RAD18 contributes to the migration and invasion of human cervical cancer cells via the interleukin-1β pathway

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Abstract. The E3 ubiquitin ligase RAD18 has been identified as an oncoprotein that exhibits prometastatic properties in various types of cancer; however, the role of RAD18 in cervical cancer (CC) remains unclear. In the present study, it was revealed that increased expression of RAD18 was associated with worse prognosis of patients with CC. Knockdown of endogenous RAD18 suppressed the motility and invasiveness of CC cells, as evaluated by Transwell assays. mRNA sequencing revealed that silencing RAD18 altered the expression profile of proinflammatory mediators, such as interleukin-1β (IL-1β). Furthermore, exogenous IL-1β treatment rescued RAD18-mediated CC cell invasion. These findings indicated an underlying mechanism via which RAD18 promotes CC progression, suggesting that RAD18 may be a potential biomarker and therapeutic target for malignant CC.

Introduction

Cervical cancer (CC) is the fourth most common malignant tumor and a leading cause of cancer-associated mortality in women, accounting for nearly 265,700 deaths globally in 2012 (1) Despite advances in early diagnosis, surgical resection, and radio- and chemotherapy, the overall survival (OS) rate of patients with CC remains low, particularly for patients with metastasis (2). The five-year survival rate of patients decreased from 85 to 50% for patients with lymph node metastasis compared with patients with cervical carcinoma *in situ* (3). The median OS of patients with bone metastasis was only 23 months (4). Consequently, identifying the molecular events involved in the invasion and metastasis of CC is required in order to develop novel therapeutic strategies and improve CC outcomes.

Cellular DNA is continuously exposed to a range of endogenous and exogenous damaging factors, which contribute to genome instability and are thus associated with carcinogenesis or cell death (5). If DNA damage occurs during replication, it will result in replication fork stalling, subsequently leading to the collapse of the replication fork and genome rearrangements (6). To avoid the occurrence of severe aberrations of the genome, cells have evolved a translesion DNA synthesis (TLS) system that employs a series of specialized DNA polymerases with low fidelity to replicate damaged DNA templates and relieve the DNA replication stress (7). Various DNA polymerases (pols), including pol ι, pol κ and pol η, belong to the Y-family, whereas pol ζ is a B-family polymerase (8). Due to the high error-proneness of these TLS polymerases, their recruitment to DNA is tightly regulated via several mechanisms. For example, proliferating cell nuclear antigen (PCNA), as a scaffold for replicative polymerases, is monoubiquitylated at Lys164 in mammalian cells when exposed to various DNA lesion-inducing agents, subsequently stalling the replication fork and mediating the access of TLS polymerases (9). The E3 ubiquitin ligase RAD18 forms a E2-E3 complex with human homolog of RAD6 (hHR6)A/hHR6B and facilitates PCNA monoubiquitination in human cells (10,11).

The TLS system is a double-edged sword in guarding the genome. It has been reported that low-fidelity DNA polymerases are involved in the accumulation of spontaneous and DNA damage-induced genomic mutations, which are recognized as a driving force for malignant transformation (12). As a key regulator of the TLS pathway, RAD18 has been identified as an oncoprotein that exhibits elevated expression in primary

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and metastatic melanoma (13). Increased expression of RAD18 is associated with worse 5-year survival in patients with melanoma (13). Zhou *et al* (14) revealed that RAD18 expression was significantly increased in esophageal squamous cell cancer (ESCC) tissue compared with adjacent normal tissues. A subsequent study by the same group demonstrated that RAD18 expression levels were inversely associated with patient survival, and that RAD18 contributed to ESCC cell proliferation and migration by activating the JNK-matrix metalloproteinase (MMP) pathway (15).

In the present study, the role of RAD18 in CC progression was investigated. It was demonstrated that upregulated expression of RAD18 was associated with worse patient prognosis. RNA sequencing (RNA-Seq) was performed in CC cells following RAD18 knockdown, revealing that silencing RAD18 altered the expression profile of proinflammatory mediators, such as interleukin-1β (IL-1β). Furthermore, exogenous IL-1β treatment rescued RAD18-mediated CC cell invasion following RAD18 knockdown.

Materials and methods

Tissue samples and cell lines. A total of 126 archival paraffin-embedded human CC tissues (29-70 years) were used in the present study. The samples were collected at the Nanjing Medical University Affiliated Suzhou Hospital between January 2010 and November 2011. Informed patient consent was obtained at the time of collection. The clinicopathological features of the patients were staged according to the 8th Union of International Control of Cancer (UICC) classification (16) and presented in Table I. The use of the tissues for the present retrospective study was approved by the Institutional Ethics Committee of the Nanjing Medical University. The human CC cell lines HeLa229 and SiHa were purchased from the Shanghai Cell Bank. Cells were cultured in DMEM (HyClone; GE Healthcare Life Sciences) supplemented with 10% FBS (HyClone; GE Healthcare Life Sciences) and antibiotics (100 μg/ml streptomycin and 100 U/ml penicillin; Corning Inc.), and incubated in humidified atmosphere with 5% CO_2 at 37˚C.

