

Ran GTPase protein promotes metastasis and invasion in pancreatic cancer by deregulating the expression of AR and CXCR4

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Ran, a member of the RasGTPase family, has been showed to function in diverse cellular processes of cancer. In the present study, we examined the effects of Ran on the cell motility in pancreatic cancer cells and explored the possible mechanism of Ran's function in the metastasis of pancreatic cancer. We demonstrated that the expression of Ran was remarkably higher in lymph node metastases than in primary pancreatic cancer tissues. In the functional studies, stable knockdown of Ran by shRNA could efficiently inhibit the migration and invasion of pancreatic cancer cells both in vitro and in vivo. By PCR array, we analyzed the differences in the expression levels of metastasis-associated genes before and after the downregulation of Ran, and it was showed that the regulation of pancreatic cancer metastasis by Ran was partially mediated by AR and CXCR4. We further confirmed that AR and CXCR4 were significantly decreased following knockdown of Ran. These data indicated that Ran could regulate the invasion and metastasis of pancreatic cancer cells through AR and CXCR4.

Introduction

Pancreatic cancer is one of the most common malignant tumors of the digestive system. It was estimated that 277 000 new cases and 266 000 deaths occurred annually worldwide.¹ Due to the lack of specific symptoms, pancreatic cancer have progressed into advanced stage in 80–85% of the patients when first diagnosed, and local invasion and distant metastasis were the main causes to disease death.^{2,3} Therefore, the 5-y survival rate of pancreatic cancers was less than 5%.⁴

The small GTPase Ran is a Ras-related GTPase that acts in diverse cellular processes. It could regulate the nucleocytoplasmic transport of molecules through the nuclear pore complex^{5,6} and modulate the cell cycle progression through the regulation of the mitotic spindle assembly and cell cycle-related proteins.^{5,7-9} It has been reported that Ran has been found constitutively activated in a wide variety of human tumor tissues and cell lines of renal cell carcinoma, ovarian cancer and colon cancer.¹⁰⁻¹² Ran could regulate a lot of oncogenes, tumor suppressors, and spindle assembly factors, and these facts suggested that Ran might play critical roles in the parthenogenesis of cancers.^{13,14}

In the previous study, we found that knockdown of Ran in pancreatic cancer cell lines could inhibit cell proliferation and

induced cell apoptosis through survivin and cell cycle proteins.⁹ In addition, Ran was frequently reported to be involved in invasion and metastasis of tumor cells.^{10,15,16} To better elucidate the roles of Ran in pancreatic cancer, we herein established cells lines with stable transfection of Ran-shRNA. We found that knockdown of Ran could significantly suppress the invasive and metastatic potential of pancreatic cancer cells, and these effects of Ran were showed to be mediated through AR and CXCR4.

Results

Ran was upregulated in pancreatic cancer tissues with high metastatic potential

The expression of Ran was examined in pancreatic cancer tissues and the matched lymph nodes by immunohistochemistry (Fig. 1). Previously, we found that the expression level of Ran in pancreatic cancer tissues was associated with the histological grade, but not statistically related to gender, age, and cancer differentiation.⁹ In the present study, further analysis showed that increased Ran expression was statistically correlated with the status of distant metastasis (Table 1, $P = 0.006$). Meanwhile, the expression level of Ran was significantly higher in metastatic

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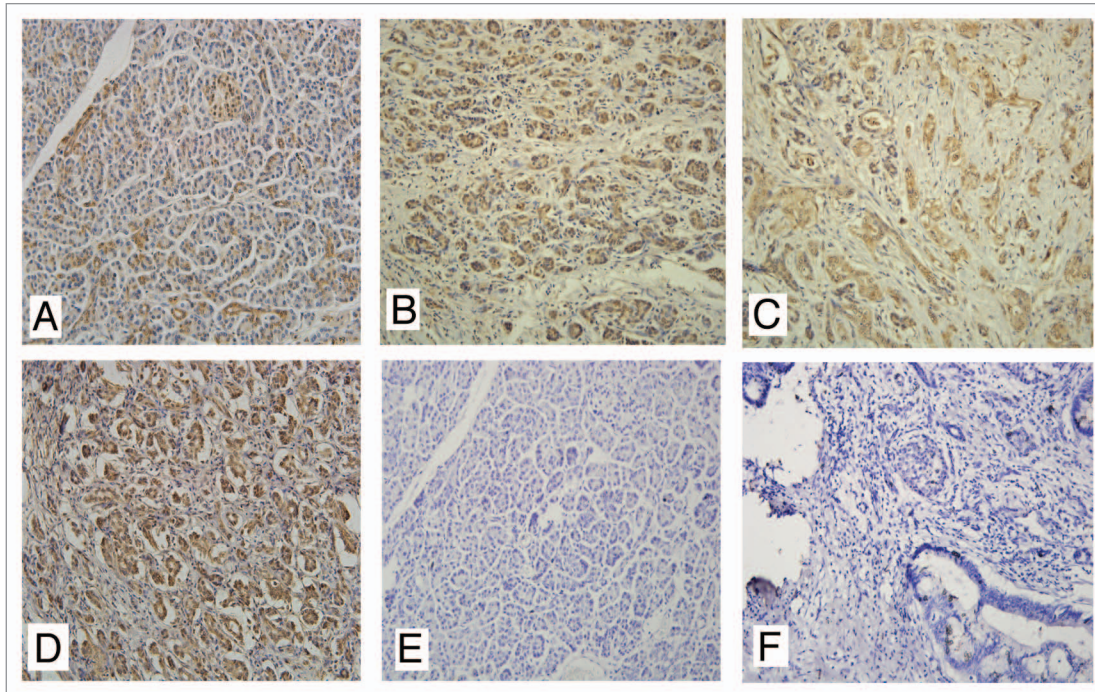


