PIK3R3 Induces Epithelial-to-Mesenchymal Transition and Promotes Metastasis in Colorectal Cancer

Guihua Wang, Xi Yang, Chuan Li, Xiaonian Cao, Xuelai Luo, and Junbo Hu

Abstract

Class IA PI3K plays an essential role in the invasion and metastasis of cancer. However, the mechanisms and specific functions of PI3K isoforms in tumor invasion and metastasis are not fully understood. We evaluated the role of PIK3R3, a PI3K regulatory subunit encoded by the PIK3R3 gene, in colorectal cancer invasion and metastasis. Clinical specimens and cell lines data show that the expression level of PIK3R3 is associated with colorectal cancer metastasis. Overexpression of PIK3R3 increases tumor migration and invasion in vitro and promotes metastasis of colorectal cancers in vivo. Furthermore, we investigated that the overexpression of PIK3R3 depends on SNAI2, inducing significant epithelial-to-mesenchymal transition (EMT). Downregulation of PIK3R3 reverses this process, which possibly contributes to the enhanced invasive and metastasizing abilities of colorectal cancer cells. In this study, we found that PIK3R3 plays an important role in colorectal cancer metastasis and might be a potential and specific target for therapies against metastatic colorectal cancer.

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Introduction

Metastasis is one of the biggest challenges in cancer therapy and is responsible for 90% of cancer patient deaths (1, 2). Targeting tumor metastasis process will improve the tumor therapy and give patients a longer overall survival (1). Activation of class IA PI3K/AKT signaling is necessary for many events involved in the metastatic pathway including escape of cells from the tumor environment, into and out of the circulation, blockade of apoptosis, activation of proliferation, and activation of angiogenesis (3–5). Targeting the PI3K/AKT pathway by PI3K inhibitors can improve the outcome of cancer, and many PI3K pathway inhibitors have been developed and are being evaluated in preclinical studies and in early clinical trials (3).

Class IA PI3Ks consist of a regulatory subunit and a catalytic subunit (6). Three mammalian genes, PIK3R1, PIK3R2, and PIK3R3, encode p85α (p85α, p55α, and p50α isoforms), p85β, and p55γ (also named p55PIK) regulatory subunits (7). The catalytic isoforms, p110α, p110β, and p110δ, are the products of 3 genes, PIK3CA, PIK3CB, and PIK3CD (8). PI3K signaling is activated in human cancers via several different mechanisms, including direct mutational activation or amplification of genes encoding key components of the PI3K pathway such as PIK3CA and AKT1 or loss of PTEN (3, 9). The role of several PI3K subunits in tumor development and metastasis has been well-investigated, including p110α, p110β, p85α, and p85β (10–12). But even now, the role and function of PIK3R3 in tumor metastasis still remain to be little known.

The PIK3R3, encoded by the PIK3R3 gene, can bind to the p110 catalytic subunit through the iSH2 domain (13). Compared with other regulatory subunits, PIK3R3 contains a unique NH2 terminal. We previously study found that the NH2 terminal of PIK3R3 mediated PIK3R3-specific functions different from other regulatory subunits, via binding to some cell growth key proteins including the retinoblastoma (RB1) protein and proliferating cell nuclear antigen (PCNA; refs. 14, 15). We have reported that the PIK3R3 regulatory subunit is important for cell proliferation and tumor growth and is overexpressed in some cancers (15–17). Zhang and colleagues also reported that the mRNA and protein levels of PIK3R3 in ovarian cancer were elevated compared with those in normal ovarian epithelia and that PIK3R3 knockdown induces ovarian cell apoptosis in vitro (18). Moreover, in our previous studies, we investigated that blocking PIK3R3 inhibits cell-cycle progression, induces cell differentiation, and inhibits tumor angiogenesis (15, 16, 19). Although PIK3R3 is especially important in the tumorigenesis, cell-cycle regulation, cell differentiation, and angiogenesis, in our previous study, blocking PIK3R3 by PIK3R3-specific inhibitor Ad-N24 can prevent colorectal cancer liver metastasis in animal model (17), which showed that PIK3R3 may play an important role in cancer metastasis, but the function and the mechanisms of PIK3R3 in tumor metastasis remain unknown.
Previous observations have indicated that epithelial-to-mesenchymal transition (EMT) is associated with tumor metastasis (20). EMT is a cellular and molecular process, whereby epithelial cells acquire mesenchymal, fibroblast-like properties and demonstrate reduced intercellular adhesion, increased cell motility, and invasion (21, 22).

