

REV3L, the catalytic subunit of DNA polymerase ζ , is involved in the progression and chemoresistance of esophageal squamous cell carcinoma

XIAOZHONG ZHU^{1*}, SHITAO ZOU^{2*}, JUNDONG ZHOU^{1,2*}, HONGSHENG ZHU³, SHUYU ZHANG⁴, ZENG FU SHANG⁴, WEI-QUN DING⁵, JINCHANG WU¹ and YIHONG CHEN⁶

¹Department of Radio-Oncology, Nanjing Medical University Affiliated Suzhou Hospital; ²Suzhou Cancer Center Core Laboratory;

³Department of Clinical Laboratory, Nanjing Medical University Affiliated Suzhou Hospital, Suzhou, Jiangsu 215001; ⁴School of Radiation Medicine and Protection, Medical College of Soochow University, Suzhou, Jiangsu 215123, P.R. China; ⁵Department of Pathology, University of Oklahoma Health Science Center, Oklahoma City, OK 73104, USA; ⁶Department of Radio-Oncology, The First Affiliated Hospital of Wannan Medical College, Wuhu, Anhui 241001, P.R. China

Received September 28, 2015; Accepted November 5, 2015

DOI: 10.3892/or.2016.4549

Abstract. Protein reversionless 3-like (REV3L), the catalytic subunit of DNA polymerase (pol) ζ , is well known to participate in error-prone translesion synthesis (TLS) with less stringent and lower processivity. Recent evidence has demonstrated that REV3L is involved in carcinogenesis and tumor progression. However, the function of REV3L remains unclear in esophageal squamous cell carcinoma (ESCC). In the present study, we examined REV3L expression in ESCC tissues and its association with clinicopathological parameters. REV3L was found to be significantly upregulated and correlated with lymph node metastasis and clinical stage in the ESCC tissues. To further investigate the potential role of REV3L in esophageal cancer, stable ESCC cell lines with suppression of REV3L expression were established. Downregulation of REV3L expression led to a decrease in cell proliferation and invasive capacity partly through suppression of cyclin D1 and survivin expression, and an increase in cellular sensitivity to 5-fluorouracil (5-FU) by induction of G1 phase arrest and apoptosis. Therefore, REV3L plays an important role in ESCC progression and chemore-

sistance, and is a potential diagnostic marker and therapeutic target for ESCC.

Introduction

Esophageal squamous cell carcinoma (ESCC) is one of the most fatal malignancies with a relatively high incidence worldwide (1,2). Clinical evidence indicates that the etiology of ESCC includes multiple factors containing both environmental and genetic determinants (3). Although a high number of genetic and epigenetic alterations has been reported in ESCC, molecular markers for early diagnosis and prognosis remain to be discovered. The majority of ESCC deaths are due to invasive disease; therefore, identification of novel genes involved in the tumorigenesis and development of ESCC could contribute to improving the outcome of this disease.

Translesion DNA synthesis (TLS) is one type of DNA damage tolerance mechanisms that allows continuing DNA synthesis even in the presence of DNA damage (4,5). Protein reversionless 3-like (Rev3L), the catalytic subunit of the DNA polymerase (pol) ζ , is well known to participate in error-prone TLS with less stringent and lower processivity (4). Rev3L maintains genomic integrity by inserting a substitute nucleotide in the opposite DNA adducts, which increases the mutation rate and contributes to carcinogenesis (6). *Rev3L* gene polymorphisms have been correlated with the risk of lung and breast cancer (7,8). Pol ζ was reported to promote tumor formation and is significantly associated with poor progression in cervical cancer (9,10). Thus, Rev3L may play an important role in carcinogenesis and tumor progression.

The *REV3L* gene appears to be ubiquitously expressed in normal and tumor tissues, while its expression pattern remains contentious in different cancer tissues (11,12). *REV3L* expression was found to be downregulated in colon, lung, gastric and renal cancer tissues as compared to adjacent tissues (13), whereas it was found to be upregulated in human glioma tissues (14). Our

Correspondence to: Professor Jinchang Wu, Department of Radio-Oncology, Nanjing Medical University Affiliated Suzhou Hospital, 16 West Baita Road, Suzhou, Jiangsu 215001, P.R. China
E-mail: wjinchang@sina.com

Dr Yihong Chen, Department of Radio-Oncology, The First Affiliated Hospital of Wannan Medical College, 2 West Zheshan Road, Wuhu, Anhui 241001, P.R. China
E-mail: cheniyihongf01@163.com

*Contributed equally

Key words: REV3L, esophageal squamous cell carcinoma, progression, chemoresistance, 5-fluorouracil

previous study indicated that the mRNA level of Rev3L was significantly elevated in ESCC when compared with the level in normal controls (15), yet its role in ESCC development is unclear. In the present study, we analyzed the expression of REV3L in ESCC and adjacent normal tissues, as well as its association with clinicopathological parameters. Furthermore, we elucidated the role of REV3L in ESCC progression and drug-resistance using well-established ESCC cell lines.

Materials and methods

Tissue samples. Human ESCC and adjacent tissues used in the present study were obtained from the Nanjing Medical University Affiliated Suzhou Hospital (Jiangsu, China). These tissues were resected from patients before chemotherapy and radiation therapy, and were immediately frozen and stored at -80°C for reverse transcriptase (RT)-PCR and real-time PCR analysis. All patients gave signed, informed consent for their tissues to be used for scientific research. The study was approved by the Institutional Ethics Committee of Nanjing Medical University Affiliated Suzhou Hospital.

