaling Technology).

Results

Furin expression varies in pancreatic cancer cells. We first used real-time PCR and western blotting to detect the expres-

sion of Furin of pancreatic cancer cells. The data showed that the Furin mRNA and protein levels remained highly abundant in BxPC3 and SW1990 cells, while had weak expression in PANC1 and PaTu8988 cells (Fig. 1A and B). Next, we transfected BxPC3 and SW1990 cells with sh-EGFP or sh-Furin, the mRNA level of Furin was decreased by at least 70% in sh-Furin group compared with sh-EGFP group (Fig. 1C), as well as its protein level (Fig. 1D). Furin overexpression was also examined, and both mRNA and protein levels of Furin were significant upregulated in Flag-Furin group compared with vector group (Fig. 1E and F).

Furin promotes the growth of pancreatic cancer cells. To assess the effect of Furin on the cell growth, we used CCK-8 assays to determine the relative proliferation rates in human pancreatic
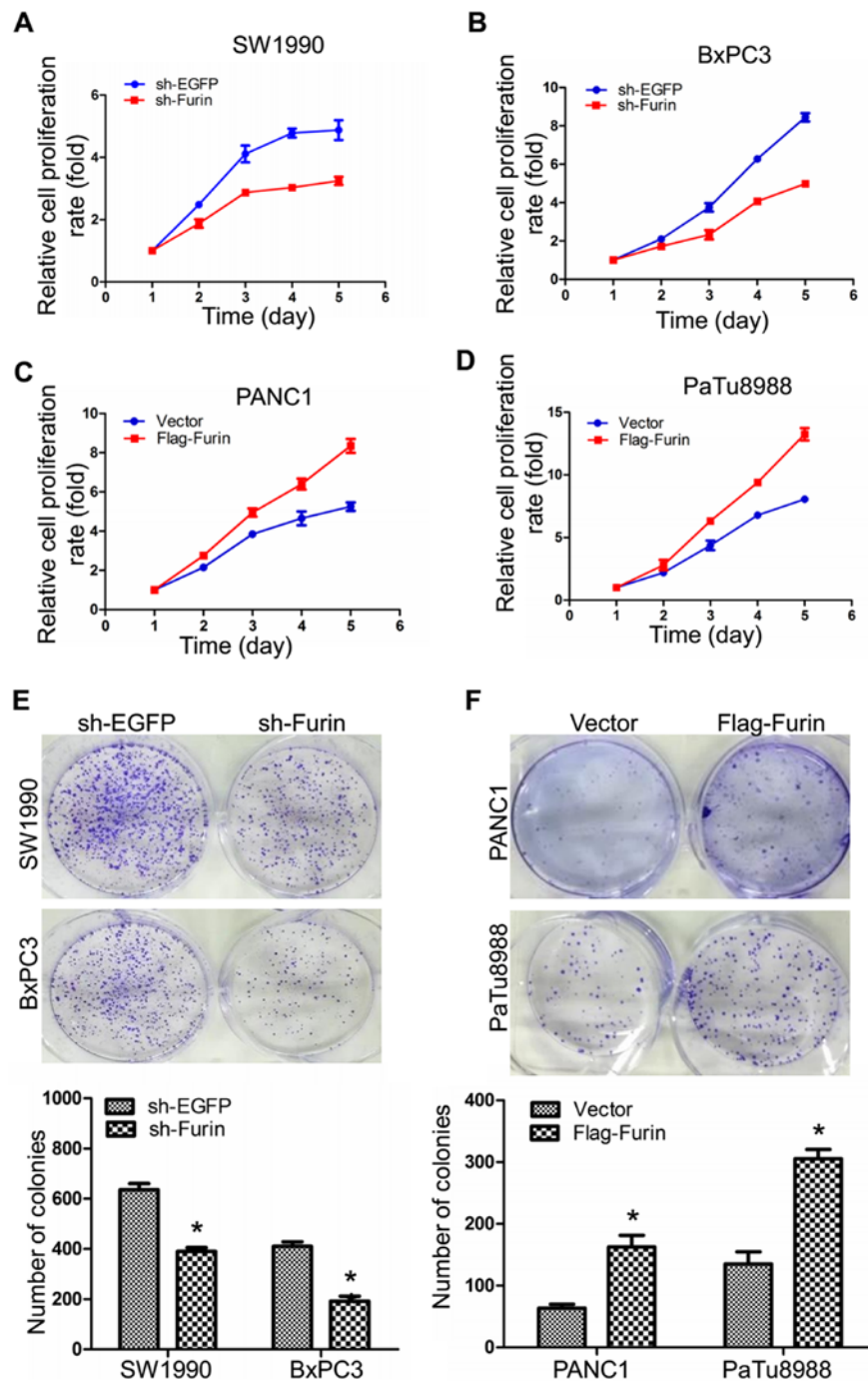
Figure 2. Furin promotes cell proliferation in pancreatic cancer cells. (A and B) CCK-8 assay showed that Furin knockdown inhibited BxPC3 and SW1990 cell growth rate, the data are presented as the mean ± SD; (n=3). (C and D) CCK-8 assay showed that Furin ovexpression promoted cell proliferation rate in PANC1 and PaTu8988 cells, the data are presented as the mean ± SD (n=3). (E) Colony forming assay showed that Furin knockdown inhibited BxPC3 and SW1990 cell anchorage-independent growth, the data are presented as the mean ± SD (Student's t-test: *P<0.05 vs. sh-EGFP). (F) Colony forming assay showed that Furin ovexpression promoted PANC1 and PaTu8988 cell anchorage-independent growth, the data are presented as the mean ± SD (Student's t-test: *P<0.05 vs. vector). The data were obtained from at least three independent experiments.