Figure 1. The expression of Ran in pancreatic cancer tissues. (A) Normal pancreatic tissues; (B) Well-differentiated; (C) Poorly-differentiated; (D) Metastatic site in the lymph node; (E and F) Negative controls of normal tissues and pancreatic cancer tissues using PBS instead of primary antibody.

Table 1. Increased Ran expression was statistically correlated with the distant metastasis

Category	Total	Ran GTPase expressions			P value
		+	++	+++	
Distant metastasis	23	2	5	16	0.006 ^a
Non-distant metastasis	39	11	15	13	

^aMann-Whitney U Test, $P < 0.05$.

Table 2. Expression of Ran in pancreatic cancer and matched lymph node metastases

Category	Total	Ran GTPase expressions			P value
		+	++	+++	
Lymph node metastasis	22	4	5	13	0.042 ^a
Primary cancer tissues	22	8	8	6	

^aMann-Whitney U Test, $P < 0.05$.

lymph nodes than that in primary cancer tissues (Table 2, $P = 0.042$).

Ran promoted the migratory, invasive, and metastatic capacities of pancreatic cancer cells

Previously, we constructed the lentivirus containing an Ran-shRNA and transfected it into PANC-1, ASPC-1, and HUVECs cells.⁹ We next explored the effect of Ran on the invasive and migratory abilities of pancreatic cancer cells using an in vitro wound-healing and transwell assays. In wound-healing assay, the downregulation of Ran resulted in the significant inhibition of the wound closure in PANC-1 and ASPC-1 cells. Meanwhile, downregulation of Ran in HUVECs did not affect cell migration

(Fig. 2A and B, $P < 0.05$). In the transwell assay, we observed similar phenomena. Downregulation of Ran could significantly suppress migration and invasion of PANC-1 and ASPC-1 cells (Fig. 2C and D, $P < 0.05$). In addition, a tail vein injection metastasis assay was performed to examine the effects of Ran on the in vivo metastasis of PANC-1 cells. PANC-1/shRan and control cells were respectively injected into the tail veins of female nude mice. The number of the metastatic tumors in the liver was significantly reduced in those received PANC-1/shRan cells compared with those received control cells (Fig. 3, $P < 0.05$). Therefore, the in vitro and in vivo data above illustrated that Ran played significant roles in pancreatic cancer metastasis.

The metastasis-related genes and transcription factors regulated by Ran were analyzed by RT² Profiler™ PCR Array analysis

To explore the mechanism of Ran's function, we employed a real-time PCR array to profile the expression of genes involved in the pancreatic cancer metastasis. The gene expression profiling was analyzed in the PANC-1/shRan and PANC-1/Ctr-shRNA. The tumor metastasis PCR array is designed to represent 84 genes known to be involved in metastasis. The human transcription factors PCR array profiles the expression of 84 genes. Genes with more than a 2-fold increase or decrease were considered to be regulated by Ran. Nine of the 84 genes were down-regulated in the tumor metastasis PCR array (Table 3). Seven of the 84 genes were downregulated in the transcription factors PCR array (Table 4). To further confirm these findings, we examined the expression of these candidate molecules by western blot and real-time PCR array. It was showed that only two of these genes, androgen receptor (AR) and chemokine (C-X-C