Immunohistochemical analyses. The expression levels of RAD18 in human CC tissues were detected using immunohistochemistry. CC tissues (4-μm sections) were deparaffinized and heat-treated at 100˚C with citrate buffer for antigen retrieval, and then incubated 0.03% hydrogen peroxide for 5 min to block endogenous peroxidases. Following blocking with 2% bovine serum albumin (Sigma-Aldrich; Merck KGaA) at 37˚C for 30 min, the tissues were incubated with anti-RAD18 antibody (1:200; cat. no. ab186835; Abcam) at 37˚C for 2 h. Horseradish peroxidase (HRP)-conjugated anti-rabbit antibody (1:200; cat. no. GP021729; Shanghai GeneTech Co., Ltd.) was then added for 1 h at 37˚C, and color was developed using 3,3'-diaminobenzidine. The tissue sections were counterstained with hematoxylin at room temperature for 5 min and washed briefly in a water bath containing drops of ammonia, prior to dehydration and mounting in Diatex. RAD18 expression levels in three randomly selected fields per sample were scored using a light microscope (magnification, x100; Leica Microsystems GmbH) as previously described (15) and defined by the final score as follows: Low expression (score ≤6, n=81) and high expression (score >6, n=45).

Stable cell line generation. Short hairpin RNA (shRNA) targeting RAD18 (shRAD18: 5'-GCTGTTTATCACGCG AAGA-3') or non-targeting negative control shRNA (shNC: 5'-TTCTCCGAACGTGTCACGT-3') were purchased from Guangzhou RiboBio Co., Ltd. The fragments were inserted into a lentiviral expression vector containing EGFP (pGLVH1/GFP+Puro; Shanghai GenePharma Co., Ltd.) and packaged into viral particles by Shanghai GenePharma Co., Ltd. A total of $2x10^6$ TU of virus particles were mixed with 5 μg/ml polybrene in 500 μl DMEM, then added to $5x10^4$ HeLa229 cells and SiHa cells in 24-well plates. The cells were then selected in medium containing 1 μg/ml Puromycin (Sigma-Aldrich; Merck KGaA) for 1 week. The stable cell lines were validated via western blotting.

Western blot analysis. Cells were harvested and lysed in mammalian protein extraction reagent (Thermo Fisher Scientific, Inc.) containing protease inhibitors (Thermo Fisher Scientific, Inc.) for 10 min on ice. The protein concentrations were measured using a bicinchoninic acid protein assay kits (Thermo Fisher Scientific, Inc.), and 20 μg/lane protein was separated via 10% SDS-PAGE and transferred to PVDF membranes (EMD Millipore). The membranes were blocked with 5% nonfat milk at room temperature for 1 h and incubated with primary antibodies targeting RAD18 (1:1,000; cat. no. ab186835; Abcam) or β-actin (1:1,000; cat. no. AA128; Beyotime Institute of Biotechnology) overnight at 4˚C. The membranes were washed and then incubated with HRP-conjugated anti-rabbit (1:2,000; cat. no. A0208) or anti-mouse secondary antibodies (1:2,000; cat. no. A0216; Beyotime Institute of Biotechnology) at room temperature for 1 h. The protein bands were visualized using High-sig ECL Western Blotting Substrate (Tanon Science and Technology Co., Ltd.).

RNA extraction and reverse transcription-quantitative PCR (RT-qPCR). Total RNA was isolated from cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocols. The concentrations of RNA were determined using a NanoDrop 2000 (Nanodrop Technologies; Thermo Fisher Scientific, Inc.). RNA (500 ng) from each sample was subjected to RT using a RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Inc.) at 60˚C for 5 min, 42˚C for 50 min and 70˚C for 15 min). The mRNA levels of target genes were measured via qPCR using QuantiFast SYBR® Green PCR kit (Qiagen, Inc.) on a StepOne Plus instrument (Applied Biosystems; Thermo Fisher Scientific, Inc.) under the following conditions: 95˚C for 2 min, then 20 or 40 cycles of 95˚C for 15 sec and 60˚C for 60 sec. The PCR products of 20 cycles were separated via electrophoresis on a 1.5% agarose gel and stained with ethidium bromide, then visualized using Bio-Rad ChemiDoc MP (Bio-Rad Laboratories, Inc.) and analyzed using Image Lab 5.2.1 software (Bio-Rad Laboratories, Inc.). The primers are listed in Table II. Relative expression levels were calculated using the $2^{-\Delta\Delta Cq}$ (17) method and normalized to β-actin expression levels.

Table I. Clinicopathological parameters of patients with cervical cancer.

Clinicopathological parameters	Cases
Age (years)	
<50	84
≥50	42
T stage	
T1	69
T2-T4	57
N stage	
N0	101
N1	25
Clinical stage	
I	69
II-IV	57

Table II. Primer sequences for reverse transcription-quantitative PCR analysis.