cancer cells. As demonstrated by Fig. 2A and B, Furin knockdown decreased the relative rates of proliferation in BxPC3 and SW1990 cells, while Furin overexpression increased in PANC1 and PaTu8988 cells (Fig. 2C and D), indicating that Furin promoted the proliferation of pancreatic cancer cells. Colony forming assay provided additional support, and the data showed that the number of colonies were 635.5±17.5 and 390±11 in sh-EGFP and sh-Furin SW1990 cells, and 410±13 and 192±14 in sh-EGFP and sh-Furin BxPC3 cells (Fig. 2E),

while the numbers of colonies were 64±5 and 165±12 in vector and Flag-Furin PANC1 cells, and 140±17 and 304±18 in vector and Flag-Furin PaTu8988 cells (Fig. 2F), suggesting that Furin promoted the ability of colony formation. All these data suggested that Furin promoted proliferation in pancreatic cancer cells.

Furin enhances migration of pancreatic cancer cells. To determine the roles of Furin in progress of pancreatic cancer cells,
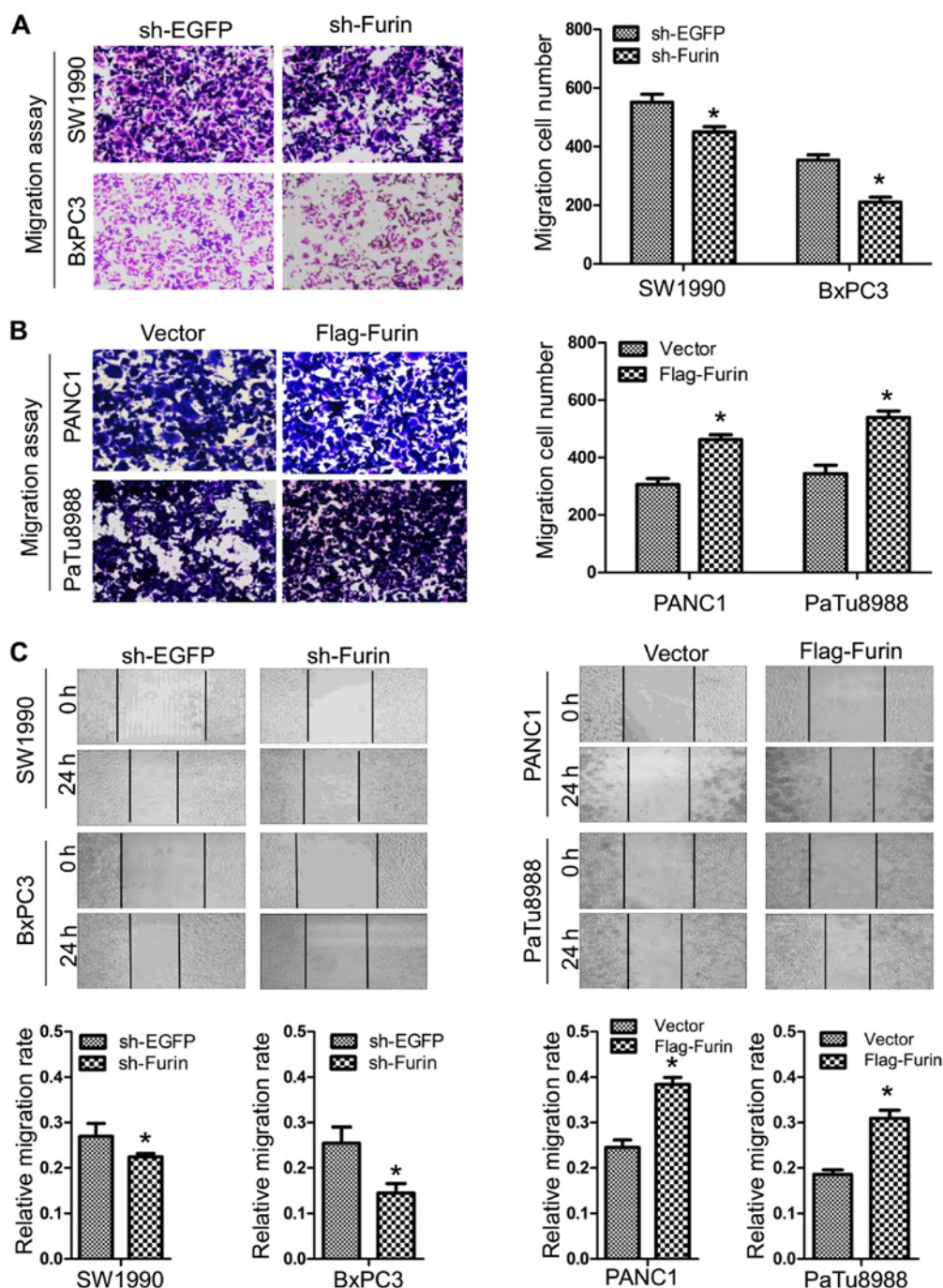
Figure 3. Furin promotes cell migration in pancreatic cancer cells. The migration changes was measured by Transwell migration assay (A and B) and wound healing assay (C), after transfected with sh-EGFP or sh-Furin plasmid in BxPC3 and SW1990 cells and vector or Flag-Furin plasmid in PANC1 and PaTu8988 cells, the data are presented as the mean ± SD (Student's t-test: *P<0.05 vs. sh-EGFP, *P<0.05 vs. vector). The data were obtained from at least three independent experiments.