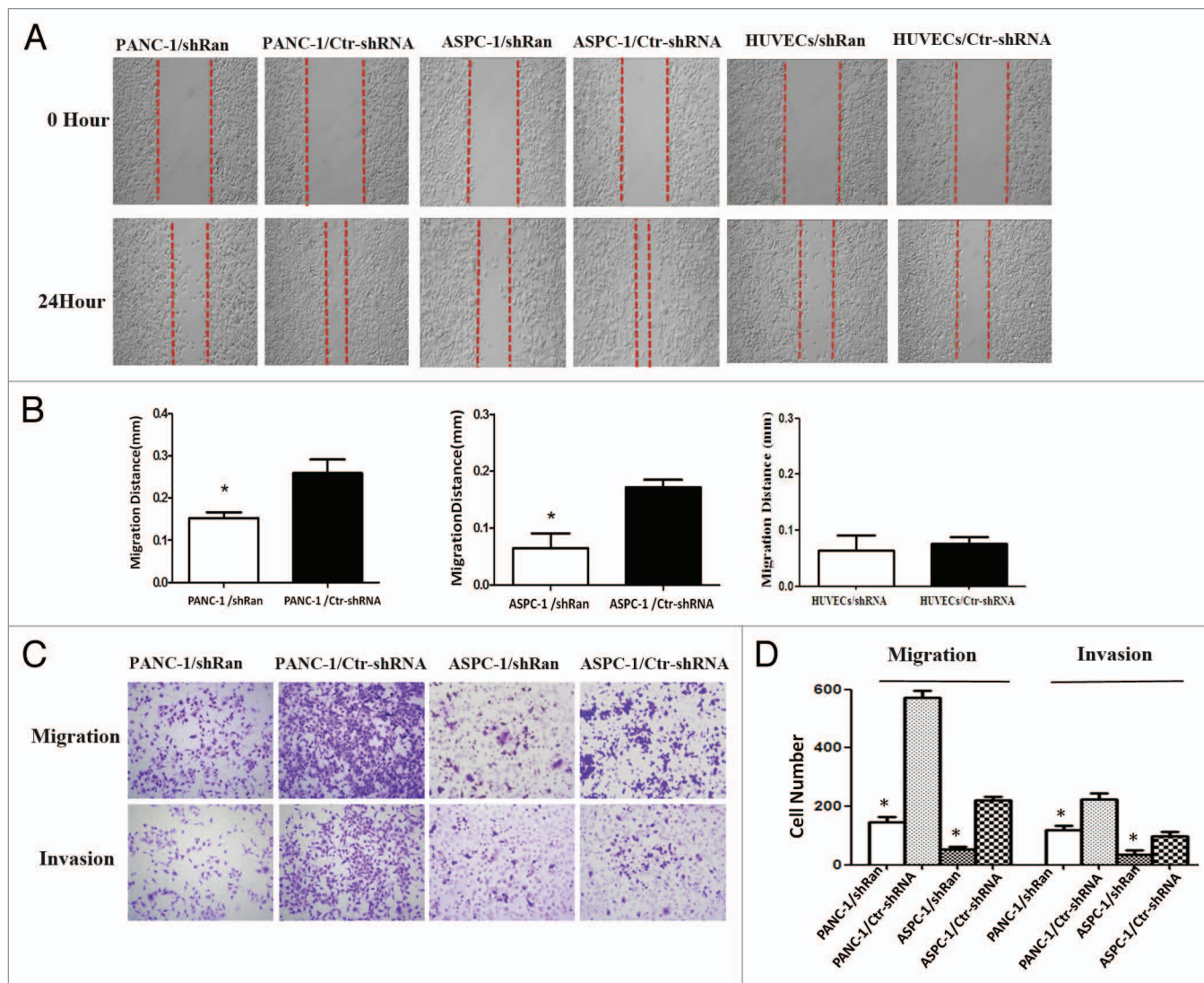


Figure 2. Ran promoted the migratory and invasive abilities of pancreatic cancer cells in vitro. (**A and B**) The migratory ability of the cells was evaluated with a wound-healing assay. The wound widths were measured at time 0 and 24 h after wounding, and the closure ratio was calculated in accordance with the following formula: wound closure (%) = (width 0 h) – width 24 h / width 0 h. These results were then compared with those of the control cells. (**C and D**) The migratory (8 μ m pore polycarbonate filters without matrigel) abilities and invasion (8 μ m pore polycarbonate filters with matrigel) abilities of cells were evaluated by transwell assays. Representative image fields of invasive cells on the membrane are shown. The values represent the mean (SEM) from at least three separate experiments, * $P < 0.05$.

motif) receptor 4 (CXCR4) were significantly decreased by Ran silencing (Fig. 4).

Ectopic expression of AR or CXCR4 could antagonize the anti-metastatic effects caused by Ran knockdown

The expression plasmids of AR or CXCR4 and their corresponding vectors were respectively transfected into PANC-1/shRan cells. The western blot results showed that AR or CXCR4 expression was elevated in PANC-1/shRan cells after the transfected in the former case (Fig. 5A). In the transwell migration assay, we found elevated expression of AR or CXCR4 could significantly raise the number of cells penetrating the chamber membrane compared with the control group (Fig. 5B, $P < 0.05$). This illustrates elevated expression of AR or CXCR4 were able to rescue migration of Ran knockdown cells.

Discussion

One of the major hallmarks of pancreatic cancer is its systemic dissemination and extraordinary local tumor progression at early stage.^{17,18} Therefore, it was urgent to explore the mechanisms of pancreatic cancer metastasis. It was reported that, Ran promoted the metastasis of renal cell cancer and colon cancer.^{10,12} In colon carcinoma, Txl-2b could interact with Ran via its RCC1 to regulating colon cancer cell metastasis.¹⁵ Ran also involved in OPN-mediated invasion and metastatic phenotype.¹⁶ However, the mechanism underlying Ran-mediated invasion and metastatic remains unclear.

In the present study, we found that the overexpression of Ran was significantly correlated with the status of metastasis of