In this study, we analyze the association of PIK3R3 with colorectal cancer metastasis and find that PIK3R3 induces EMT in colorectal cancer cells and promotes colorectal cancer metastasis in vitro and in vivo. Furthermore, we elucidate the mechanism for PIK3R3 in regulating colorectal cancer metastasis and inducing EMT process. Our data show that PIK3R3 may be also a potential and specific target for therapies against metastatic colorectal cancer, which may also give a new sight for specific PI3K inhibitor development.

Materials and Methods

Cell lines

The colorectal cancer cell lines SW480, SW48, HT-29, HCT-116, LoVo, and SW620 were purchased from the Type Culture Collection cell bank (Chinese Academy of Sciences, Beijing, PR China) in 2012. All cell lines were cultured at 37 °C under a humidified atmosphere containing 5% CO₂ and authenticated by short tandem repeat DNA profiling.

Reagents

DMEM, Leibovitz medium L-15, McCoy 5a medium, DMEM/F12, FBS, and 0.25% trypsin were purchased from HyClone. Antibodies against CDH1 (E-cadherin), CDH2 (N-cadherin), VIM (vimentin), SNAI1 (Snail), and SNAI2 (Slug) were from Cell Signaling Technology, Inc. Antibodies against PIK3R3 and GAPDH were from Santa Cruz Biotechnology. Lentivirus plasmids were purchased from Addgene and other chemical reagents were purchased from Good Time Biotech or Promoter Company.

Plasmid generation and RNAi

Full-length human PIK3R3 was isolated as described previously (23) and cDNA subcloned into pCDNA3 plasmids. Sequence-verified constructs were used in all experiments. The target sequence of the PIK3R3 siRNA was GGA CTT GCT TTA TTA GAA A. The si-h-PIK3R3-sense (5'-GGA CUU GCU UUA UGG GAA A dTdT-3') and si-h-PIK3R3-antisense (3'-dTdT CCU GAA CGA AAU ACC CUU U-5') sequences were purchased from RiboBio, with siRNA used as the control. We cloned the same sequence as an shRNA into the pLKO1.0 plasmid for generation of lentiviruses.

Cell culture and lentivirus infections

The SW480 and SW620 cells were cultured in L-15 medium supplemented with 10% FBS. HT-29 and HCT-116 cells were cultured in McCoy 5a complete medium. LoVo cells were cultured in DMEM/F12 supplemented with 10% FBS. Lentiviruses were produced by co-transfecting HEK293T cells with lentiviral packaging plasmids (2.2 μg of pCMV-dR8.91, 0.25 ng of VSV-G/pMD2G, and 2.5 μg of Hairpin-pLKO.1). The supernatants were applied to LoVo cells at an equivalent titer. After 72 hours, infected cells were selected with puromycin for 5 days, and at least 500 resistant clones from each group were pooled.

Transfection and monoclonal cell screening

Lipofectamine2000 reagent and Opti-MEM (Invitrogen) were used for cell transfection. After transfection, cells were incubated at 37 °C/5% CO₂ for 24 to 48 hours before testing for transgene expression. For stable transfection, cells were transfected using Lipofectamine 2000; at 24 hours posttransfection, 500 μg/mL G418 (Sigma-Aldrich) was applied. After 6 weeks, clones that over-expressed PIK3R3 were identified and collected. Cancer cells were transfected with siRNAs using Lipofectamine RNAiMAX reagent (Invitrogen) and incubated for 48 hours at 37 °C/5% CO₂. The inhibition efficiency of siRNAs on PIK3R3 was determined by Western blotting.