Cell culture. Human esophageal cancer cell lines ECA-109 and TE-1 were obtained from the Shanghai Cell Bank (Shanghai, China). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (both from HyClone) in a humidified atmosphere, with 5% CO₂ at 37°C.

Generation of stable cell lines. The shRNA construct against REV3L (shREV3L) and the control plasmid (shNC) were purchased from Shanghai GenePharma (Shanghai, China). The shREV3L construct or control vector was transfected into ECA-109 and TE-1 cells using Lipofectamine 2000 (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's protocols. Stable clones were selected in medium containing G418 (500 µg/ml) (Sigma-Aldrich, St. Louis, MO, USA) for 4 weeks. Individual clones were isolated and expanded for further characterization.

Reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted using TRIzol reagent (Invitrogen Life Technologies) following the manufacturer's instructions. For reverse transcription, 1.0 µg of RNA/sample was reverse transcribed using an oligo(dT)₁₂ primer and SuperScript II reverse transcriptase (Invitrogen Life Technologies). The primers for REV3L and β-actin were as follows: forward, 5'-C GCGTCAGTTGGGACTTAAG-3' and reverse, 5'-ACTATCG CCAACCTCAATGC-3' (REV3L); forward, 5'-AGCGAGCA TCCCCAAAGTT-3' and reverse, 5'-GGGCACGAAGGCT CATCATT-3' (β-actin). RT-PCR was conducted using the 2X Taq PCR Master Mix (Takara Bio, Dalian, China) according to the manufacturer's instructions. The PCR products were separated by electrophoresis on 1.5% agarose gels, and the quantification of each band was performed using Quantity One software (Bio-Rad).

Immunofluorescence. Cells were fixed with 4% paraformaldehyde, washed with phosphate-buffered saline (PBS) and permeabilized with 1% Triton X-100 in PBS. Samples were

then blocked with blocking solution (PBS containing 10% BSA and 1% Triton X-100) and incubated overnight at 4°C with the REV3L antibody (1:1,000; Abnova, Taiwan, China). After washing, the Rhodamine-labeled goat anti-rabbit antibody (1:100; KPL, Gaithersburg, MD, USA) was added and incubated for 1 h at room temperature. Nuclear counterstaining was performed using 4',6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich). Cells were visualized under a fluorescence microscope (Eclipse 80i; Nikon, Tokyo, Japan).

Cell proliferation and cell viability assays. Cell proliferation was measured using Cell Counting Kit-8 (CCK-8) (Beyotime Biotechnology, China). A total of 2x10³ cells/well were seeded in triplicate into 96-well plates for 1-6 days. Then, 10 µl of CCK-8 solution was added to each well, and incubated for 2 h at 37°C. The optical density (OD) was measured at 450 nm with a microplate reader (Thermo, Waltham, MA, USA). The viability index was calculated as the experimental OD value/the control OD value. For 5-fluorouracil (5-FU) treatment experiments, cells (5x10³) were initially plated in quadruplicate into 96-well plates. Twenty-four hours later, the cells were treated with various concentrations of 5-FU for 48 h. Cell viability was measured as described above. Three independent experiments were performed.

Cell invasion assay. The invasive potential of cells was evaluated using 24-well Transwell inserts (Costar, New York, NY, USA). The inserts were pre-coated with 40 µl Matrigel (1:4 dilution; BD Biosciences, San Jose, CA, USA). Then, 1x10⁵ cells (200 µl) in serum-free medium were added to the upper chambers. The lower chambers were filled with medium that contained 10% FBS. After incubation for 24 h, the inserts were fixed with 3.7% paraformaldehyde/PBS and stained with 2% crystal violet. The cells remaining in the upper chambers were scraped off, and the invading cells in the lower chambers were photographed under a microscope.

Flow cytometric analysis of cell cycle and apoptosis. A total of 5x10⁵ cells/well were seeded in triplicate into 6-well plates. Twenty-four hours later, the cells were treated with 50 and 100 µM 5-FU for 24 or 48 h. Cell cycle analysis was performed using the propidium iodide (PI) single staining method. Cells were collected and fixed with 70% ice-cold ethanol at 4°C overnight. Then, the cells were washed with PBS and stained with PI (100 µg/ml) for 30 min in the dark before analysis. The cell cycle profiles were assayed using a FACScan flow cytometry, and data were analyzed using MultiCycle software (both from BD Biosciences). Cell apoptosis was measured using the PE Annexin V apoptosis detection kit (BD Biosciences). After 48 h of culture, the cells were harvested and processed as described in the Annexin V-PE apoptosis detection kit and analyzed on a FACScan flow cytometry.