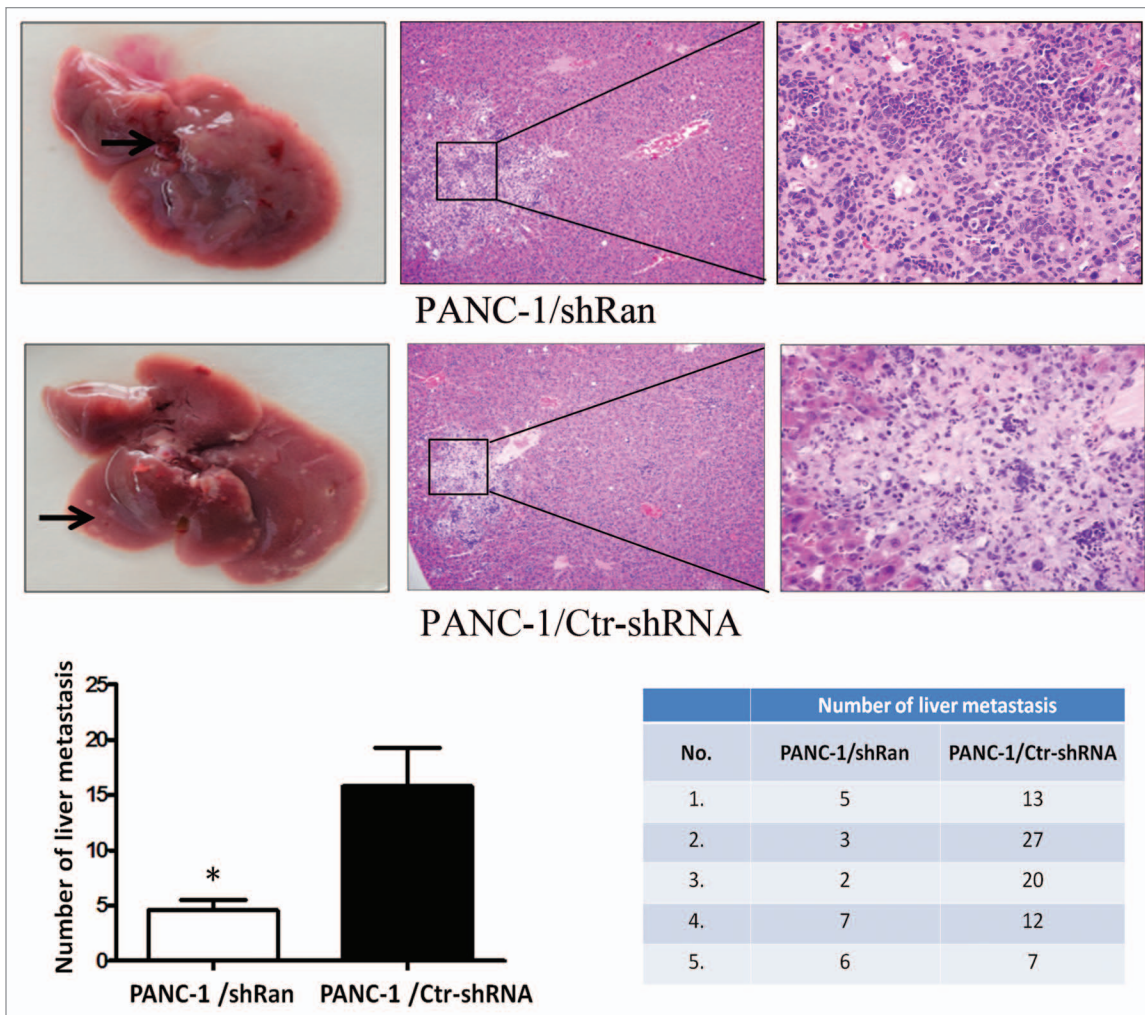


Figure 3. Ran promotes the metastatic ability of pancreatic cancer cells in vivo. Nude mice were tail vein-injected with 1×10^6 PANC-1/ shRan cells and PANC-1/Ctr-shRNA cells. All mice were sacrificed 42 d later after injection of tumor cells and examined for metastatic liver nodules. Representative images of liver tumor metastases. Metastatic loci were identified and marked by arrows; the liver tissues were sectioned serially and then stained with H&E. The number of liver metastasis data are shown in histograms. The values represent the mean (SEM) from at least three separate experiments. $*P < 0.05$.

Table 3. mRNA expression of metastasis-related genes after downregulation of Ran

No.	Gene name	Description	Fold change
1	CCL7	Chemokine (C-C motif) ligand 7	-4.95
2	CXCR4	Chemokine (C-X-C motif) receptor 4	-3.94
3	COL4A2	Collagen, type IV, α 2	-3.77
4	CDH1	Cadherin 1, type 1, E-cadherin (epithelial)	-2.83
5	TIMP3	TIMP metalloproteinase inhibitor 3	-2.66
6	KISS1	KISS-1 metastasis-suppressor	-2.59
7	MMP7	Matrix metalloproteinase 7 (matrilysin, uterine)	-2.27
8	FGFR4	Fibroblast growth factor receptor 4	-2.07
9	KISS1R	KISS1 receptor	-2.02

Table 4. mRNA expression of transcription factors after downregulation of Ran

No.	Gene name	Description	Fold change
1	AR	Androgen receptor	-16.66
2	ATF3	Activating transcription factor 3	-3.49
3	GTF2F1	General transcription factor IIF, polypeptide 1, 74 kDa	-2.60
4	EGR1	Early growth response 1	-2.36
5	HNF4A	Hepatocyte nuclear factor 4, α	-2.24
6	POU2AF1	POU class 2 associating factor 1	-2.15
7	CEBPA	CCAAT/enhancer binding protein (C/EBP), α	-2.06

pancreatic cancer, and Ran was more highly expressed in lymph node metastases than in primary tissues. These data suggested that Ran might serve as an oncogene in pancreatic cancer and played a positive role in cancer metastasis. We used wound-healing, transwell, and tail vein injection assays to observe the migration and invasion of pancreatic cancer cell lines with knockdown of Ran. In this study, we demonstrate that Ran could promote the invasion and metastasis of pancreatic cancer cells. To identify the downstream effectors of Ran contributing to its function, we employed a real-time PCR array to profile the expression of metastasis related genes and transcription factors. Nine of the 84 genes with more than 2-fold alteration were downregulated when Ran was knocked down and 7 of the 84 transcription factors altered >2-fold were downregulated after the knockdown of Ran. However, only AR and CXCR4 were further confirmed by western blotting and qRT-PCR. We also found elevated expression of AR and CXCR4 were able to rescue migration of Ran knockdown cells.