Colonies formation in soft agar

A soft agar assay was used to determine whether PIK3R3 increases anchorage-independent growth of tumor cells. SW480-control and SW480-PIK3R3 cells were incubated in DMEM supplemented with 1% (w/v) Bactoagar, 10% FBS, penicillin (100 U/mL), and streptomycin (100 μg/mL). Cells were then plated on a semisolid medium [DMEM with 0.5% (w/v) Bacto-agar, 10% FBS, and penicillin/streptomycin]. DMEM containing 10% FBS was added to wells after 2 days. After incubation for 14 days at 37 °C/5% CO₂, colonies with a diameters greater than 50 μm were counted under a dissecting microscope at 400× magnification.

Cell motility and migration assays

The effects of PIK3R3 on the migratory and invasive capabilities of LoVo and SW480 cells were determined using a Transwell polycarbonate membrane (3422, Costa) and the Boyden chamber assay. Briefly, 100,000 SW480 cells in 200 μL of serum-free DMEM were added to the upper portion of the inserts, with 50,000 LoVo cells added to each well. The lower chambers were filled with DMEM supplemented with 10% FBS and incubated at 37 °C/5% CO₂ for 48 hours. The Transwell membrane was fixed using 4% paraformaldehyde for 10 minutes at room temperature, washed 3 times with PBS, and then stained with 0.1% (w/v) crystal violet in 20% (v/v) ethanol for 30 minutes. Cells that did not migrate were removed from the inserts using cotton swabs. Migrating cells were counted under a microscope and quantified. For the invasion assay, the membrane was coated with Matrigel (BD Catalog) before cells were seeded.

RNA isolation and quantitative PCR assays

RNA was extracted using TRIzol reagent (Invitrogen), and cDNA was prepared from isolated RNA with a Superscript Reverse Transcriptase Kit (Transgene) following the
boiled for 5 minutes at 95°C such that its final concentration was 1/C2.

We added an appropriate volume of 5/C2 loading buffer to each sample to carry out the Western blotting (Fig. 1C and D). Results from the BrdUrd incorporation assays are presented in Fig. 1E. The DNA incorporation rate in cells overexpressing PIK3R3 was 75% ± 5% compared with 48% ± 4.5% (P = 0.003) in control cells.

### Western blotting

Cells were lysed in NP-40 lysis buffer containing 10 nmol/L of phenylmethylsulfonylfluoride and protease inhibitor. The lysate contents were measured using a bicinchoninic acid assay kit (Thermo). We added an inhibitor. The lysate contents were measured using a nmol/L of phenylmethylsulfonylfluoride and protease inhibitor. Three different protein concentrations were loaded to each well. SDS-PAGE was performed for 2 hours at 150 V. Following SDS-PAGE, proteins were transferred to polyvinylidene difluoride membranes for immunoblotting. Blots were probed with primary antibodies against AKT, p-AKT (s473), PIK3R3, GAPDH, CDH1, CDH2, VIM, SNAI1, and SNAI2. The appropriate secondary antibodies were then used to detect the presence of various proteins in samples.

### Proliferation assays

Proliferation was determined by cell counting and measurement of bromodeoxyuridine (BrdUrd) incorporation. BrdUrd incorporation was conducted using a CASY TT-cell counter FACSCalibur from BD Biosciences according to the manufacturer’s instructions. For BrdUrd measurements, cells were seeded and treated in 6-well plates and then incubated with BrdUrd (final concentration of 10 μmol/L) and added to the culture medium for 30 minutes. The distribution of staining was calculated with ModFit LT software (Becton Dickinson).

### IHC

Sections of paraffin-embedded tissues were dewaxed and then subjected to SDS-PAGE. Following SDS-PAGE, proteins were transferred to polyvinylidene difluoride membranes for immunoblotting. We determined the effects of PIK3R3 on metastasis using a liver metastasis assay. Our study was performed in accordance with institutional guidelines and was approved by the Animal Experimentation Committee of the Central Institute for Experimental Animals, Tongji Hospital (Wuhan, China). We bred female BALB/c NOD mice and used them once they reached 4 to 5 weeks of age. Colon cancer cells were harvested with 0.25% trypsin and suspended in serum-free medium at a concentration of 2 × 10⁵ cells/mL. Experimental liver metastases were generated by an intrasplenic injection of 1 × 10⁶ colon cancer cells (50 μL of cell suspension) and splenectomy. Mice were sacrificed 6 weeks later and liver metastases enumerated immediately without fixation. The numbers of metastatic livers clones in each liver and small foci were compared in the 2 groups.