Western blot analysis. Western blotting was carried out on whole-cell extracts which were lysed in RIPA lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100 and 1% sodium deoxycholate, 0.1% SDS) that contained protease inhibitors for 20 min at 4°C. The proteins were separated by 10% SDS-PAGE and transferred to PVDF membranes (Millipore, Bedford, MA, USA). After blocking with 5%

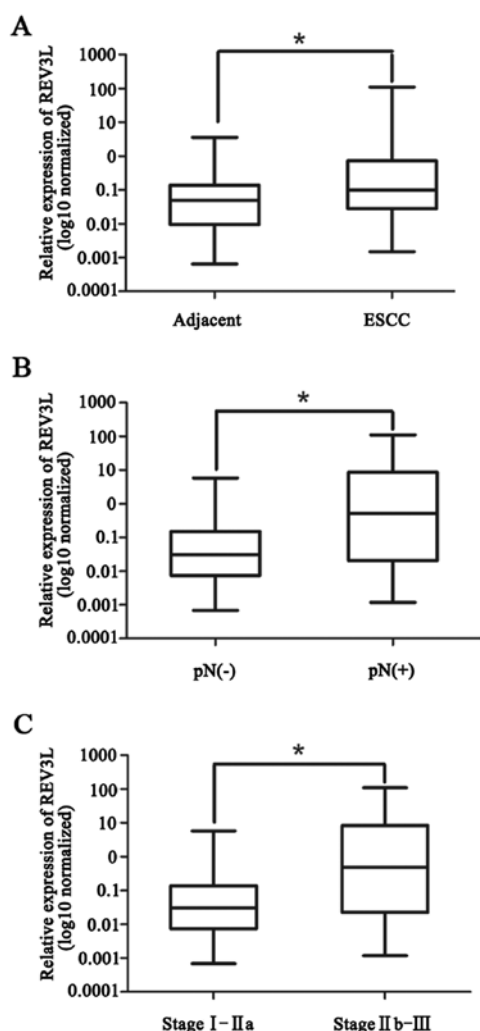


Figure 1. Overexpression of REV3L mRNA in ESCC tissues. (A) Real-time PCR assay was performed to detect the mRNA expression of REV3L in ESCC and adjacent tissues (* $P<0.05$). (B) Correlation between REV3L expression and lymph node metastasis (* $P<0.05$). (C) Correlation between REV3L expression and clinical stage (* $P<0.05$).

non-fat milk in PBS-Tween-20 for 1 h at room temperature, the membranes were incubated with primary antibodies targeting β -actin, survivin, cyclin D1 and PARP (Abcam Inc., Cambridge, MA, USA). After washing three times with TBST, the membranes were incubated with a horseradish peroxidase (HRP)-conjugated anti-rabbit or anti-mouse secondary antibody (Beyotime Biotechnology) for 2 h. The proteins were visualized using enhanced chemiluminescence (ECL; Beyotime, Nantong, China).

Statistical analysis. Results are expressed as means \pm standard error of the mean (SEM). Statistical analyses were performed using SPSS 17.0 software (IBM, Armonk, NY, USA). P -value <0.05 was considered to indicate a statistically significant result.

Results

Elevated REV3L mRNA expression level in ESCC and its correlation with lymph node metastasis and clinical stage. To investigate REV3L gene expression in ESCC and adjacent

Table I. Relationship between REV3L expression and clinicopathological parameters of the esophageal squamous cell carcinoma cases ($n=68$).

Clinicopathological parameters	Relative REV3L expression (relative to GAPDH)		
	Cases	Median (Q3-Q1)	P-value
Age (years)			
<60	28	0.031 (6.499)	0.151
≥ 60	40	0.120 (0.773)	
Gender			
Male	50	0.094 (3.768)	0.149
Female	18	0.030 (0.970)	
Histological grade			
Poor	9	0.016 (0.032)	0.946
Moderate	33	0.080 (9.232)	
Well	26	0.143 (2.389)	
pT			
pT1-2	25	0.077 (1.297)	0.900
pT3-4	43	0.080 (4.047)	
pN			
(-)	28	0.031 (0.143)	0.013
(+)	40	0.511 (8.480)	
Stage			
I-IIa	26	0.031 (0.132)	0.019
IIb-III	42	0.485 (8.147)	

tissues, the mRNA level of REV3L was analyzed by qRT-PCR using 68 ESCC and 48 adjacent tissues. As shown in Fig. 1A, the expression of REV3L in the ESCC tissues was significantly higher than that in the adjacent tissues ($P<0.05$). To further evaluate the correlation of REV3L mRNA expression and clinicopathological features, the characteristics of 68 ESCC patients included in the present study were analyzed (Table I). As shown by Mann-Whitney U test, REV3L mRNA expression was positively correlated with lymph node metastasis (Fig. 1B; $P<0.05$) and clinical stage (IIb and III) (Fig. 1C; $P<0.05$). In contrast, REV3L mRNA expression was not correlated with pT, gender, age and histological grade among the groups of patients. Collectively, these findings indicate that overexpression of REV3L in ESCC is associated with lymph node metastasis and tumor progression.

Establishment of REV3L-knockdown cell lines. To study the potential role of REV3L in esophageal cancer, stable cell lines with REV3L knockdown were established using the ECA-109 and TE-1 cells. REV3L mRNA and protein expression levels were analyzed by RT-PCR and immunofluorescence to verify the successful knockdown of REV3L in these cells. The results revealed that REV3L expression at the mRNA and protein levels was significantly lower ($P<0.05$) in the cells stably transfected with the shREV3L construct as compared to the cells transfected with the control vector (shNC), indicating effective knockdown of REV3L expression (Fig. 2A and B).