Androgen receptor (AR) is a ligand-dependent transcription factor that belongs to the nuclear receptor superfamily.¹⁹ In prostate cancer AR could activate the matrix metalloproteinase 2 (MMP-2) and MMP-9 or regulate the CCL2/CCR2-STAT3 axis to promote invasion and metastasis of tumors.^{20,21} Recent studies showed that IL-6 also enhanced pancreatic cancer cell migration in the presence of AR.²² On the basis of what we have observed, we speculated that Ran could promote pancreatic cancer cell metastasis by regulating the downstream gene AR. CXCR4 was highly expressed in a variety of tumors and it played an important role in tumor proliferation, infiltration, vascularization, and metastasis.²³ Recently, some researches showed that the SDF-1/CXCR4 axis and CXCL12/CXCR4 biology axis played important roles in the invasion and metastasis of pancreatic cancer cells.^{24,25} The CXCL12/CXCR4-PI3-MAPK-NFκB signaling pathways were essential for the tumor lymphatic metastasis,²⁶ and NFκB was unable to enter the nucleus, probably because of the lack of RanGTP production.²⁷ As described above, we demonstrated that Ran enhanced pancreatic cancer cell invasion and metastasis and these effects were at least partly mediated by AR and CXCR4. The detailed mechanisms responsible for these pathways need to be further studied.

Materials and Methods

Ethics statement

For tissue specimens, all patients were informed consent to use excess pathological specimens for research purposes. The protocols used in this study were approved by the hospital's Protection of Human Subjects Committee. The use of human tissues was approved by the institutional review board of Xijing Hospital and was conformed to the Helsinki Declaration, and to local legislation. Patients offering samples for the study signed informed consent forms. For animal research, all procedures for animal experimentation were performed in accordance with the

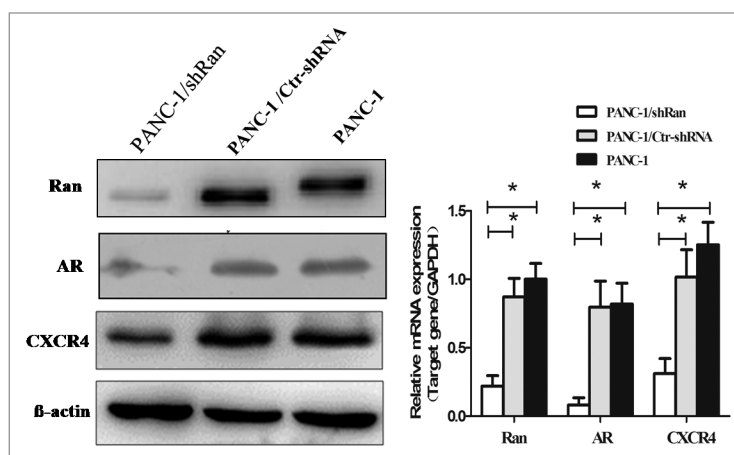


Figure 4. Alteration of gene expression profiling by Ran knockdown in PANC-1 cells. The expression of Ran, AR, and CXCR4 in pancreatic cancer cells was evaluated by western blotting and real-time PCR. β -actin and GAPDH were used as the internal control. * $P < 0.05$.

Institutional Animal Care and Use Committee Guidelines of the Experiment Animal Center of the Fourth Military Medical University. The approval ID for using the animals was No. 12477 by Experiment Animal Center of the Fourth Military Medical University.

Cell culture and tissue collection

The human pancreatic cancer cell lines PANC-1 and ASPC-1; human umbilical vein endothelial cells (HUVECs); stably infected Ran-shRNA cells PANC-1/shRan, ASPC-1/shRan, HUVECs/shRan cells; infected scrambled non-target shRNA cells PANC-1/Ctr-shRNA, ASPC-1/Ctr-shRNA, and HUVECs/Ctr-shRNA were conserved in our institute. All of the cells were cultured in DMEM high glucose medium (Hyclone) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 mg/mL streptomycin (Hyclone). The pancreatic cancer tissues and lymph node metastases specimens were collected from 62 patients who underwent surgery in our hospital between 2009 and 2011. In total, 38 male, 24 female; aged from 30 to 79; 16 cases were well-differentiated, 34 moderately differentiated, and 12 poorly differentiated. All cases of pancreatic cancer and adjacent non-tumor tissues were diagnosed clinically and pathologically.

Immunohistochemistry

Immunohistochemistry analysis was performed as described previously.⁹ Primary antibodies used were polyclonal antibodies against Ran (1:500; Santa Cruz Biotechnology). The ratio of positive cells per specimen was evaluated quantitatively and scored as follows: 0, staining of $\leq 1\%$; 1, staining of 2–25%; 2, staining of 26–50%; 3, staining of 51–75%; and 4, staining of >75% of examined cells. Staining intensity was divided into four groups: 0, no signal; 1, weak staining; 2, moderate staining; and 3, strong staining. A total score of 0–12 was determined as follows: total score = ratio of positively staining cells (score) \times intensity of immunoreactivity (score). Scores were graded as negative (–; score: 0–1), weak (+; score: 2–4), moderate (++; score: 5–8) or strong (+++; score: 9–12).