Genes	Primers
BIRC3	5'-AGTTCATCCGTCAAGTT-3' sense 5'-ATCTCCTGGGCTGTC-3' antisense
CXCL2	5'-TGCGCTGCCAGTGCTTG-3' sense 5'-TGGCTATGACTTCGGTTTGG-3' antisense
CXCL8	5'-GCCTTCCTGATTTCTGC-3' sense 5'-CCCTCTGCACCCAGTT-3' antisense
CXCL16	5'-AGATGGGACGGGACTTGC-3' sense 5'-GGAGCTGGAACCTCGTGTAG-3' antisense
EGR1	5'-CACGAACGCCCTTACGC-3' sense 5'-TTCATCGCTCCTGGCAAAC-3' antisense
ICAM1	5'-TGCAAGAAGATAGCCAACCA-3' sense 5'-GCCAGTTCCACCCGTTC-3' antisense
IL-1α	5'-GCCAAAGTTCCAGACA-3' sense 5'-GGTTGCTACTACCACCA-3' antisense
IL-1β	5'-ACAGTGGCAATGAGGATG-3' sense 5'-TGTAGTGGTGGTCGGAGA-3' antisense
IL-6	5'-CCCTGAGAAAGGAGACA-3' sense 5'-CAAATCTGTTCTGGAGGT-3' antisense
STAT1	5'-TGAACTTACCCAGAATGCC-3' sense 5'-TGCTGCCGAACTTGC-3' antisense
β-actin	5'-AGCGAGCATCCCCCAAAGTT-3' sense 5'-GGGCACGAAGGCTCATCATT-3' antisense

BIRC3, baculoviral IAP repeat containing 3; CXCL, C-X-C motif chemokine ligand; EGR1, early growth response 1; ICAM1, intercellular adhesion molecule 1; IL, interleukin.

RNA-Seq transcriptome analysis. Total RNA from SiHa shNC/shRAD18 was prepared and stored at -80˚C. The RNA quality was determined using a Bioanalyzer 2200 (Agilent Technologies, Inc.), and an RNA integrity number >8.0 was considered acceptable for cDNA library construction. Sequencing and bioinformatic analysis were performed by Vazyme Biotech Co., Ltd. Gene expression quantification was performed using Cufflinks 2.2.1 (http://cole-trapnell-lab.github.io/cufflinks/releases/v2.2.1/). Genes were considered to be significantly differentially expressed between groups when $P<0.05$ and the fold change of expression was >1.5. Gene Ontology (GO) and pathway analyses were performed using the GO (http://geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases (https://www.genome.jp/kegg/kegg1.html).

Cell migration and invasion analysis. Cell migration was analyzed using Transwell chambers (Corning Inc.). Cells [$5x10^4$; treated with/without 10 ng/ml IL-1β (PeproTech, Inc.) for 24 h] were suspended in 200 μl serum-free medium and seeded in the upper chamber. The lower chambers were filled with 600 μl media containing 10% FBS. Following incubation for 24 h, the cells remaining in the upper chambers were scraped off, and the migrating cells were fixed with 3.7% paraformaldehyde and stained with Giemsa (both at room temperature for 5 min), then imaged under a light microscope (magnification, x100; Leica Microsystems GmbH) in three randomly selected fields per sample.

Cell invasion was assessed using Matrigel-coated Transwell chambers (Corning Inc.). A total of $1x10^5$ cells treated with/without IL-1β (10 ng/ml) for 24 h were suspended in 200 μl serum-free media and plated in the upper chamber, which was pre-coated with 60 μl Matrigel (1:6 dilution; BD Biosciences). The lower chambers were filled with 600 μl media containing 10% FBS. Following incubation for 48 h, the cells that invaded through the Matrigel to the lower surface of the filter were fixed with 3.7% paraformaldehyde and stained with Giemsa as aforementioned. The penetration of cells through the membrane was determined under a light microscope (magnification, x100; Leica Microsystems GmbH) in three randomly selected fields per sample.

Statistical analysis. Data are presented as mean ± standard deviation (n=3). Statistical analyses were performed using SPSS 19.0 software (IBM Corp.). Kaplan-Meier analysis and the log-rank test were used to calculate overall survival and disease-free survival of patients. A Cox proportional hazards model was used for multivariate survival analysis. Student's t-test and one-way analysis of variance followed by Tukey's post hoc test were used to compare the differences between two groups and >2 groups, respectively. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Expression of RAD18 is positively associated with poor prognosis in patients with CC. To investigate the potential role of RAD18 in the progression of CC, RAD18 expression was determined in 126 CC tissues that were collected from Nanjing Medical University Affiliated Suzhou Hospital using immunohistochemistry (Fig. 1A). Kaplan–Meier analysis and log-rank tests indicated that patients with higher RAD18 expression