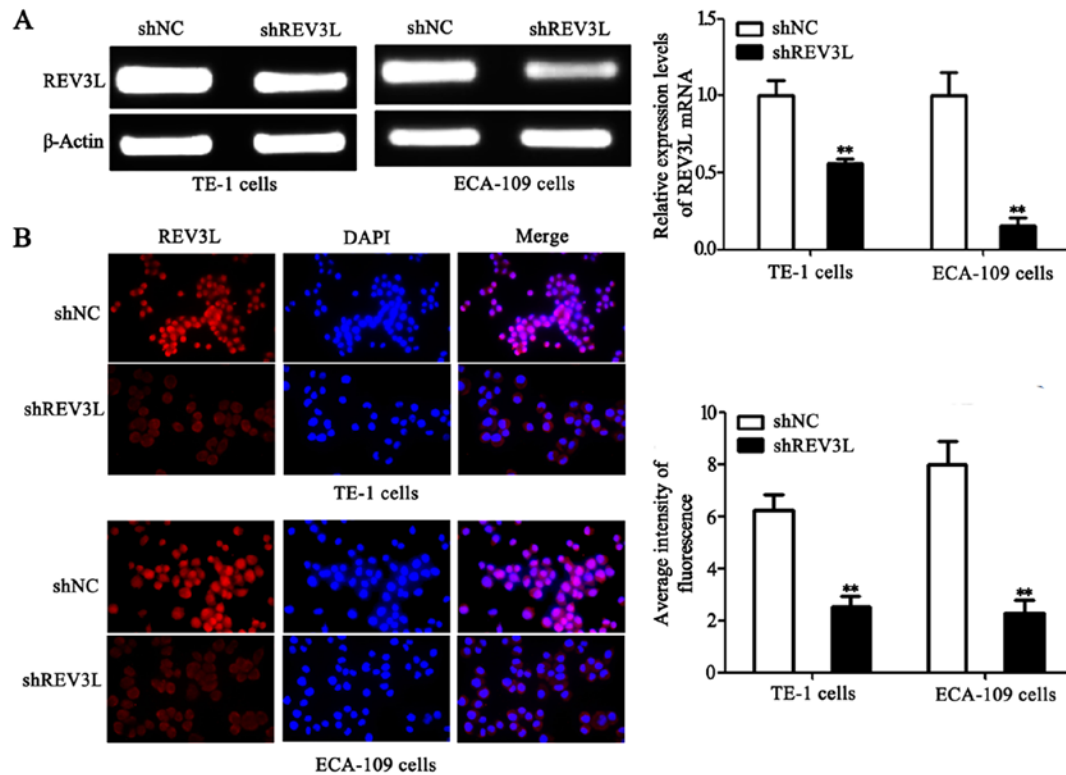


Figure 2. Generation of REV3L-knockdown cell lines. (A) RT-PCR assay was performed to detect REV3L mRNA expression in THE TE-1 and ECA-109 cells stably transfected with the shREV3L construct and control vector. β -actin level served as the loading control (** $P < 0.05$). (B) Protein expression of REV3L was analyzed by immunofluorescence analysis (** $P < 0.05$).

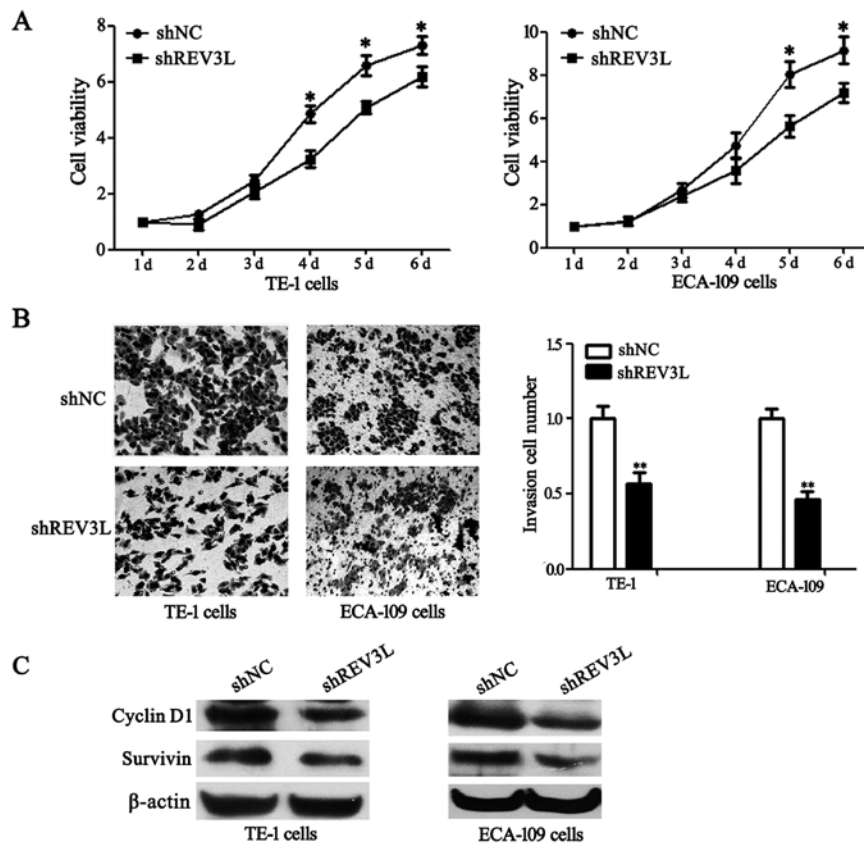


Figure 3. REV3L knockdown inhibits ESCC cell proliferation and invasion. (A) TE-1 and ECA-109 cells and their transfectants were cultured in 96-well plates at 2×10^3 /well for 1-6 days. The cell proliferation was assessed by CCK-8 assay. (B) Cell invasion was detected using a Transwell chamber assay, which showed that THE shREV3L group was less invasive than the control group in both cell lines ($P < 0.05$). (C) Western blot assay of the expression of cyclin D1 and survivin in the control and REV3L-knockdown cells. β -actin level served as the loading control.