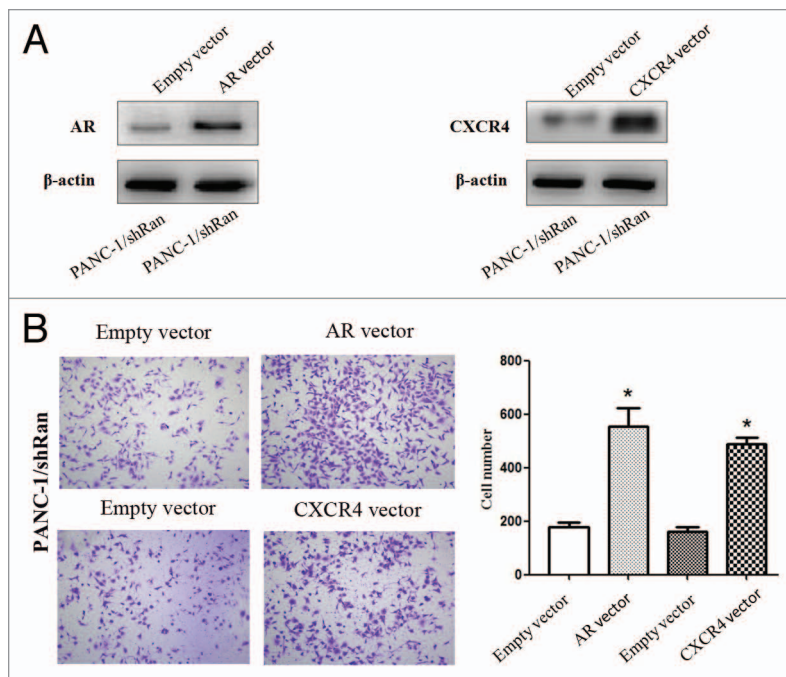


Figure 5. The migratory ability of pancreatic cancer cells after transfection of AR or CXCR4 plasmid into PANC-1/shRan cells. **(A)** Immunoblotting analysis of AR and CXCR4 expression after transfection of plasmid into cells. β -actin was used as a loading control. **(B)** Cell migration was increased following transfection of AR and CXCR4 plasmid into PANC-1/shRan cells. * $P < 0.05$.

Table 5. Quantitative PCR primers

Gene	Forward primer	Reverse primer	T °C
RAN	TACTGGAAAA ACGACCTT	TCCATACAT TGAACCTTA	55
CXCR4	TATCCTGCCT GGTATTGTC	GGAAATCATC AAGCAAGGG	50
AR	GGACAGTACC AGGGACCATG	TCCGTAGTGA CAGCCAGAAG	60
GAPDH	GAAGGTGAAG GTCGGAGT	GAAGATGGTG ATGGGATTTC	60

Western blot analysis

Western blot analysis was performed as described previously.⁹ Primary antibodies used were as follows: polyclonal antibodies against Ran (dilution 1:500, Santa Cruz Biotechnology), monoclonal antibodies against β -actin (dilution 1:2000, Sigma), androgen receptor (AR) (diluted 1:500; Cell Signaling Technology), and CXCR4 (diluted 1:500; Abcam).

Real-time PCR

Real-time PCR analysis was performed as described previously.⁹ The primers used were shown in Table 5. The PCR conditions were as follows: 95 °C for 10 min followed by 40 cycles at 95 °C for 15 s and 1 min annealing. Optimal annealing temperature was determined for each primer pair. All reactions were performed in triplicate.

Wound healing assay

Cells were plated in 6-well plates at a density of 5×10^5 cells per mL and cultured to form confluent cell monolayers. Then cells were scraped with a 10 μ L pipette tip and washed with PBS to remove nonadherent cells, followed by incubation in medium

containing 1% FBS at 37 °C for 24 h. The wound areas were calculated and photographed under a phase contrast microscopy at 200 \times magnifications (Olympus, JP). The migration distance was quantified by subtracting the width of the wounds at each time point from the width of the initial wounds. All experiments were repeated three times.

Transwell assay

The migratory abilities and invasion abilities of cells were evaluated by transwell assays. A total of 4×10^4 cells in 200 μ L serum free medium was added into the transwell inserts with 8 μ m pore polycarbonate filters that were coated with (invasion) or without matrigel (migration) (BD Biosciences). After 24 h (migration) or 72 h (invasion) incubation, non-migrated cells on the top of the membrane were removed with a cotton swab. Cells migrated to the bottom sides of the membrane were fixed and stained. The number of migrated cells on the membrane was then counted in 3 randomly selected fields and taken the picture under a light microscope (Olympus) at 200 \times magnification. All the experiments were performed at least three times.

Tail vein injection

Four-week-old female nude mice obtained from the Shanghai Institute for Biological Sciences. The PANC-1/shRan cells and PANC-1/Ctr-shRNA cells were injected into tail vein with 10^6 cells in 0.2 mL of PBS medium. All mice were sacrificed 42 d later after injection of tumor cells and examined for metastatic liver nodules. The liver tissues from different models were subjected to histopathological analysis.

