Sex determining region Y-box 2 inhibits the proliferation of colorectal adenocarcinoma cells through the mTOR signaling pathway

HUI LIU[1*], LUTAO DU[1*], ZHIHUA WEN[2], YONGMEI YANG[1], JUAN LI[1], ZHAOGANG DONG[1], GUIXI ZHENG[1], LILI WANG[1], XIN ZHANG[1] and CHUANXIN WANG[1]

[1]Department of Clinical Laboratory and [2]Institute of Basic Medical Sciences, Qilu Hospital, Shandong University, Jinan, Shandong 250012, P.R. China

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Abstract. Sex determining region Y-box 2 (SOX2), is a high mobility group box transcription factor involved in the maintenance of pluripotency and the self-renewal of embryonic and neuronal stem cells, which also plays differential roles in the cell proliferation of several tumors. However, its role in colorectal adenocarcinoma cell proliferation and the underlying mechanisms remain unclear. The mammalian target of rapamycin (mTOR) signaling pathway has recently emerged as an important regulator of cell proliferation in many types of cancer. In this study, we examined the effect of SOX2 on the proliferation of colorectal adenocarcinoma cells and evaluated the role of the mTOR pathway in this process. Our results indicated that the overexpression of SOX2 significantly inhibited the proliferation of colorectal adenocarcinoma cells. Of note, mechanistic investigations revealed that SOX2 inhibited the activation of the mTOR pathway in HT-29 cells. We then examined the effect of SOX2 on the cell cycle, and the results revealed that SOX2 downregulated cyclin D1 expression and induced G0/G1 arrest in the HT-29 cells. Moreover, we also analyzed the correlation between SOX2 expression and patient clinicopathological characteristics in colorectal adenocarcinoma tissues, as well as the level of phospho-S6 (S235/236), phospho-Akt (S473) and cyclin D1. The results revealed a significant negative correlation between SOX2 expression and tumor size and the levels of phospho-S6 (S235/236), phospho-Akt (S473) and cyclin D1. Taken together, to our knowledge, our findings suggest for the first time that SOX2 suppresses colorectal adenocarcinoma cell proliferation through the inhibition of the mTOR pathway.

Correspondence to: Professor Chuanxin Wang, Department of Clinical Laboratory, Qilu Hospital, Shandong University, 107 Wenhua West Road, Jinan, Shandong 250012, P.R. China
E-mail: cxwang@sdu.edu.cn

*Contributed equally

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Introduction

Cell deviation from the canonical cell cycle constitutes a critical event in the development of cancer, and the dysregulation of cell proliferation may result in colonic and rectal tumorigenesis (1,2). It is known that cancer is most likely to be caused by the deregulation of transcription factors that affect cell fate and proliferation (3,4). Therefore, it is worthwhile to explore the transcription factors involved in the proliferation of colorectal adenocarcinoma cells and the underling mechanisms, so as to gain important insight into effective therapeutic strategies.

Sex determining region Y-box 2 (SOX2), which belongs to group B of the SOX family, is a high mobility group box transcription factor involved in the maintenance of pluripotency and the self-renewal of embryonic and neuronal stem cells (5,6). SOX2, as well as other SOX family factors, plays a critical role in cell fate determination, differentiation and proliferation (7-9). Recent studies have reported that the anomalous expression of SOX2 is associated with cell proliferation in several types of human cancer (5,10-13). For instance, SOX2 has been shown to promote cell proliferation in lung and esophageal squamous cell carcinomas (5,10,11). However, it has also been shown to inhibit cell proliferation in gastric cancers (13). To date, the effect of SOX2 on the proliferation of colorectal adenocarcinoma cells and the underlying mechanisms remain unclear.

The mammalian target of rapamycin (mTOR) is an evolutionarily conserved Ser/Thr kinase that belongs to the family of the phosphatidylinositol 3-kinase-related kinases (PIKKs) (14). It is upregulated in many types of cancer, including colorectal adenocarcinoma, contributing to the dysregulation of cell proliferation, growth, differentiation and survival (15). mTOR forms two complexes, termed mTOR complex 1 (mTORC1) and complex 2 (mTORC2). mTORC1 elicits its pleiotropic function mainly through controlling protein synthesis through the phosphorylation of the eukaryotic initiation factor 4E-binding protein 1 (4E-BP1), p70 ribosomal S6 kinase 1 (S6K1) and ribosomal protein S6 (S6) (16,17). Its activity is sensitive to

rapamycin. mTORC2, which phosphorylates (Ser473) and activates Akt, is known to be rapamycin-insensitive (14,18). A number of recent studies have suggested that many factors affect the proliferation of tumor cells through the regulation of mTOR activity (19-22). However, little information is available on the effect of SOX2 on the mTOR pathway.

In this study, in order to elucidate the role of SOX2 on the proliferation of human colorectal adenocarcinoma cells, we performed cell proliferation assay using three cell lines, HT-29, SW480 and LoVo, after which we selected the HT-29 cells for further study. Firstly, we investigated whether the mTOR pathway is involved in the SOX2-induced inhibition of HT-29 cell proliferation. We also assessed the effects of SOX2 on the cell cycle progression of HT-29 cells. Moreover, we examined the expression of SOX2 and downstream targets of the mTOR pathway in colorectal adenocarcinoma tissues.