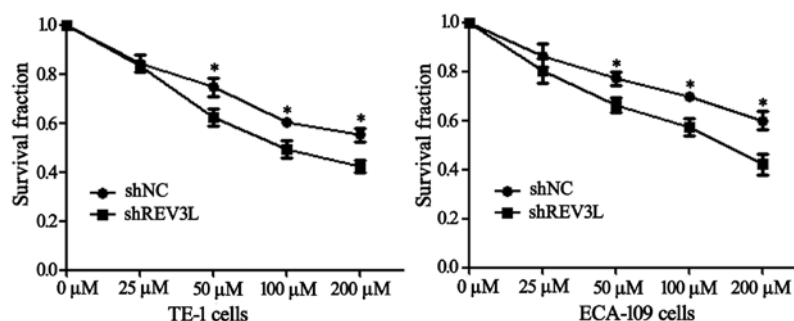


Figure 4. REV3L knockdown sensitizes ESCC cells to 5-FU. Cell viability was determined by the CCK-8 assay. Suppression of REV3L increased ESCC cell sensitivity to the cytotoxic effect of 5-FU (* $P < 0.05$).

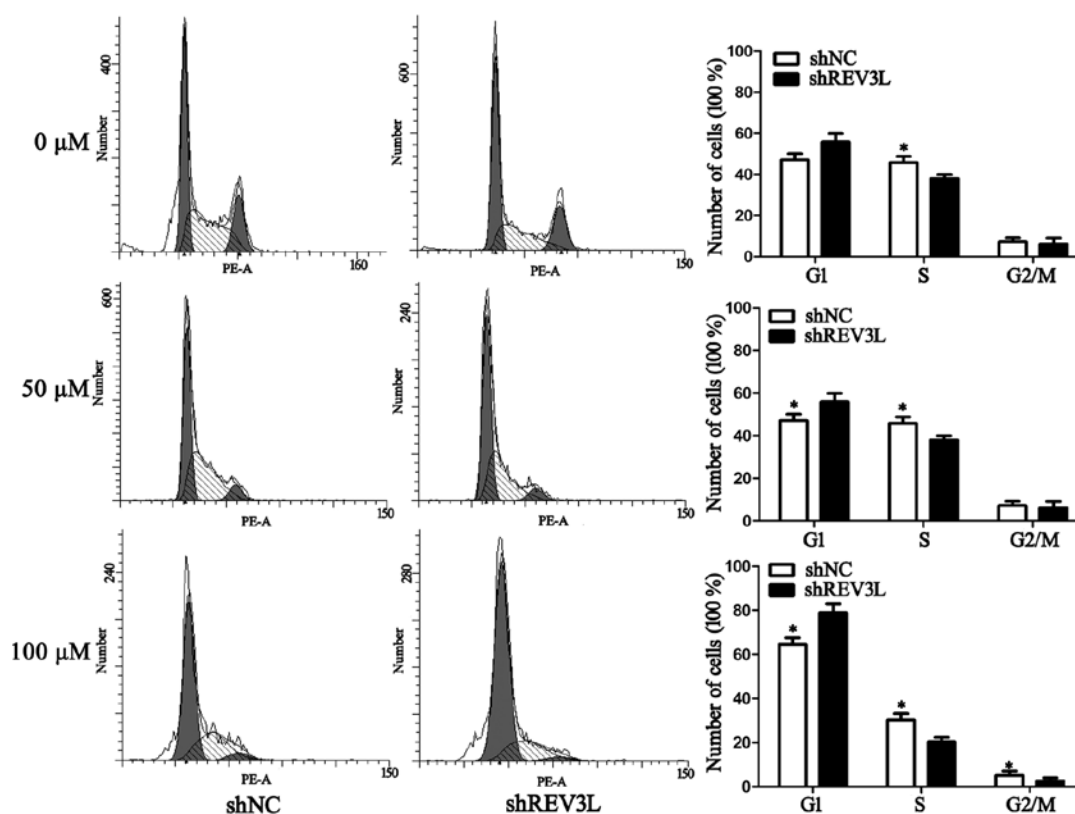


Figure 5. REV3L knockdown sensitizes ECA-109 cells to 5-FU-induced G1 phase arrest. ECA-109 cells were treated with 50 and 100 μ M 5-FU for 24 h, and the phase distribution of the cell cycle was determined using flow cytometry. Downregulation of REV3L markedly promoted 5-FU-induced G1 phase arrest of ECA-109 cells (* $P < 0.05$).

Effect of REV3L knockdown on the proliferation and invasion of esophageal cancer cells in vitro. To examine whether the modulation of REV3L expression affects the tumorigenic properties of the esophageal cancer *in vitro*, we measured the abilities of cell proliferation and invasion using CCK-8 assay and Transwell analysis, respectively. As shown in Fig. 3, the cell proliferation (Fig. 3A) and invasion (Fig. 3B) were both decreased in the REV3L-knockdown cells (TE-1/shREV3L and ECA-109/shREV3L cells) compared to the control cells (TE-1/shNC and ECA-109/shNC cells) ($P < 0.05$). These results suggest that REV3L plays an important role in esophageal cancer progression.