RT² Profiler™ PCR array

To profile the gene expression associated with tumor metastasis and transcription factors, we employed the Tumor Metastasis RT² Profiler™ PCR Array and the Human Transcription Factors RT² Profiler™ PCR Array (SuperArray). RNA isolation, DNase treatment, and RNA clean-up were performed according to the manufacturer's protocol (Qiagen). The isolated RNA was reverse transcribed into cDNA using the RT² FirstStrand Kit (Invitrogen). PCR was performed using the RT² SYBRGreen qPCR Master Mix (Invitrogen) on an ABI PRISM7900 instrument (Applied Biosystems). Data normalization was based on correcting all Ct values for the average Ct values of several constantly expressed housekeeping genes present on the array. The analysis was completed by ShanghaiKangChen Bio-tech Company.

Construction of expression plasmids and transient transfection

The full-length pcDNA3.1 (Invitrogen) AR or CXCR4 vector was made by cloning of the full-length PCR product of AR or CXCR4 with PFU DNA polymerase (Invitrogen). All the plasmid sequences were confirmed by DNA sequencing. For transient transfection experiments, cells were plated in a 24-well plate at a density of 2×10^5 24 h before transfection. Lipofectamine™2000

(Invitrogen) was used to perform transfection with 2.0 mg pcDNA3.1 AR or CXCR4 vector or 2.0 mg pcDNA3.1 empty vector (as control) according to the manufacturer's protocol. After 24 h, transfected cells were used in transwell migration assays.

Statistical analysis

All data were analyzed using SPSS version 17.0 software (SPSS) including the Mann–Whitney U test and Student *t* test. The minimal level of significance was defined as $P < 0.05$.

References

1. Pandolfi S, Gukovskaya A, Eddekerkaoui M, Dawson D, Eibl G, Lugea A. Epidemiology, risk factors, and the promotion of pancreatic cancer: role of the stellate cell. *J Gastroenterol Hepatol* 2012; 27(Suppl 2):127-34; PMID:22320930; <http://dx.doi.org/10.1111/j.1440-1746.2011.07013.x>
2. Vincent A, Herman J, Schulick R, Hruban RH, Goggins M. Pancreatic cancer. *Lancet* 2011; 378:607-20; PMID:21620466; [http://dx.doi.org/10.1016/S0140-6736\(10\)62307-0](http://dx.doi.org/10.1016/S0140-6736(10)62307-0)
3. Stathis A, Moore MJ. Advanced pancreatic carcinoma: current treatment and future challenges. *Nat Rev Clin Oncol* 2010; 7:163-72; PMID:20101258; <http://dx.doi.org/10.1038/nrclinonc.2009.236>
4. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012; 62:10-29; PMID:22237781; <http://dx.doi.org/10.3322/caac.20138>
5. Dasso M. Running on Ran: nuclear transport and the mitotic spindle. *Cell* 2001; 104:321-4; PMID:11239388; [http://dx.doi.org/10.1016/S0092-8674\(01\)00218-5](http://dx.doi.org/10.1016/S0092-8674(01)00218-5)
6. Güttler T, Görlich D. Ran-dependent nuclear export mediators: a structural perspective. *EMBO J* 2011; 30:3457-74; PMID:21878989; <http://dx.doi.org/10.1038/emboj.2011.287>
7. Dasso M. The Ran GTPase: theme and variations. *Curr Biol* 2002; 12:R502-8; PMID:12176353; [http://dx.doi.org/10.1016/S0960-9822\(02\)00970-3](http://dx.doi.org/10.1016/S0960-9822(02)00970-3)
8. Sazer S, Dasso M. The ran decathlon: multiple roles of Ran. *J Cell Sci* 2000; 113:1111-8; PMID:10704362
9. Deng L, Lu Y, Zhao X, Sun Y, Shi Y, Fan H, Liu C, Zhou J, Nie Y, Wu K, et al. Ran GTPase protein promotes human pancreatic cancer proliferation by deregulating the expression of Survivin and cell cycle proteins. *Biochem Biophys Res Commun* 2013; 440:322-9; PMID:24076388; <http://dx.doi.org/10.1016/j.bbrc.2013.09.079>
10. Abe H, Kamai T, Shirataki H, Oyama T, Arai K, Yoshida K. High expression of Ran GTPase is associated with local invasion and metastasis of human clear cell renal cell carcinoma. *Int J Cancer* 2008; 122:2391-7; PMID:18241036; <http://dx.doi.org/10.1002/ijc.23400>
11. Barrès V, Ouellet V, Lafontaine J, Tonin PN, Provencher DM, Mes-Masson AM. An essential role for Ran GTPase in epithelial ovarian cancer cell survival. *Mol Cancer* 2010; 9:272; PMID:20942967; <http://dx.doi.org/10.1186/1476-4598-9-272>

Disclosure of Potential Conflicts of Interest

The authors have no conflict of interest or financial interest to disclose.