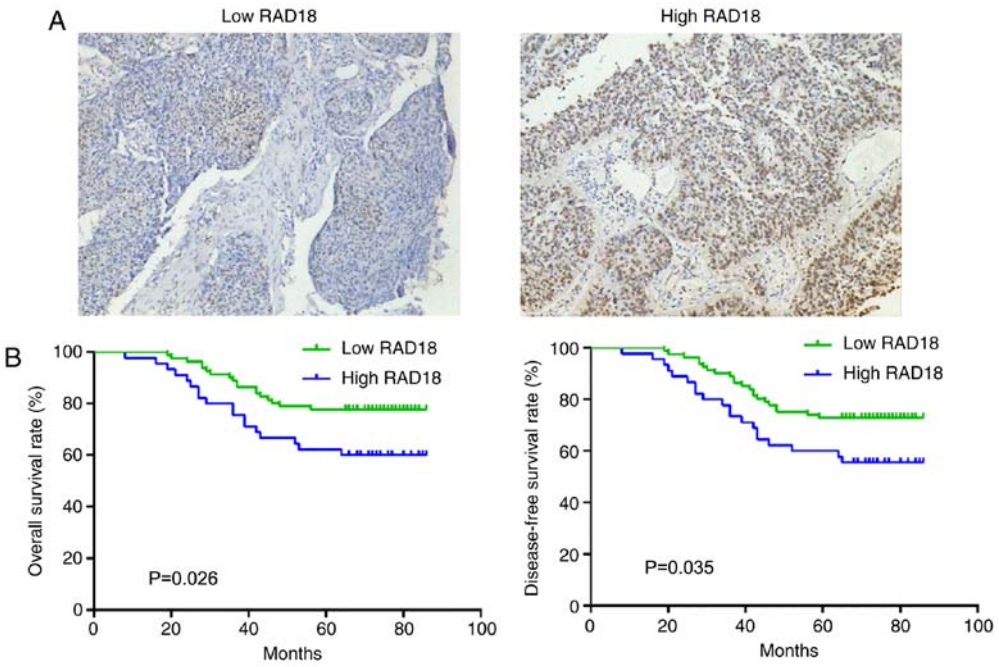

Figure 1. RAD18 expression is positively associated with the poor prognosis of patients with CC. (A) Immunohistochemical staining of RAD18 in 126 CC tissues (magnification, x100; Low expression=81, High expression=45). (B) Kaplan-Meier survival analysis of RAD18 expression and overall (P=0.026) or disease-free survival (P=0.035) of patients with CC. CC, cervical cancer.

(n=45) exhibited significantly shorter OS (P=0.026) and disease-free survival (DFS; P=0.035) compared with patients with low RAD18 expression (n=81; Fig. 1B). Additionally, multivariate Cox regression analysis was performed to determine three of the main OS and DFS-associated factors. It was revealed that RAD18 was an independent prognostic marker for low OS (HR=2.125; 95% CI=1.101-4.101; P=0.025) and DFS (HR=2.031; 95% CI=1.103-3.740; P=0.023; Table III). Collectively, these results indicated that RAD18 may serve an important role in promoting the progression of CC.

RAD18 promotes CC cell migration and invasion in vitro. To investigate how RAD18 regulates CC progression, the stable cell lines that RAD18 knockdown cell models were generated using RAD18-targeting shRNA. Western blotting was performed to detect changes in RAD18 expression (Fig. 2A). The results demonstrated that shRAD18 markedly suppressed RAD18 expression in HeLa229 and SiHa cells, indicating that the RAD18 knockdown cell models were successfully generated. As cell migration and invasion are important properties for cell metastasis (18), the cell lines were subjected to migration and invasion assays. Knockdown of RAD18 significantly inhibited the migratory and invasive abilities of HeLa229 and SiHa cells (Fig. 2B and C). In conclusions, these results suggested a potential role for RAD18 in CC cell invasion.

Differential gene expression in SiHa cells following RAD18 knockdown. To explore the potential mechanisms of the RAD18-mediated migration and invasion of CC cells, RNA-Seq transcriptome analysis was performed in SiHa-shRAD18 and SiHa-shNC cells. The data revealed that 349 genes exhibited a 1.5-fold or higher change in expression in the RAD18 knockdown SiHa cells compared with the control group. Among them, 95 genes were upregulated and 254 genes were down-regulated (Fig. 3A). GO analysis revealed that the differentially expressed genes were involved in biological processes such as inflammatory and immune responses, exhibited molecular functions including chemokine activity, cytokine activity and receptor binding, and were distributed in extracellular space, extracellular regions, and the cell surface and cytoplasm (Fig. 3B). KEGG pathway analysis revealed that RAD18 knockdown altered the expression of genes associated with several key metastasis-associated signaling pathways, including NF-κB (19), nucleotide-binding oligomerization domain (NOD)-like receptor (20) and Toll-like receptor signaling pathways (21) (Fig. 3C).