To further identify the mechanisms by which the silencing of REV3L inhibited esophageal cancer cell proliferation

and invasion, we analyzed the expression levels of cyclin D1 and survivin proteins due to their established roles in cell proliferation and invasion. Western blot analysis revealed that REV3L knockdown significantly downregulated cyclin D1 and survivin protein expression in the esophageal cancer cells (Fig. 3C).

Knockdown of REV3L enhances 5-FU-induced cytotoxicity in the esophageal cancer cells. To study the possible role of REV3L in affecting the sensitivity of esophageal cancer cells to 5-FU, several concentrations of 5-FU were used to treat ECA-109 and TE-1 cells for 48 h. The cell viability was determined by the CCK-8 assay. As shown in Fig. 4, a dose-dependent inhibition in cell growth was observed in

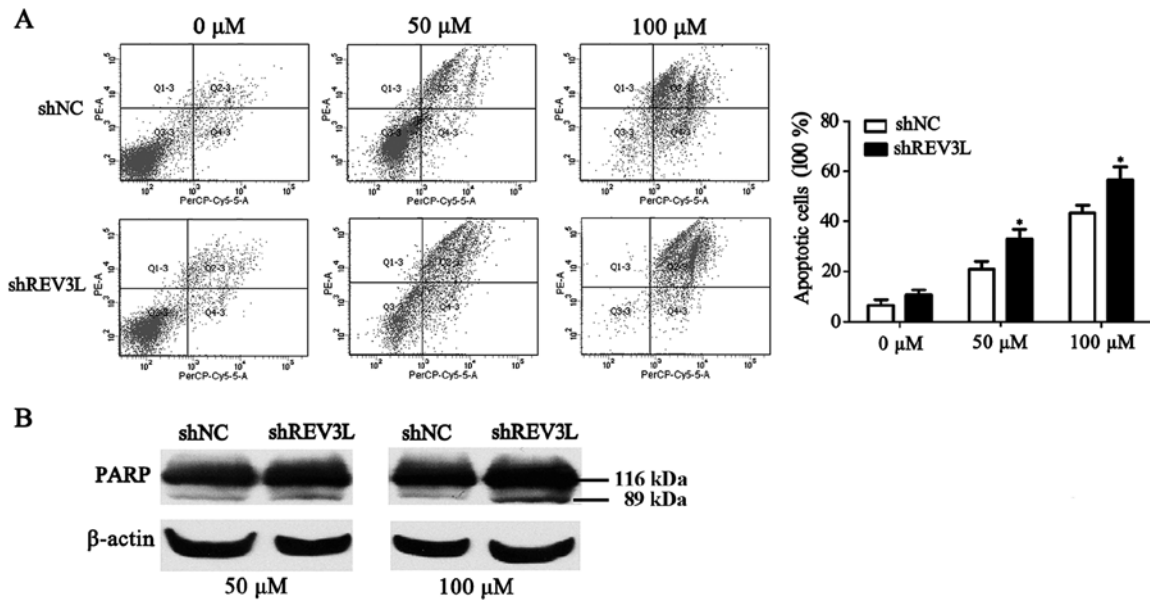


Figure 6. REV3L knockdown sensitizes ECA-109 cells to 5-FU-induced apoptosis. (A) ECA-109 cells were treated with 50 and 100 μ M 5-FU for 48 h, and apoptosis was examined by Annexin V-PE staining and flow cytometry. The percentage of Annexin V-PE stained cells was increased in the REV3L-knockdown cells (* P <0.05). (B) PARP cleavage was increased in the REV3L-knockdown cells as detected by western blotting following treatment with 50 or 100 μ M 5-FU for 48 h. β -actin level served as the loading control.

the 5-FU-treated TE-1 and ECA-109 cells, and the REV3L-knockdown cells were more sensitive to the cytotoxic effect of 5-FU. The results from the cell cycle analysis using PI staining showed that the number of cells in the G1 phase in the ECA-109/shREV3L cells was significantly higher than the number in the ECA-109/shNC cells after treatment with different doses of 5-FU for 24 h (Fig. 5). Together, these results indicate that REV3L plays a role in esophageal cancer resistance to 5-FU.

REV3L plays a critical role in esophageal cancer resistance to 5-FU-induced apoptosis. Next, we investigated whether the increased 5-FU cytotoxicity observed in the REV3L-knockdown cells was related to apoptosis. The extent of apoptosis was determined by measuring the percentage of Annexin V stained cells, which detect both early- and late-stage apoptosis. As shown in Fig. 6A, the apoptotic rates in the ECA-109/shREV3L cells were significantly higher than the rates in the ECA-109/shNC cells in response to different doses of 5-FU for 48 h. We then performed western blotting to analyze the cleavage of PARP protein, another established marker of apoptosis. As shown in Fig. 6B, ECA-109 REV3L-knockdown cells exhibited increased PARP cleavage compared with the control cells after treatment with 50 or 100 μ M 5-FU for 48 h.