we used Transwell migration assays and wound healing assays to examine the ability of migration of pancreatic cancer cells. As showed in Fig. 3A and B, the numbers of migrated cells was 551±20 and 450±13 in sh-EGFP and sh-Furin SW1990 cells, and 354±13 and 540±16 in sh-EGFP and sh-Furin BxPC3 cells, while 309±15 and 463±12 in vector and Flag-Furin PANC1 cells, and 344±21 and 211±12 in vector and Flag-Furin PaTu8988 cells. Consistently, the migration rate was 0.27±0.02 and 0.225±0.005 in sh-EGFP and sh-Furin SW1990 cells, and 0.255±0.025 and 0.145±0.015 in sh-EGFP and sh-Furin BxPC3 cells, while 0.2455±0.0115 and 0.384±0.011 in vector and

Flag-Furin PANC1 cells, and 0.186±0.007 and 0.3095±0.0125 in vector and Flag-Furin PaTu8988 cells (Fig. 3C). The data suggested that Furin promoted migration of pancreatic cancer cells.

Furin activates the ability of invasion of pancreatic cancer cells. Then, we examined the effect of Furin on the invasive abilities of the pancreatic cancer cells using Transwell invasion assay. As shown in Fig. 4A and B, the number of invaded cells was 474±15 and 238.5±12.5 in sh-EGFP and sh-Furin SW1990 cells, and 320.5±14.5 and 204±8 in sh-EGFP and
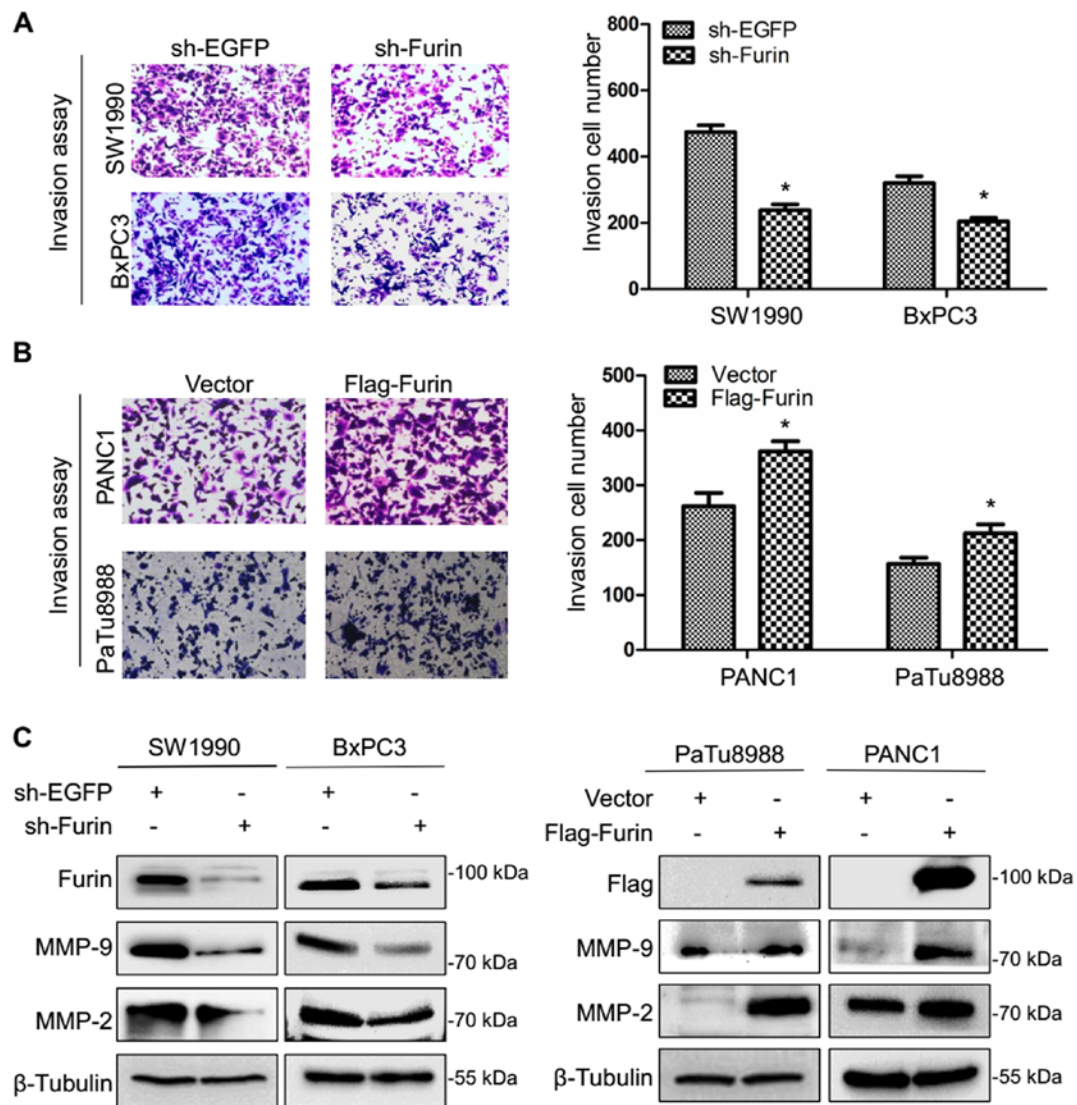
Figure 4. Furin promotes cell invasion in pancreatic cancer cells. The invasion changes were measured by Transwell invasion assay. After transfected with sh-EGFP or sh-Furin plasmid in BxPC3 and SW1990 cells (A) and vector or Flag-Furin plasmid in PANC1 and PaTu8988 cells (B), the data are presented as the mean ± SD (Student's t-test: *P<0.05 vs. sh-EGFP, *P<0.05 vs. vector). The protein levels of MMP-2 and MMP-9 were measured by western blotting (C). The data were obtained from at least three independent experiments.