RAD18 increases the migration and invasion of CC cells by promoting IL-1β expression. The expression levels of 10 representative genes were validated via RT-qPCR analysis, and those of baculoviral IAP repeat containing 3, C-X-C chemokine ligand (CXCL)8, IL-1α, IL-1β and intercellular adhesion molecule 1 were downregulated, consistent with microarray data, whereas STAT1, CXCL16 and CXCL2 were upregulated, the opposite of the microarray data (Fig. 3D). IL-1β is a pleiotropic cytokine that serves major roles in the regulation of immune and inflammatory responses (22). In addition, IL-1 participates in a wide range of processes, including regulation of the growth and metastasis of melanoma, glioma, meningioma, breast, cervical, thyroid and ovarian cancer cells (23). The mRNA and protein levels of IL-1β were further validated in SiHa-shRAD18 and SiHa-shNC cells using RT-qPCR and western blot analyses, with the observed results consistent with the previous analysis (Fig. 4A and B). To determine the potential role of IL-1β in RAD18-induced CC cell invasion, SiHa-shRAD18 cells were exposed to exogenous IL-1β. As presented in Fig. 4C, IL-1β treatment rescued CC cell invasion in SiHa-shRAD18 cells, as determined by Transwell assays.

Table III. Multivariate Cox proportional hazards analysis for candidate variables.

	Overall survival			Disease-free survival		
Variables	HR	95% CI	P-value	HR	95% CI	P-value
T stage	12.646	4.210-37.983	<0.001	9.209	3.846-22.050	<0.001
N stage	1.322	0.659-2.652	0.433	1.091	0.559-2.129	0.799
RAD18 (high vs. low)	2.125	1.101-4.101	0.025	2.031	1.103-3.740	0.023

CI, confidence interval; HR, hazard ratio.