Discussion

Pol ζ is an error-prone DNA polymerase involved in TLS that is characterized by a less-stringent active site and a lower processivity compared with the high-fidelity replicative DNA polymerases (16). As the catalytic subunit of the pol ζ , REV3L is thought to be one of the major components of error-prone TLS and may play a significant role in tumor mutagenicity, progression, cytotoxicity and chemoresistance (17). In the present study, the expression of REV3L was demonstrated

to be significantly upregulated and positively correlated with lymph node metastasis and clinical stage in ESCC tissues. These findings indicate that overexpression of Rev3L may accumulate genetic damages that are involved in the tumorigenesis and progression of ESCC.

There have been various controversial studies regarding the effect of REV3L on cancer cell growth. On the one hand, REV3L was established to be necessary for proliferation of mouse embryonic fibroblasts, and inhibition of REV3L expression resulted in a pronounced growth arrest in Burkitt lymphoma, lung, breast, mesothelioma and colon cancer cells (6,18). In contrast, suppression of REV3L expression did not alter cell growth/survival in HCT116, U2OS and HeLa cancer cell lines (13,19). In the present study, we found that downregulation of REV3L expression decreased cell growth, migratory and invasive potential of ESCC cells. This favors the notion that REV3L plays an important role in the tumorigenesis and progression of ESCC.

To elucidate the molecular mechanisms whereby REV3L modulates growth and invasion of esophageal cancer cells, we examined the expression of cyclin D1 and survivin, key regulators of cancer cell proliferation and therapeutic resistance. Cyclin D1, a key cell cycle regulator for cancer cell growth, is frequently overexpressed in a variety of human types of cancers including ESCC (20). Elevated levels of cyclin D1 have been found to correlate with early cancer onset, tumor progression, increased metastasis and reduced survival of cancer patients (21-23). Similarly, survivin, a member of the inhibitor of apoptosis protein family, functions to inhibit the mitochondrial pathway of apoptosis (24). Overexpression of survivin in ESCC was found to be associated with poor prognosis and resistance to radio-chemotherapy (25). Our data indicated that cyclin D1 and survivin were significantly decreased in ESCC cell lines following REV3L knockdown. These findings indicate that

cyclin D1 and survivin are involved in the REV3L actions in promoting esophageal cancer cell growth and invasion.

5-Fluorouracil (5-FU), a pyrimidine analogue of a broad-spectrum anticancer drug, is widely prescribed for ESCC patients (26). The anticancer activity of 5-FU is through the inhibition of thymidylate synthase (TS) and incorporation of its metabolites into RNA and DNA molecules (27), which result in failed DNA synthesis leading to cell apoptosis (28). Thus, suppression of DNA replication signaling is a potential approach to increase 5-FU sensitivity. REV3L has been shown to play a critical role in preventing cisplatin cytotoxicity (9,14), yet its role in 5-FU-resistance has not been previously reported. In the present study, we found that inhibition of REV3L expression increased the sensitivity of esophageal cancer cells to 5-FU by inducing G1 phase arrest and apoptosis. These results indicate that inhibition of REV3L is likely a new strategy to overcome 5-FU-resistance in esophageal cancer.

In summary, the present study demonstrated that REV3L expression is significantly upregulated in ESCC tissues compared with adjacent tissues, and is positively correlated with lymph node metastasis and clinical stage. Inhibition of REV3L expression reduces ESCC cell proliferation and invasion at least in part through suppression of cyclin D1 and survivin expression. Furthermore, we demonstrated that REV3L functions to confer chemoresistance to 5-FU treatment via regulation of the cell cycle and apoptosis. These findings illustrate a role of REV3L in human ESCC progression and chemoresistance, and provide a potential new diagnostic marker and therapeutic target for ESCC.

Acknowledgements

The present study was supported by the National Natural Science Foundation of China (81372433 and 81472919), the Natural Science Foundation of Jiangsu Province of China (BK20131149), and the Suzhou Administration of Science and Technology (SYS201360, SYS201254 and SYS201571).