Materials and methods

Cell lines and tissue samples. The colorectal adenocarcinoma cell lines, HT-29, SW480 and LoVo, were provided by the Institute of Basic Medical Sciences, Qilu Hospital of Shandong University, Jinan, China. The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and cultured in a 37˚C humidified atmosphere containing 95% air and 5% CO_2. A total of 101 patients with colorectal adenocarcinoma were selected from the Department of General Surgery, Qilu Hospital of Shandong University, between October 2010 and April 2012. None of the patients had received pre-operative adjuvant therapy after being diagnosed with colorectal adenocarcinoma. The colorectal adenocarcinoma tissues were subpackaged and frozen at -80˚C immediately after surgical removal and maintained at -80˚C for future use. Informed consent was obtained from each patient, and tissues were collected using protocols approved by the Ethics Committee of Shandong University. Histological grading was performed according to the WHO criteria and staging was based on the TNM classification system, in affiliation with the International Union against Cancer.

Quantitative real-time polymerase chain reaction (qRT-PCR). Total RNA was extracted from the tumor tissues and cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA (1 μg) was used for reverse transcription using a SuperScript kit (Toyobo, Osaka, Japan). All real-time PCR analyses were performed in triplicate using LightCycler® FastStart DNA Master SYBR-Green I (Roche Diagnostics, Mannheim, Germany) on a Roche LightCycler 2.0. β-actin as used the internal control. The primer sequences were as follows: SOX2, 5'-CATGCACCGCTACGACGTGAG-3' (forward) and 5'-TGGGAGGAAGAGGTAACCACAGG-3' (reverse); cyclin D1, 5'-CCGTCCATGCGGAAGATC-3' (forward) and 5'-ATGGCCAGCGGGAAGAC-3' (reverse); β-actin, 5'-TGACGTGGACATCCGCAAAG-3' (forward) and 5'-CTGGAAGGTGGACAGCGAGG-3' (reverse). We analyzed the results using LightCycler software version 4.0 (Roche Diagnostics).

Plasmid DNA and transfection. PCMV-HA-SOX2, the eukaryotic expression plasmid encoding SOX2, was kindly provided by Dr Dongshi Guan from the Department of General Surgery, Provincial Hospital Affiliated to Shandong University. The colorectal adenocarcinoma cells were transfected with PCMV-HA-SOX2 and PCMV-HA using Lipofectamine 2000 (Invitrogen) at the optimal multiplicity of infection (MOI) according to the manufacturer's instructions. The messenger RNA (mRNA) and protein expression of SOX2 was characterized using qRT-PCR and western blot analysis.

Cell proliferation assay. HT-29, SW480 and LoVo cells were plated at $2x10^3$ cells/well in 96-well plates. Following overnight incubation, the cells were transfected with PCMV-HA-SOX2 or PCMV-HA using Lipofectamine 2000 at the optimal MOI. The culture medium was changed to DMEM with 5% FBS at 4-6 h after transfection. Cell proliferation was evaluated on days 1, 3 and 5 after transfection using the Cell Counting kit-8 (CCK-8) (Dojindo Laboratories, Kumamoto, Japan) at 450 and 630 nm dual wavelengths. The experiments were performed in duplicate and repeated three times. All operations were performed according to the manufacturer's instructions.

Western blot analysis. The HT-29 cells were plated at $7x10^4$ cells/well in 24-well plates. Cell lysates were harvested at 48 and 72 h after transfection with PCMV-HA-SOX2 or PCMV-HA. Tumor tissues were resuspended in ice-cold buffer for lysis. The lysates were then resolved in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellulose membranes. Proteins were detected with specific antibodies and revealed using the enhanced chemiluminescence (ECL) kit (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). The primary antibodies specific for SOX2, cyclin D1, phospho-mTOR (S2448), phospho-4E-BP1 (T37/46), phospho-S6K1 (T389), phospho-S6 (S235/236) and phospho-Akt (S473) were purchased from Cell Signaling Technology, Inc. (Beverly, MA, USA). The primary antibodies specific for mTOR, 4E-BP1, S6K1, S6 and Akt were purchased from Santa Cruz Biotechnology, Inc. β-actin antibody was purchased from Bioworld Technology, Inc. (St. Louis Park, MN, USA).

Cell cycle analysis. HT-29 cells were plated at $7x10^4$ cells/well in 24-well plates. At 48 and 72 h after transfection with PCMV-HA-SOX2 or PCMV-HA, the cells were harvested and fixed with 70% ethanol overnight at 4˚C. The following day, the cells were resuspended in phosphate-buffered saline (PBS) containing 50 μg/ml propidium iodide and 10 μg/ml RNase A, and incubated for 30 min at room temperature in the dark. We measured the cell cycle progression using flow cytometry (Becton-Dickinson, San Jose, CA, USA). The percentages of cells in the G0/G1, S and G2/M phases were analyzed using FCS Express softwarea and ModFit LT.