sh-Furin BxPC3 cells, while the number of invaded cells was 262±17 and 362±13 in vector and Flag-Furin PANC1 cells, and 157±8 and 212.5±11.5 in vector and Flag-Furin PaTu8988 cells suggesting that Furin promoted the invasion ability of pancreatic cancer cells.

As MMP-2 and MMP-9 possess the ability to hydrolyze components of the basement membrane and regulate various aspects of tumor growth and metastasis, we determined the effects of Furin on expression of MMP-2 and MMP-9. The results indicated that Furin overexpression led to the increase of the protein level of MMP-2 and MMP-9, and Furin knockdown resulted in the opposite effects (Fig. 4C). The above data suggested that Furin promoted the invasive ability of pancreatic cancer cells.

Furin induces EMT in pancreatic cancer cells. EMT is thought to be a key mechanism in which primary tumor cells are capable of metastasizing (19). To determine whether EMT is involved in Furin-induced migration and invasion, we first detected the expression of EMT markers at protein levels. The data showed that Furin knockdown resulted in the downregulation of N-cadherin and Vimentin and in the upregulation of E-cadherin in SW1990 and BxPC3 cells. In PANC1 and PaTu8988 cells, Furin overexpression remarkably led to the opposite effects (Fig. 5A). Our data confirmed that Furin promoted EMT in pancreatic cancer cells.