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12. Fan H, Lu Y, Qin H, Zhou Y, Gu Y, Zhou J, Wang X, Fan D. High Ran level is correlated with poor prognosis in patients with colorectal cancer. *Int J Clin Oncol* 2013; 18:856-63; PMID:22956174; <http://dx.doi.org/10.1007/s10147-012-0465-x>
13. Clarke PR, Zhang C. Spatial and temporal coordination of mitosis by Ran GTPase. *Nat Rev Mol Cell Biol* 2008; 9:464-77; PMID:18478030; <http://dx.doi.org/10.1038/nrm2410>
14. Kau TR, Way JC, Silver PA. Nuclear transport and cancer: from mechanism to intervention. *Nat Rev Cancer* 2004; 4:106-17; PMID:14732865; <http://dx.doi.org/10.1038/nrc1274>
15. Lu Y, Zhao X, Li K, Luo G, Nie Y, Shi Y, Zhou Y, Ren G, Feng B, Liu Z, et al. Thioredoxin-like protein 2 is overexpressed in colon cancer and promotes cancer cell metastasis by interaction with ran. *Antioxid Redox Signal* 2013; 19:899-911; PMID:23311631; <http://dx.doi.org/10.1089/ars.2012.4736>
16. Kurisetty VV, Johnston PG, Johnston N, Erwin P, Crowe P, Fernig DG, Campbell FC, Anderson IP, Rudland PS, El-Tanani MK. RAN GTPase is an effector of the invasive/metastatic phenotype induced by osteopontin. *Oncogene* 2008; 27:7139-49; PMID:18794800; <http://dx.doi.org/10.1038/onc.2008.325>
17. Neoptolemos JP, Dunn JA, Stocken DD, Almond J, Link K, Beger H, Bassi C, Falconi M, Pedersoli P, Dervenis C, et al.; European Study Group for Pancreatic Cancer. Adjuvant chemoradiotherapy and chemotherapy in resectable pancreatic cancer: a randomised controlled trial. *Lancet* 2001; 358:1576-85; PMID:11716884; [http://dx.doi.org/10.1016/S0140-6736\(01\)06651-X](http://dx.doi.org/10.1016/S0140-6736(01)06651-X)
18. Keleg S, Büchler P, Ludwig R, Büchler MW, Friess H. Invasion and metastasis in pancreatic cancer. *Mol Cancer* 2003; 2:14; PMID:12605717; <http://dx.doi.org/10.1186/1476-4598-2-14>
19. Mellado B, Codony J, Ribal MJ, Visa L, Gascón P. Molecular biology of androgen-independent prostate cancer: the role of the androgen receptor pathway. *Clin Transl Oncol* 2009; 11:5-10; PMID:19155198; <http://dx.doi.org/10.1007/s12094-009-0304-3>
20. Hara T, Miyazaki H, Lee A, Tran CP, Reiter RE. Androgen receptor and invasion in prostate cancer. *Cancer Res* 2008; 68:1128-35; PMID:18281488; <http://dx.doi.org/10.1158/0008-5472.CAN-07-1929>
21. Izumi K, Fang LY, Mizokami A, Namiki M, Li L, Lin WJ, Chang C. Targeting the androgen receptor with siRNA promotes prostate cancer metastasis through enhanced macrophage recruitment via CCL2/CCR2-induced STAT3 activation. *EMBO Mol Med* 2013; 5:1383-401; PMID:23982944; <http://dx.doi.org/10.1002/emmm.201202367>
22. Okitsu K, Kanda T, Imazeki F, Yonemitsu Y, Ray RB, Chang C, Yokosuka O. Involvement of interleukin-6 and androgen receptor signaling in pancreatic cancer. *Genes Cancer* 2010; 1:859-67; PMID:21779469; <http://dx.doi.org/10.1177/1947601910383417>
23. Balkwill F. The significance of cancer cell expression of the chemokine receptor CXCR4. *Semin Cancer Biol* 2004; 14:171-9; PMID:15246052; <http://dx.doi.org/10.1016/j.semcancer.2003.10.003>
24. Roy I, Zimmerman NP, Mackinnon AC, Tsai S, Evans DB, Dwinell MB. CXCL12 chemokine expression suppresses human pancreatic cancer growth and metastasis. *PLoS One* 2014; 9:e90400; PMID:24594697; <http://dx.doi.org/10.1371/journal.pone.0090400>
25. Li X, Ma Q, Xu Q, Liu H, Lei J, Duan W, Bhat K, Wang F, Wu E, Wang Z. SDF-1/CXCR4 signaling induces pancreatic cancer cell invasion and epithelial-mesenchymal transition in vitro through non-canonical activation of Hedgehog pathway. *Cancer Lett* 2012; 322:169-76; PMID:22450749; <http://dx.doi.org/10.1016/j.canlet.2012.02.035>
26. Lu DY, Tang CH, Yeh WL, Wong KL, Lin CP, Chen YH, Lai CH, Chen YF, Leung YM, Fu WM. SDF-1alpha up-regulates interleukin-6 through CXCR4, PI3K/Akt, ERK, and NF-kappaB-dependent pathway in microglia. *Eur J Pharmacol* 2009; 613:146-54; PMID:19285061; <http://dx.doi.org/10.1016/j.ejphar.2009.03.001>
27. Wong CH, Chan H, Ho CY, Lai SK, Chan KS, Koh CG, Li HY. Apoptotic histone modification inhibits nuclear transport by regulating RCC1. *Nat Cell Biol* 2009; 11:36-45; PMID:19060893; <http://dx.doi.org/10.1038/ncb1810>