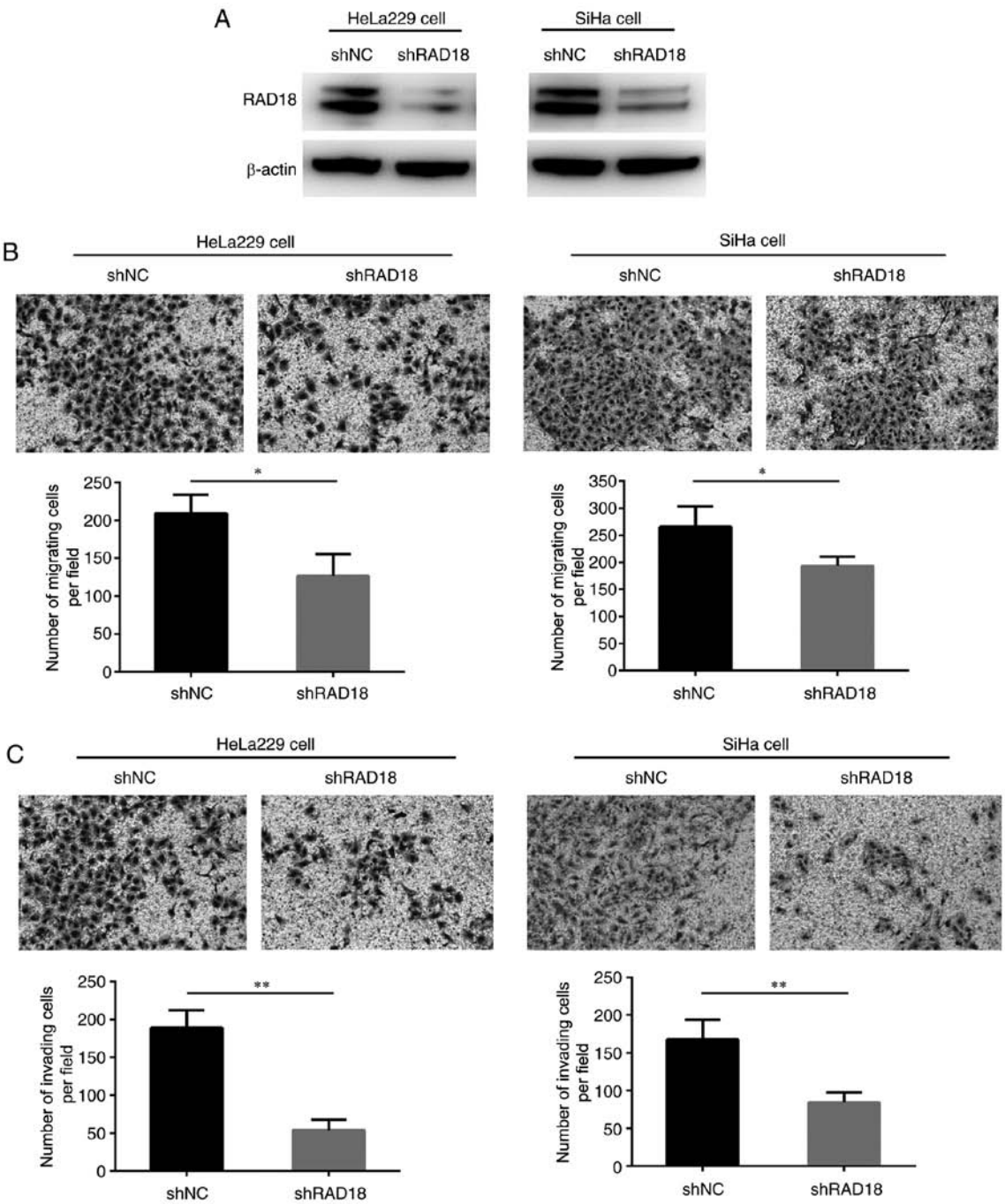

Figure 2. Knockdown of RAD18 inhibits CC cell migration and invasion *in vitro*. (A) Western blotting was performed to detect the RAD18 protein levels in CC cells transfected with a RAD18 shRNA lentivirus (n=3). (B) Transwell assays were performed to evaluate cell migration. RAD18 knockdown significantly reduced CC cell migration (magnification x100). N=3. *P<0.05. (C) Matrigel Transwell assays were performed to evaluate cell invasion. RAD18 knockdown significantly suppressed the invasive abilities of cervical cancer cells (magnification x100). N=3. **P<0.01. CC, cervical cancer; sh, short hairpin RNA.

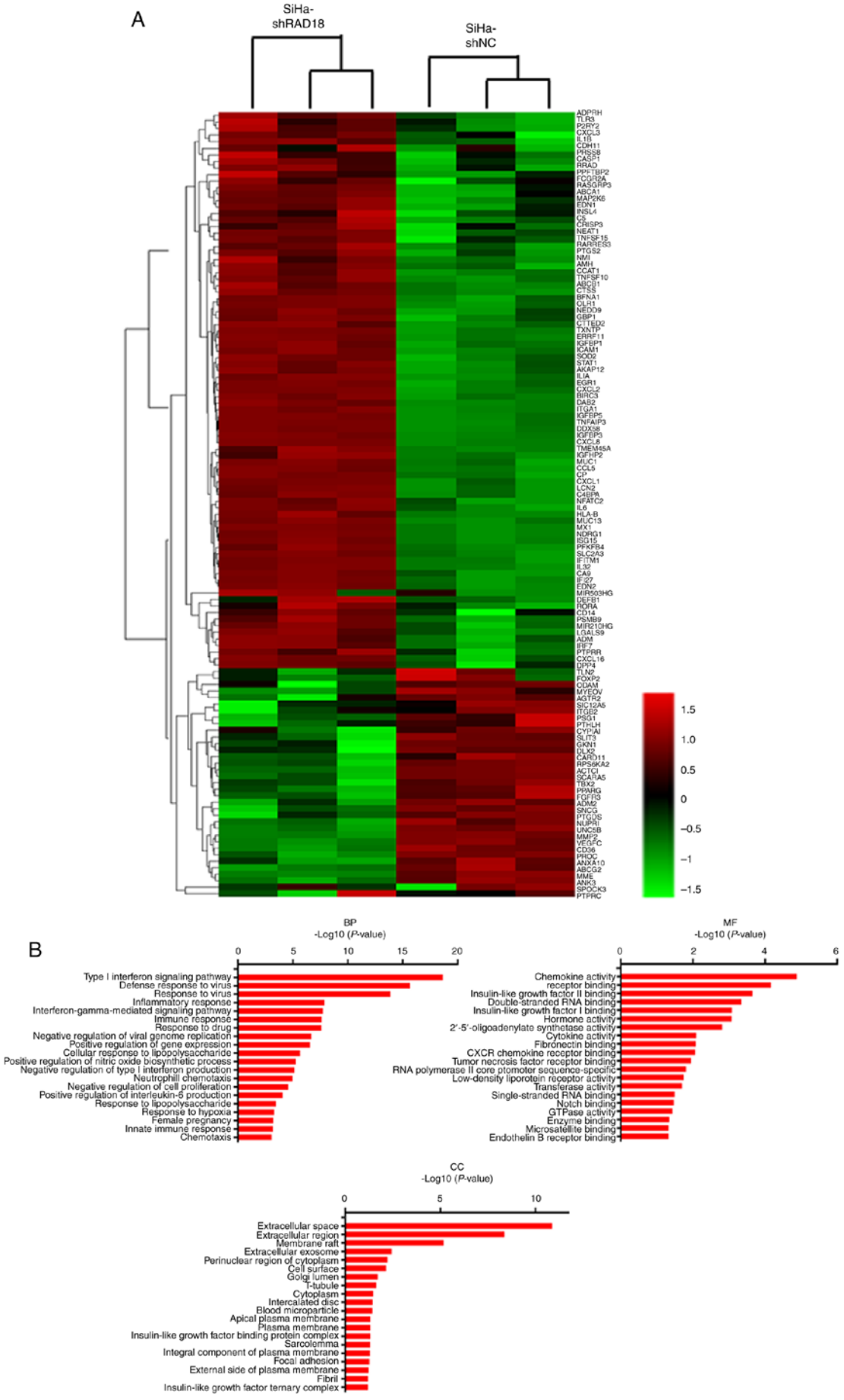

Figure 3. RAD18 regulatory network analysis. Differential gene expression in SiHa-shRAD18 cells. RNA isolated from RAD18 knockdown cells and control cells were analyzed by RNA sequencing. (A) Heat map of differentially expressed transcripts. (B) Gene Ontology based on all identified transcripts.