Furin affects the Hippo-YAP pathway in pancreatic cancer cells. Previous studies identified that EMT can be regulated via Hippo-YAP signaling (20-22). To explore whether YAP is functional in Furin driving EMT in pancreatic cancer cells, we detected the expression of the relevant proteins in classic Hippo-YAP pathway, such as Mob1, p-Mob1, YAP and p-YAP. Our results revealed that Furin knockdown suppressed the expression of total YAP and p-Mob1, and upregulated p-YAP and Mob1 level, while Furin overexpression resulted in the opposite effects (Fig. 5B). These data suggested that Furin affected Hippo-YAP pathway in pancreatic cancer cells.
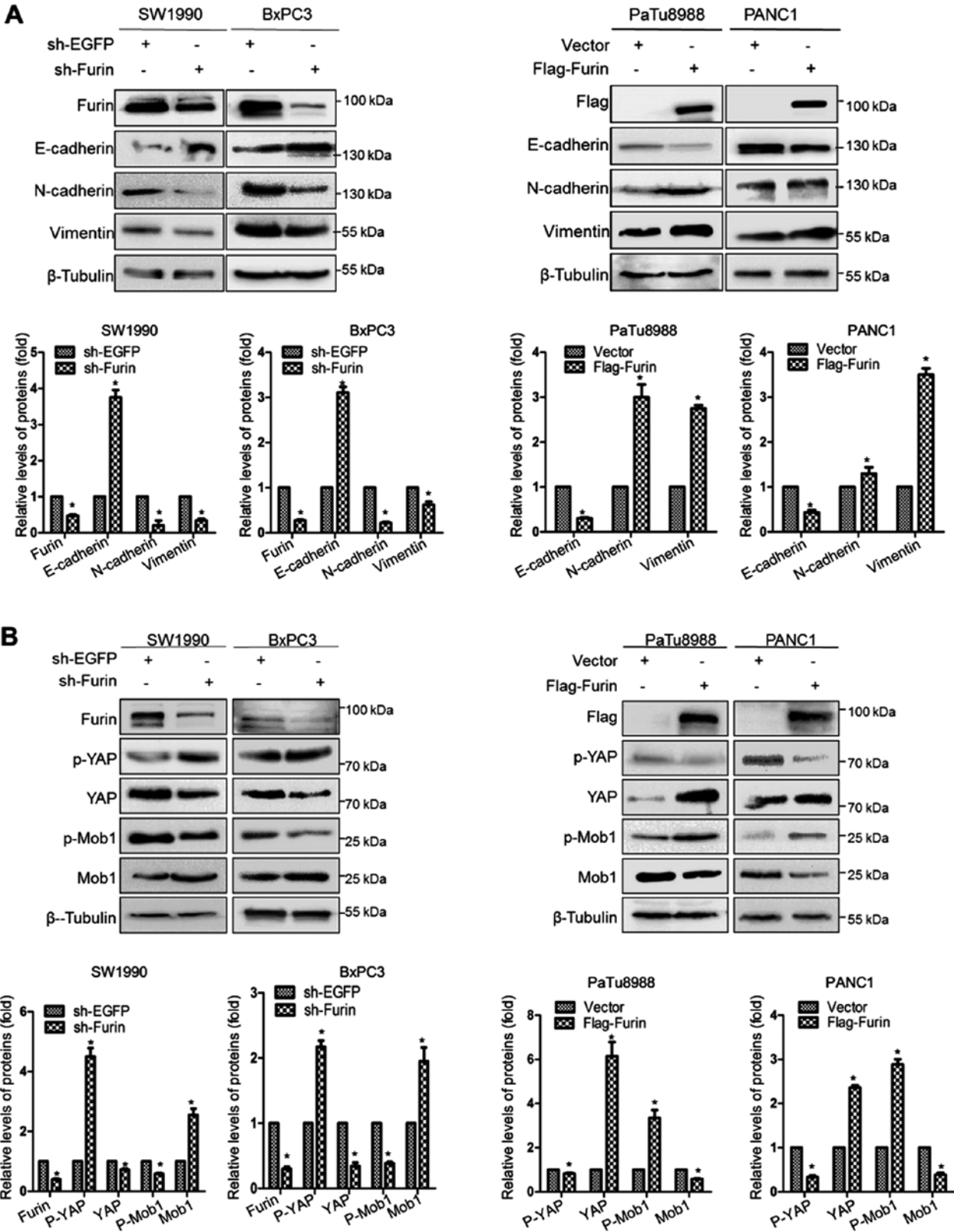
Figure 5. Furin affects EMT and Hippo-YAP signal pathway in pancreatic cancer cells. (A) The protein levels of E-cadherin, N-cadherin and Vimentin were measured by western blotting, after transfected with sh-EGFP or sh-Furin plasmid in BxPC3 and SW1990 cells and vector or Flag-Furin plasmid in PANC1 and PaTu8988 cells. (B) The protein levels of YAP, p-YAP, Mob1, p-Mob1 were measured by western blotting, after transfected with sh-EGFP or sh-Furin plasmid in BxPC3 and SW1990 cells and vector or Flag-Furin plasmid in PANC1 and PaTu8988 cells. The histograms show the gray intensity analyses to the bands of western blotting, and the data are presented as the mean ± SD (Student's t-test: *P<0.05 vs. sh-EGFP, *P<0.05 vs. vector). The data were obtained from at least three independent experiments.