References

- Jemal A, Siegel R, Ward E, Hao Y, Xu J and Thun MJ: Cancer statistics, 2009. *CA Cancer J Clin* 59: 225-249, 2009.
- Enzinger PC and Mayer RJ: Esophageal cancer. *N Engl J Med* 349: 2241-2252, 2003.
- Talukdar FR, Ghosh SK, Laskar RS and Mondal R: Epigenetic, genetic and environmental interactions in esophageal squamous cell carcinoma from northeast India. *PLoS One* 8: e60996, 2013.
- Friedberg EC: A history of the DNA repair and mutagenesis field: I. The discovery of enzymatic photoreactivation. *DNA Repair* 33: 35-42, 2015.
- Gan GN, Wittschleben JP, Wittschleben BO and Wood RD: DNA polymerase zeta (pol ζ) in higher eukaryotes. *Cell Res* 18: 174-183, 2008.
- Knobel PA, Kotov IN, Felley-Bosco E, Stahel RA and Marti TM: Inhibition of REV3 expression induces persistent DNA damage and growth arrest in cancer cells. *Neoplasia* 13: 961-970, 2011.
- Zhang S, Chen H, Zhao X, Cao J, Tong J, Lu J, Wu W, Shen H, Wei Q and Lu D: REV3L 3'UTR 460 T>C polymorphism in microRNA target sites contributes to lung cancer susceptibility. *Oncogene* 32: 242-250, 2013.
- Varadi V, Bevier M, Grzybowska E, Johansson R, Enquist K, Henriksson R, Butkiewicz D, Pamula-Pilat J, Tecza K, Hemminki K, *et al*: Genetic variation in genes encoding for polymerase ζ subunits associates with breast cancer risk, tumour characteristics and survival. *Breast Cancer Res Treat* 129: 235-245, 2011.
- Yang L, Shi T, Liu F, Ren C, Wang Z, Li Y, Tu X, Yang G and Cheng X: REV3L, a promising target in regulating the chemosensitivity of cervical cancer cells. *PLoS One* 10: e0120334, 2015.
- Shi TY, Yang L, Yang G, Tu XY, Wu X, Cheng X and Wei Q: DNA polymerase ζ as a potential biomarker of chemoradiation resistance and poor prognosis for cervical cancer. *Med Oncol* 30: 500, 2013.
- Kawamura K, O-Wang J, Bahar R, Koshikawa N, Shishikura T, Nakagawara A, Sakiyama S, Kajiwarra K, Kimura M and Tagawa M: The error-prone DNA polymerase ζ catalytic subunit (Rev3) gene is ubiquitously expressed in normal and malignant human tissues. *Int J Oncol* 18: 97-103, 2001.
- Xiao W, Lechler T, Chow BL, Fontanie T, Agustus M, Carter KC and Wei YF: Identification, chromosomal mapping and tissue-specific expression of hREV3 encoding a putative human DNA polymerase ζ . *Carcinogenesis* 19: 945-949, 1998.
- Brondello JM, Pillaire MJ, Rodriguez C, Gourraud PA, Selves J, Cazaux C and Piette J: Novel evidences for a tumor suppressor role of Rev3, the catalytic subunit of Pol ζ . *Oncogene* 27: 6093-6101, 2008.
- Wang H, Zhang SY, Wang S, Lu J, Wu W, Weng L, Chen D, Zhang Y, Lu Z, Yang J, *et al*: REV3L confers chemoresistance to cisplatin in human gliomas: The potential of its RNAi for synergistic therapy. *Neuro Oncol* 11: 790-802, 2009.
- Zhou J, Zhang S, Xie L, Liu P, Xie F, Wu J, Cao J and Ding WQ: Overexpression of DNA polymerase ι (*Pol*) in esophageal squamous cell carcinoma. *Cancer Sci* 103: 1574-1579, 2012.
- Waters LS, Minesinger BK, Wiltout ME, D'Souza S, Woodruff RV and Walker GC: Eukaryotic translesion polymerases and their roles and regulation in DNA damage tolerance. *Microbiol Mol Biol Rev* 73: 134-154, 2009.
- Gibbs PE, McGregor WG, Maher VM, Nisson P and Lawrence CW: A human homolog of the *Saccharomyces cerevisiae* REV3 gene, which encodes the catalytic subunit of DNA polymerase ζ . *Proc Natl Acad Sci USA* 95: 6876-6880, 1998.
- Gueranger Q, Stary A, Aoufouchi S, Faili A, Sarasin A, Reynaud CA and Weill JC: Role of DNA polymerases η , ι and ζ in UV resistance and UV-induced mutagenesis in a human cell line. *DNA Repair* 7: 1551-1562, 2008.
- Hicks JK, Chute CL, Paulsen MT, Ragland RL, Howlett NG, Guéranger Q, Glover TW and Canman CE: Differential roles for DNA polymerases eta, zeta, and REV1 in lesion bypass of intra-strand versus interstrand DNA cross-links. *Mol Cell Biol* 30: 1217-1230, 2010.
- Sarbia M, Stahl M, Fink U, Heep H, Dutkowski P, Willers R, Seeber S and Gabbert HE: Prognostic significance of cyclin D1 in esophageal squamous cell carcinoma patients treated with surgery alone or combined therapy modalities. *Int J Cancer* 84: 86-91, 1999.
- Diehl JA: Cycling to cancer with cyclin D1. *Cancer Biol Ther* 1: 226-231, 2002.
- Jares P, Colomer D and Campo E: Genetic and molecular pathogenesis of mantle cell lymphoma: Perspectives for new targeted therapeutics. *Nat Rev Cancer* 7: 750-762, 2007.
- Thomas GR, Nadiminti H and Regalado J: Molecular predictors of clinical outcome in patients with head and neck squamous cell carcinoma. *Int J Exp Pathol* 86: 347-363, 2005.
- Altieri DC: Survivin, cancer networks and pathway-directed drug discovery. *Nat Rev Cancer* 8: 61-70, 2008.
- Kato J, Kuwabara Y, Mitani M, Shinoda N, Sato A, Toyama T, Mitsui A, Nishiwaki T, Moriyama S, Kudo J *et al*: Expression of survivin in esophageal cancer: Correlation with the prognosis and response to chemotherapy. *Int J Cancer* 95: 92-95, 2001.
- Wang L, Feng J, Chen X, Guo W, Du Y, Wang Y, Zang W, Zhang S and Zhao G: Myricetin enhance chemosensitivity of 5-fluorouracil on esophageal carcinoma in vitro and in vivo. *Cancer Cell Int* 14: 71, 2014.
- Pinedo HM and Peters GF: Fluorouracil: Biochemistry and pharmacology. *J Clin Oncol* 6: 1653-1664, 1988.
- Bijnsdorp IV, Peters GJ, Temmink OH, Fukushima M and Kruijff FA: Differential activation of cell death and autophagy results in an increased cytotoxic potential for trifluorothymidine compared to 5-fluorouracil in colon cancer cells. *Int J Cancer* 126: 2457-2468, 2010.