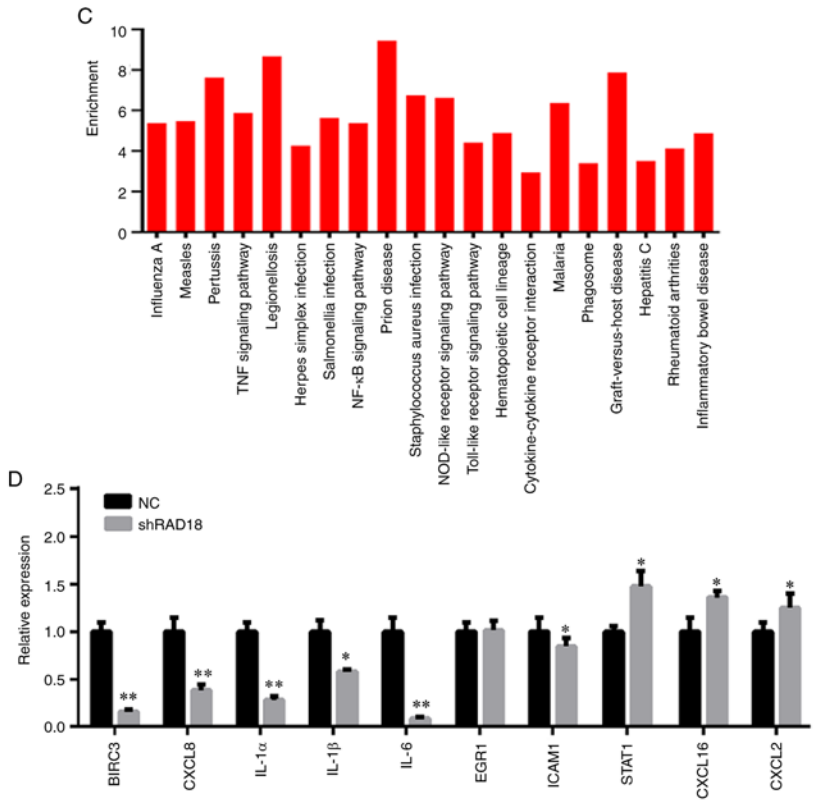

Figure 3. Continued. (C) Pathway analysis, based on all identified transcripts. (D) Differentially expressed transcripts were validated via reverse transcription-quantitative PCR analysis in SiHa-shRAD18 cells. N=3. *P<0.05, **P<0.01. BP, biological process; CC, cellular component; MF, molecular function; sh, short hairpin RNA.

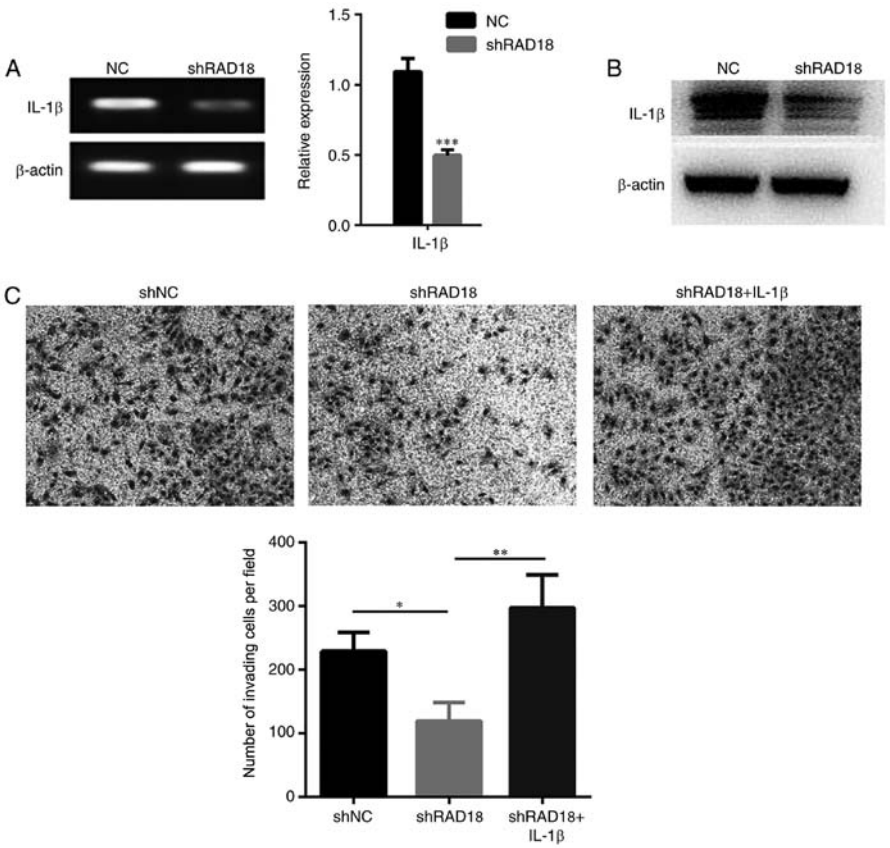

Figure 4. Exogenous IL-1β treatment rescues the invasion of cervical cancer cells following RAD18 knockdown. (A) Reverse transcription-quantitative PCR analysis was performed to determine IL-1β mRNA expression. The products of PCR were separated by electrophoresis on an agarose gel and stained with ethidium bromide to visualize the bands. N=3. ***P<0.001 (B) Western blot analysis were performed to validate the expression of IL-1β in SiHa-shRAD18 cells. (C) SiHa-shRAD18 cells were pretreated with IL-1β (10 ng/ml) for 24 h, and Transwell assays was performed to determine cell invasion (magnification x100). N=3. *P<0.05, **P<0.01. sh, short hairpin RNA.