Furin inhibitor D6R suppresses the proliferation, migration and invasion of BxPC3 cells. Furin inhibition seems to be a

logical route to inhibiting the activation of its substrates, many of which are essential components of the invasive/metastatic
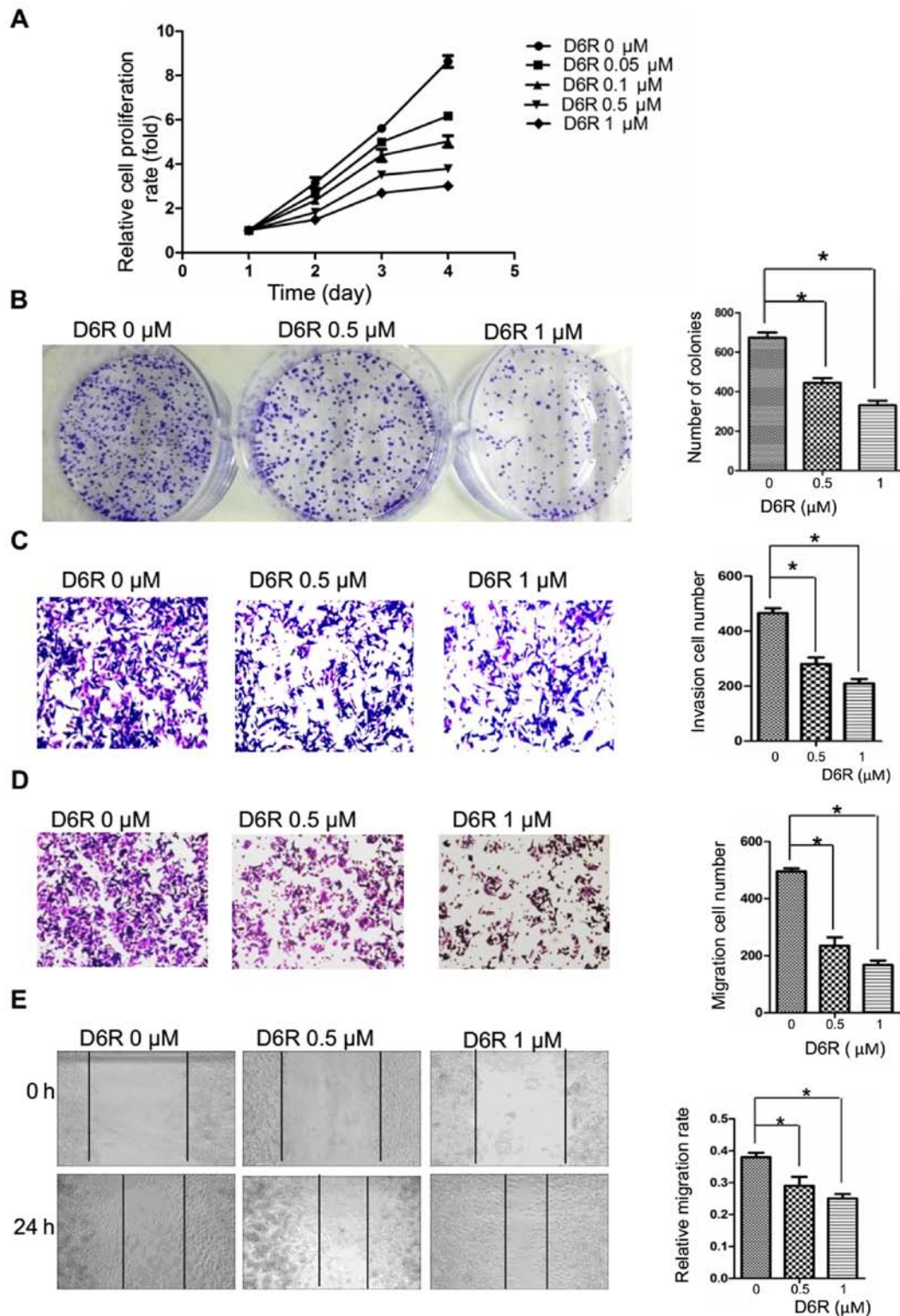
Figure 6. Furin inhibitor D6R prohibits the ability of proliferation, migration and invasion in BxPC3 cells. (A) CCK-8 assay showed that D6R inhibited BxPC3 cell growth rate and it was concentration dependent. (B) Anchorage-independent growth was evaluated by colony formation assay, the data are presented as the mean ± SD (Student's t-test: *P<0.05 vs. D6R-free group). (C) The ability of invasiveness was detected by Transwell assay, the data are presented as the mean ± SD (Student's t-test: *P<0.05 vs. D6R-free group). (D and E) The ability of migration was detected by Transwell assay and wound healing assay, the data are presented as the mean ± SD (Student's t-test: *P<0.05 vs. D6R-free group). The data were obtained from at least three independent experiments.