### Statistical analysis

All data were expressed as the mean ± SEM. Between-group and among-group comparisons were conducted using Student t tests and ANOVA, respectively. Differences were considered statistically significant when P value was less than 0.05.

### Results

#### PIK3R3 enhances cell proliferation and anchorage-independent growth of colorectal cancer cells

Our previous study showed that blocking PIK3R3 by PIK3R3-specific inhibitor Ad-N24 could prevent colorectal cancer liver metastasis in animal model (17). To confirm the relationship of expression levels of PIK3R3 with cancer metastasis, we detected the expression level of PIK3R3 in 5 colorectal cancer cell lines with different metastatic abilities (HCT-116, HT-29, SW480, SW48, SW620, and LoVo). Of these 5 cell lines, SW620 and LoVo were derived from metastases. Data showed that in SW620 and LoVo cells, both mRNA and protein levels of PIK3R3 were higher than in the other 3 that were derived from primary tumors cell lines (Fig. 1A). IHC analysis of 2 colorectal cancer liver metastasis samples showed that the expression level of PIK3R3 was higher in liver metastases (Fig. 1B). Then, we generated PIK3R3-overexpressing and PIK3R3-knockdown cell lines in HT-29 and LoVo cells. Stable PIK3R3 was generated and confirmed by Western blotting (Fig. 1C and D). Results from the BrdUrd incorporation assays are presented in Fig. 1E. The DNA incorporation rate in cells overexpressing PIK3R3 was 75% ± 5% compared with 48% ± 4.5% (P = 0.003) in control cells.
then, we detected the anchorage-independent growth of colorectal cancer cells after changing the expression level of PIK3R3. As shown in Figure 1, the HT-29 cells overexpressing PIK3R3 (97.5 ± 5.7 colonies/well) formed more colonies than control cells (52.5 ± 15.7; P = 0.001; Fig. 1F). PIK3R3 knockdown suppressed colony formation in LoVo cells (48.5 ± 9.3 vs. 73.5 ± 8.7 colonies/well; PIK3R3-knockdown vs. control cells; P = 0.002; Fig. 1G).

**PIK3R3 enhances colorectal cancer cell mobility, migration, and invasion**

The ability of a cancer cell to undergo mobility and migration allowed it to change position within the tissues; this process allows cancer cells to leave from primary tumor (21). To investigate the effects of PIK3R3 on cell mobility and migration, we seeded HT-29 or SW48 cells in modified Boyden chambers and assessed cell migration 48 hours later. Cells with high PIK3R3 levels showed an increase in migration in both HT-29 and SW48 cell lines (Fig. 2A and B). The effects of PIK3R3 on cell migration were also examined via scratch wound assays in HT-29 and SW48 cells. After 24 and 48 hours, cells overexpressing PIK3R3 migrated more distance than vector control cells (Fig. 2C and D).

Cell invasion is an intrinsic cellular pathway whereby cells respond to extracellular stimuli to migrate through and modulate the structure of their extracellular matrix (ECM; ref. 21). In cancer, cell invasion allows neoplastic cells to enter lymphatic and blood vessels for dissemination into the circulation and then undergo metastatic growth in distant organs (24, 25). To investigate the effect...
of PIK3R3 on cell invasion, we used the Transwell assay for detection. The membrane was coated with matrix-gel (BD Catalog) before cells were seeded. Data showed that HT-29 and SW48 cells overexpressing PIK3R3 migrated through membranes exhibiting higher rates than the control cells (Fig. 2E and F).

Knockdown of PIK3R3 inhibits colorectal cancer cell migration and invasion

To confirm the effects of PIK3R3 on colorectal cancer cell migration and invasion, we silenced PIK3R3 by transfecting PIK3R3 siRNA in SW48 and LoVo cells. PIK3R3 silencing significantly decreased LoVo and SW48 cell migration (Fig. 3A and B). In invasion assays, knockdown of PIK3R3 abrogated the invasion compared with control group in both LoVo and SW48 cells (Fig. 3C and D).