Collectively, these results revealed that RAD18 enhanced the invasive abilities of CC cells, at least partially by regulating the expression of IL-1β.

Discussion

In the present study, the expression of RAD18 was analyzed in 126 CC samples, and it was found that the expression levels of RAD18 were negatively associated with the prognosis of patients with CC. High RAD18 expression was associated with reduced 5-year OS and DFS in patients, from 76.5 and 72.8%, respectively, for patients with low expression to 62.2 and 60%, respectively, for patients with high expression. Furthermore, multivariate Cox regression analysis revealed that high RAD18 expression may be an independent prognostic indicator of survival in patients with CC. Metastasis is the leading reason for the mortality of patients with cancer (24). The 5-year survival rate of patients with CC reduced from 80-95% in patients without lymph node metastases to 50-65% in patients with lymph nodes metastases (25). Using CC cell lines, it was demonstrated that knockdown of RAD18 significantly suppressed the invasiveness of CC cells *in vitro* (Fig. 2). Consistent with the present study, Shi *et al* (26) reported a positive association between positive expression of DNA pol ζ, another TLS member, and the depth of the cervical stromal invasion of CC cells. They also observed that pol ζ-positive expression was highly associated with the poor prognosis of patients with cervical squamous cell carcinoma that underwent adjuvant concurrent chemoradiation therapy following radical surgery treatment, indicating that pol ζ may be a potential biomarker of chemoradioresistance in CC (26). Yang *et al* (27) reported that protein reversionless 3-like (REV3L), the catalytic subunit of pol ζ, also exhibited elevated expression in CC tissues compared with adjacent normal tissues. Furthermore, they found that overexpression of REV3L promoted chemoresistance in CC cells by inhibiting apoptosis.

To further investigate the molecular mechanisms underlying RAD18-mediated CC invasion, gene expression analysis was performed using an mRNA microarray, which identified 95 upregulated and 254 downregulated genes. Pathway analysis revealed that these genes were enriched in several metastasis-associated signaling pathways, including NF-κB, NOD-like receptor and Toll-like receptor signal pathways (20). Although infection with human papilloma virus is considered to be the major event leading to CC, viral infection alone is not sufficient for CC progression (28). A number of studies reported that chronic inflammation serves a crucial role in the development of various types of cancer, including colon cancer and CC (29,30). As an important inflammatory cytokine, IL-1α is produced by numerous types of cells, including macrophages, fibroblasts and cervical epithelium (31). Emerging evidence suggests that the secretion of IL-1α within tumor microenvironments promotes the expression of a large number of growth factors, including IL-6 and vascular endothelial growth factor, in addition to the metastasis-associated MMPs (32,33). MMPs are a group of enzyme that enhance the progression of the epithelial-to-mesenchymal transition (34,35) by degrading various components of extracellular matrix proteins. Therefore, IL-1 has been proposed to facilitate the invasion and metastasis of cancers. Song *et al* (36) reported that IL-1 expression was associated with lymph node metastasis and stromal invasion in CC. IL-1β is a member of interleukin 1 family cytokines; the activation of IL-1β is involved in a variety of cellular process, including cell proliferation, differentiation and apoptosis (37). In the present study, it was demonstrated that RAD18 regulated the expression of IL-1β in CC. Furthermore, it was revealed that the exogenous IL-1β treatment can promote invasion in CC cells following RAD18 knockdown.

How RAD18 regulates the transcription of IL-1β remains unclear; however, it has been reported that the transcriptional factor NF-κB can transactivate IL-1α and IL-1β following influenza virus infection (38). Zou *et al* (15) reported that RAD18 activated the JNK signal pathway, which is involved in the regulation of NF-κB transcriptional activity. The potential interaction of RAD18 with the JNK and NF-κB pathways requires further investigation.

Collectively, the present findings demonstrated that elevated RAD18 expression was positively associated with the poor prognosis of patients with CC. RAD18 promoted CC cell migration and invasion by upregulating IL-1β. These data indicated that RAD18 may be a potential biomarker and/or therapeutic target in malignant CC.

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Availability of data and materials

All data used or analyzed during the current study are included in this published article.

Authors' contributions

PL and SZ performed the majority of experiments and drafted the manuscript. JZ and ZS designed the research and assisted in drafting the manuscript. CH, AG and SH contributed to data collection and interpretation, and critically reviewed the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Institutional Ethics Committee of the Nanjing Medical University**.** Informed

consent was obtained from patients at the time of sample collection.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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