cascade (e.g. TGF-β and MT-MMPs). The above data confirm that the expression levels of Furin affected EMT probably via Hippo-YAP pathway in pancreatic cancer cells. It is worth assessing whether the activity of Furin similarly affects their growth and EMT. Therefore, we first treated BxPC3 cells with different doses of Furin inhibitor D6R (0, 0.05, 0.1, 0.5 and 1 μM). The data indicated that D6R significantly inhibited the cell proliferation in dose-dependent manner (Fig. 6A), and the half maximal inhibitory concentration (IC$_{50}$) of D6R is between 0.5 and 0.6 μM. Therefore, the concentration of 0,
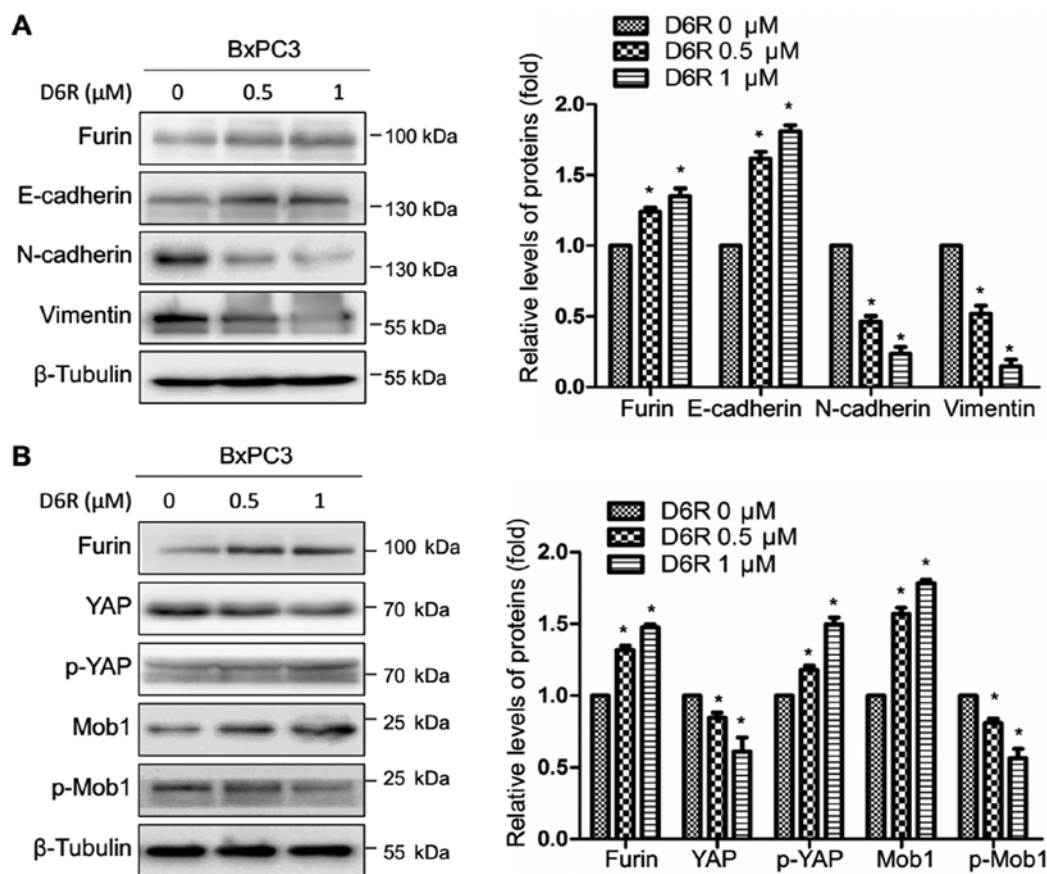
Figure 7. Furin inhibitor D6R affects the EMT and Hippo-YAP pathway in BxPC3 cells. (A) The protein levels of E-cadherin, N-cadherin and Vimentin were measured by western blotting. (B) The protein levels of YAP, p-YAP, Mob1, p-Mob1 were measured by western blotting. The histograms show the gray intensity analyses to bands of western blotting, and the data are presented as the mean ± SD (Student's t-test: *P<0.05 vs. D6R-free group). The data were obtained from at least three independent experiments.

0.5 and 1 μM was selected for further study. We found that compared to the D6R-free group, the abilities of proliferation, migration and invasion were significantly suppressed in the D6R groups (Fig. 6B-E), indicating that the activity of Furin actually affects the biological behavior of pancreatic cancer cells.