**PIK3R3 promotes colorectal cancer growth and metastasis in vivo**

To confirm the effects of PIK3R3 on tumor metastasis in vivo, we injected HT-29 cells overexpressing PIK3R3 into mice and control mice were injected with an empty vector. In the control group, no visible metastases formed in any of the mice; however, we did observe liver metastases in 2 mice from the experimental group (Fig. 4A, Table 1). Metastatic nests were found in all mice from the PIK3R3-overexpressing group and in 3 mice from the control group. Using H&E staining, we found that the metastatic foci in the PIK3R3-overexpressing group were

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Figure 2. PIK3R3 enhances colorectal cancer cell mobility, migration, and invasion. A, in Transwell migration assays, migrated HT-29 cells were stained with crystal violet. PIK3R3 overexpression significantly increased cell migration at 48 hours and quantified data are shown on the right. B, in Transwell migration assays, migrated SW48 cells were stained with crystal violet. PIK3R3 overexpression significantly increased cell migration at 48 hours and quantified data are shown on the right. C, PIK3R3 expression increased colorectal cancer cell migration ability in HT-29 cells. Confluent HT-29 cells cultured in 96-well plates were carefully wounded using sterile pipette tips. The cell migration was photographed under a phase contrast microscope. The distance of cells migrating into the initial wound area was measured and expressed as mean ± SEM; P < 0.05 versus the control group. D, PIK3R3 expression increased colorectal cancer cell migration ability in SW48 cells. The distance of cells migrating into the initial wound area was measured and expressed as mean ± SEM; P < 0.05 versus the control group. E, in Transwell invasion assays, invaded HT-29 cells were stained with crystal violet. PIK3R3 overexpression significantly increased cell invasion at 48 hours and quantified data are shown on the right. F, SW48 Transwell invasion assay is shown and quantified data are shown on the right. The results above were reproduced in 3 independent experiments.
larger than those in the controls (Fig. 4B). Our IHC results showed that PIK3R3 was expressed in metastatic nests and tiny foci of the experimental group; however, this staining was absent when examining the control group (Fig. 4C).

PIK3R3 mediates EMT in colorectal cancer

The EMT is considered to be a significant step in the invasive cascade (20). We showed that the HT-29 cell line stably overexpressing PIK3R3 had a mesenchymal phenotype compared with the control cell line (Fig. 5A). Cells overexpressing PIK3R3 became fibroblast-like in appearance and exhibited decreased levels of the epithelial marker CDH1 but increased the expression levels of the mesenchymal markers CDH2 and VIM (Fig. 5B). SNAI1 and SNAI2 were the key transcription factors in EMT progression. Our data also showed that the expression of SNAI2 was enhanced with PIK3R3 overexpression, whereas there was no significant change in SNAI1 expression (Fig. 5B). To verify these findings at the protein level, molecular analyses involving qPCR assays were conducted on HT-29 cells (Fig. 5C).

To further confirm the relationship between PIK3R3 and EMT, several markers were analyzed by Western blotting in the stable LoVo PIK3R3-knockdown cell line. Silencing of PIK3R3 induced an increase of CDH1 and a decrease of VIM, CDH2, and SNAI2 (Fig. 5D). The qPCR results revealed that CDH1 mRNA levels were elevated, whereas VIM and SNAI2 levels were reduced. SNAI1 and ZEB1 levels remained unaltered (Fig. 5E).

PIK3R3 depends on SNAI2, regulating CDH1 expression and promoting EMT

Signaling pathways involved in EMT are complex, but the hallmark of EMT in cancer is the downregulation of CDH1, which is also thought to be a repressor of invasion and metastasis (22, 26). Several transcription factors have been implicated in the transcriptional repression of CDH1, including zinc finger proteins, SNAI1, SNAI2, ZEB1ZEB1, and so on (26–28). We constructed the CDH1 promoter vector pGL-E-cad promoter and transfected the vector into HT-29 PIK3R3-overexpressing stable cells and LoVo PIK3R3 siRNA stable cells. The data showed that when PIK3R3 was upregulated, the CDH1 was transcriptionally activated, and when PIK3R3 was downregulated, CDH1 was transcriptionally repressed. The same results were obtained in SW48 cells (Fig. 6A). Then, we constructed a mutated CDH1 promoter vector pGL-E-cad promotermut, which contained the SNAI2-binding domain mutation, and finally we transfected the vector into HT-29 PIK3R3-overexpressing stable cells or LoVo PIK3R3 siRNA stable cells. The data showed there were no changes on relative luciferase activation upon changing PIK3R3 expression level (Fig. 6B). To further confirm whether PIK3R3 regulated CDH1 expression, we knocked down SNAI2 in HT-29 PIK3R3-overexpressing cells. The data also showed that CDH1 transcriptional activity was reduced. SNAI1 and ZEB1 levels remained unaltered (Fig. 5E).
reversed (Fig. 6C). We further detected the protein level changes after downregulation of SNAI2 in HT-29 PIK3R3-overexpressing cells, which showed that downregulation of SNAI2 reversed PIK3R3-induced EMT (Fig. 6D).

**Discussion**

It was originally believed that PIK3R3 was a regulatory subunit of PI3K (13, 29). Some studies have showed that PIK3R3 is overexpressed in ovarian cancers and other cells (18, 30). Statistical evidence suggested that PIK3R3 is related to tumor malignance (30). We previously found that the PIK3R3 regulatory subunit was important for cell proliferation and tumor growth via its NH2 terminal binding to RB1 and PCNA (14, 15, 17). Moreover, from our previous study, we can conclude that blocking PIK3R3 by PIK3R3-specific inhibitor Ad-N24 can prevent colorectal cancer liver metastasis in animal models (17), but the role of PIK3R3 in cancer metastasis still remains unclear.

In this study, we found that the PIK3R3 expression level in colorectal cancer tissues was higher than that in normal colon mucosa, especially during metastasis. Two cell lines derived from metastases showed higher PIK3R3 levels, suggesting that PIK3R3 is related to tumorigenesis and metastasis. Further supporting this notion, we generated stable cell lines where PIK3R3 was overexpressed or knocked down. The anchorage-independent growth of colorectal cancer cells was significantly promoted by PIK3R3 overexpression. We observed migration and invasion between cells with different PIK3R3 levels; PIK3R3 induced more cells to infiltrate through the Transwell membrane. These results indicated that PIK3R3 promotes the migration and invasion of colorectal cancer cells.

**Table 1.** Numbers of liver metastases and micrometastases after intrasplenic injection

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<tr>
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<th>Vector</th>
<th>PIK3R3</th>
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<tr>
<td>Plant</td>
<td>8</td>
<td>8</td>
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<tr>
<td>Micrometastasis formation</td>
<td>3</td>
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<td>Metastasis clone formation</td>
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Figure 4. PIK3R3 promotes colorectal cancer growth and metastasis in vivo. In the HT-29 model, PIK3R3 promoted the metastasis and macrometastasis of HT-29 cells. HT-29 cells were injected intrasplenically into female BABL/C NOD mice, and mice were killed 6 weeks later. Tumor islets were observed in livers by staining sections with H&E staining. A, typical views of liver presenting macroscopic metastases at week 6. Arrows, metastatic niche. B, H&E staining of metastatic liver tumor tissues of nude mouse models. C, PIK3R3 staining of metastatic liver tumor tissues of nude mouse models. All experiments were performed and analyzed in triplicate.
Knockdown of PIK3R3 inhibited these processes, demonstrating that these effects are mediated by PIK3R3. The effects of PIK3R3 on colorectal cancer metastasis were studied in a hepatic metastasis mouse model. We saw that PIK3R3 induced the colorectal cancers to form more and larger colonies in the liver, indicating that PIK3R3 promotes colorectal cancer metastasis by positively regulating cell migration and invasion.