Furin inhibitor D6R affects EMT and Hippo-YAP pathway in pancreatic cancer cells. Next, we examined the effects of D6R on EMT and Hippo-YAP pathway. As shown in Fig. 7A, D6R resulted in the downregulation of N-cadherin, Vimentin and in the upregulation of E-cadherin. Also, D6R resulted in the downregulation of total YAP, p-Mob1 and in the upregulation of p-YAP, Mob1 (Fig. 7B). It is suggested that Furin inhibitor D6R affects EMT and Hippo-YAP pathway in pancreatic cancer cells.

Discussion

We identified that Furin functions as an oncogene in pancreatic cancer cells. Furin promotes proliferation, migration and invasion in pancreatic cancer cells. Importantly, Furin promotes EMT probably via the Hippo-YAP signal pathway.

There is evidence connecting Furin with tumorigenicity of a wide spectrum of human tumors. Furin promotes cell proliferation, tumorigenicity and invasiveness of head and neck squamous cell carcinoma (HNSCC) cells *in vitro* and *in vivo* (11,15). Increasing Furin expression enhanced skin tumor development and growth (13). Inhibition of Furin suppressed the IGF-1 receptor which affected IRS-1 and Akt phosphorylation and showed a significantly reduced ability to form liver metastases *in vivo* (14). In this study, we find that Furin activates oncogenic activities such as the proliferation and the ability of colony formation in pancreatic cancer cells are consistent with previous studies in other tumors and further support its oncogenic potential.

Furthermore, Furin can process a group of notorious molecules involved directly or indirectly in tumor growth and progression, such as vascular endothelial growth factor (VEGF), insulin-like growth factor-1 receptor (IGF-1R), transforming growth factor-β (TGF-β), insulin-like growth factor 2 (IGF-2) and membrane type 1 matrix metalloproteinase (MT1-MMP), which contributes to aggression and metastatic potential of cancer cells (23-27). Accumulating evidence reveal that Furin-processed substrate molecules, including TGF-β and MT1-MMP, are critical for enhancing invasion metastasis and promoting EMT (24,25). Hence, it would be logical to conclude that Furin played a central role in EMT. In the present study, we proved that Furin knockdown resulted in downregulation of N-cadherin and Vimentin, and upregulation of E-cadherin while Furin overexpression remarkably led to the opposite effects. Moreover, the fact that Furin enhanced

the ability of migration and invasion in pancreatic cancer cells, was consistent with previous studies and further supported the significant role of Furin in EMT. As a precursors cleaved by Furin, some investigations showed that N-cadherin rendered a substantial decrease in cell migration (28), whereas other studies linked the NCAD activated by Furin to intestinal tumorigenesis (29). These conflicting data show that Furin function and/or expression in cancer cells varies in a tumor-specific fashion. It is reported that Furin activated NCAD at site RQKR↓DW161 and a second putative PC-processing site RIRSDR↓DK189 located in the first extracellular domain. Cleavage at the second site would inactive NCAD because of the loss of the critical Trp161 (30). We surmised that the activated/inactivated NCAD exist in a balance. In our model, we speculated that Furin may mainly activate NCAD in pancreatic cancer cells. Furthermore, Furin activated precursors implicated in epithelial to mesenchymal transition (31), an ECAD-to-NCAD transition, such as TGF-β (25).

YAP, as a direct downstream effector of the Hippo pathway, is found to play an important role in EMT (20). A previous study confirms that YAP and KRAS converge on the FOS to regulate EMT (21). YAP and TAZ likely together with the co-factor Tead2 provoke the induction of EMT (20,22). These observations suggested that YAP1 interacted with specific transcription factors to regulate EMT. Our results demonstrated conclusively that Furin-induced regulation of EMT accompanied with the alterations of YAP phosphoration level and total YAP protein level. This effect strongly suggests that Furin might have a role in cleaving the upstream proteins of Hippo-YAP pathway. For example, Furin is probably involved in the activation of growth factors and adhesion molecules which may influence the subcellular localization of YAP, such as TGF-β and E-cadherin (28,32). The possible divergence in function of Furin leads to a link with YAP. However, further investigations are required to elucidate the role of Furin processing in the regulation of EMT via Hippo-YAP pathway.

In conclusion, inhibition or depletion of Furin results in a marked reduction of proliferation, migration and invasiveness of pancreatic cancer cells. Our results indicate that Furin promotes EMT in pancreatic cancer cells may be through affecting the Hippo-YAP pathway. Further study is needed on the mechanisms of Furin. Taken together, this information indicates that inhibition or depletion of Furin may be a viable route to ameliorate the malignant phenotype of pancreatic cancer cells.

Acknowledgements

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