EMT had been implicated as the key step in the progression of tumors toward metastasis and invasion (21, 26, 31). Cancer cells undergoing EMT lost proper target recognition and avoided apoptosis (26). Epithelial cells undergoing EMT rearranged their cytoskeletons and developed features of mesenchymal cells, losing cell–cell adhesion structures and polarity (32). In EMT changes, mesenchymal cell–specific marker proteins including CDH2 and VIM were upregulated, and tight- or adherens-junction proteins such as CDH1 were downregulated (20, 26). In this study, we found that upregulating PIK3R3 induced colorectal cancer cells EMT; when we knockdown PIK3R3 in cells, this was reversed. This showed that PIK3R3 promoted the metastasis of colorectal cancer via inducing the EMT process of colorectal cancer cells.

CDH1 was a cell–cell adhesion molecule, which formed epithelial adherent junctions and sequestrated CTNNB1 (33, 34). CDH1-lost expression was often linked to a higher incidence of metastasis and tumor recurrence (34, 35). The loss of CDH1 and the resulting suppression of cell–cell adhesion had been regarded as a crucial step in the EMT process (34). Generally, loss of CDH1 expression in human tumors was most commonly caused by methylation of its promoter or upregulation of the transcriptional repressors SNAI1, SNAI2, and ZEB1, which targeted the CDH1 promoter (33). In our study, we noticed that PIK3R3 overexpression can decrease CDH1 transcriptional...
activation; moreover, we found that PIK3R3 upregulated the transcription of CDH1 mediated by SNAI2 and that SNAI2 was downregulated in cells where PIK3R3 was knocked down. This finding demonstrated that SNAI2 is required for PIK3R3-induced EMT.

Aspirin and other NSAIDs had been reported to affect microenvironment, inflammation, and EMT and were associated with longer survival among patients with mutated PIK3CA colorectal cancer but not among patients with wild-type PIK3CA cancer (36, 37). In this study, our results demonstrate that PIK3R3 is expressed in colorectal cancer colorectal cancer cells and that its activation leads to phenotypic changes associated with tumor progression and metastasis. Although we showed that treatment-targeting PIK3R3 inhibits tumor growth, the roles of PIK3R3 in colorectal cancer metastasis indicate novel functions for this molecule in tumorigenesis. The expression of PIK3R3 in colorectal cancer cells serves as a "predictor" for PIK3R3-targeted antineoplastic regimens. Comprehensive

Figure 6. PIK3R3 depends on SNAI2 regulating CDH1 expression and promoting EMT. A, HT-29–overexpressing PIK3R3 cells, SW48-overexpressing PIK3R3 cells, LoVo-knockdown PIK3R3 cells, and SW48-knockdown PIK3R3 cells were transfected with the CDH1 promoter vector pGL-E-cad promoter. Relative luciferase activation was detected. B, HT-29–overexpressing PIK3R3 cells, SW48-overexpressing PIK3R3 cells, LoVo-knockdown PIK3R3 cells, and SW48-knockdown PIK3R3 cells were transfected with the mutated CDH1 promoter vector pGL-E-cad promoter. Relative luciferase activation was detected. C, inhibition of SNAI2 reverses the CDH1 transcriptional activation caused by PIK3R3. Stably overexpressing PIK3R3 or control HT-29 cells were transfected with si-SNAI2 or si-Con and then the relative luciferase activation of CDH1 promoter was detected. D, inhibition of SNAI2 reverses the EMT caused by PIK3R3. Stably overexpressing PIK3R3 or control HT-29 cells were transfected with si-SNAI2 or si-Con and the SNAI2, CDH1, and VIM were detected by Western blotting. All experiments were performed and analyzed in triplicate.

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understanding of the expression and functions of PIK3R3 in colorectal cancer, and their associated biologic markers, will facilitate the development of better targeted therapies against colorectal cancer. But there are still minor defects in this study; we did not clear the mechanism of PIK3R3 regulating SNAI2 expression, which will be the main work in our future study.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Disclaimer
The authors had no role in study design, data collection and analysis, decision to publish, or preparation of the article.

Authors' Contributions
Conception and design: J. Hu
Development of methodology: G. Wang, X. Yang, X. Cao

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