Immunohistochemical analysis. Resected fresh tissues from 67 patients with colorectal adenocarcinoma were formalin-fixed and paraffin-embedded, and cut into 4-μm-thick sections using a microtome. Following deparaffinization, antigen retrieval was performed in 10 mM sodium citrate buffer and endogenous peroxidase was eliminated using 3% H_2O_2. All sections were then blocked with goat serum and incubated with specific antibodies overnight at 4˚C. The primary antibodies

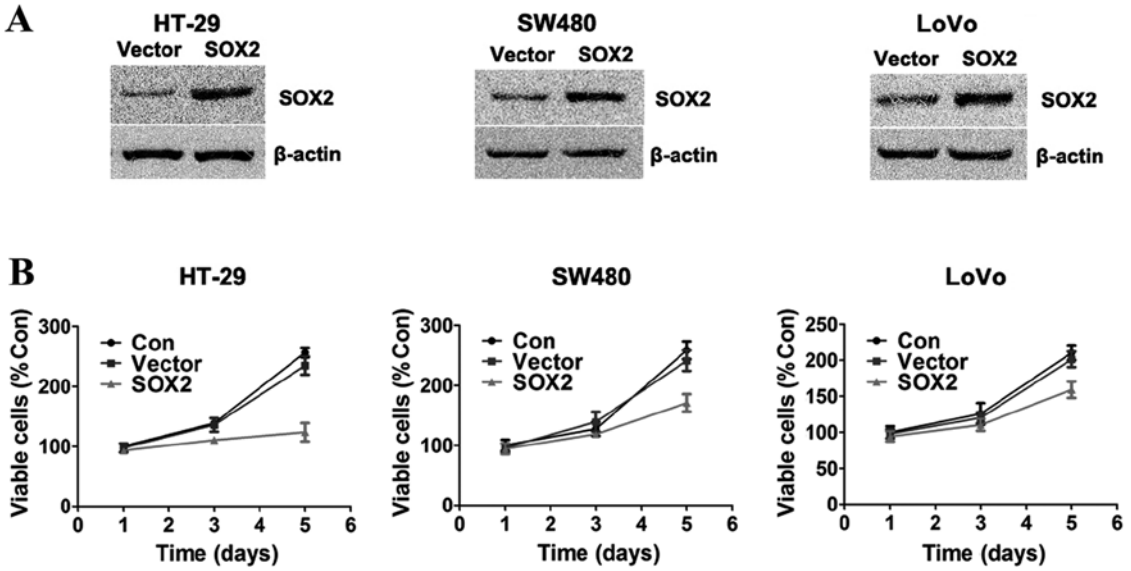

Figure 1. SOX2 inhibits the proliferation of colorectal adenocarcinoma cells. (A) Western blot analysis of SOX2 protein expression after the transfection of HT-29, SW480 and LoVo cells. (B) The number of viable cells was measured using CCK-8 assay on days 1, 3 and 5 after the transfection of HT-29, SW480 and LoVo cells. The assay was performed in triplicate, and bars indicate the means ± SD (all at $P<0.05$).

used were anti-SOX2, phospho-S6 (S235/236), phospho-Akt (S473) and cyclin D1 (Cell Signaling Technology, Inc.). The following day, horseradish peroxidase conjugated IgG and 3,3-diaminobenzidine solution (Vector Laboratories, Burlingame, CA, USA) were used to visualize the antibody binding. All sections were counterstained with hematoxylin. After dehydration and mounting with neutral gummi, the results were observed under a microscope.

The immunoreactivity in the carcinoma tissues was graded semiquantitatively according to the intensity and the percentage of positive cells. The intensity of the samples was scored as 0, no staining; 1, weak staining; 2, moderate staining; or 3, strong staining. The percentage of positive cells was scored as 0, <5%; 1, 5-25%; 2, 26-50%; 3, 51-75%; or 4, >75%. The sum of the score was then graded as -, 0-1; +, 2-3; ++, 4-5; or +++, 6-7. We defined '-' as negative, and '+, ++ and +++' as positive.

Statistical analysis. The SPSS software package (version 13.0; SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Correlations of SOX2 protein expression with clinicopathological factors were examined using the χ^2 test. Spearman's rank correlation coefficient test was performed to assess the correlation between SOX2 and cyclin D1. Other data from experiments were analyzed using the Student's t-test or analysis of variance (ANOVA) where appropriate. Data are presented as the means ± standard deviation (SD) of more than three separate experiments. A P-value <0.05 was considered to indicate a statistically significant difference.

Results

SOX2 inhibits the proliferation of colorectal adenocarcinoma cells. To evaluate the effect of SOX2 on the proliferation of colorectal adenocarcinoma cells, the colorectal adenocarcinoma cells (HT-29, SW480 and LoVo) were transiently transfected with PCMV-HA-SOX2 or PCMV-HA. The expression of SOX2 was confirmed by western blot analysis (Fig. 1A). Cell proliferation was evaluated on days 1, 3 and 5 following transfection with CCK-8. Cell growth curves were made based on the acquired data (Fig. 1B). As demonstrated by our results, SOX2-overexpressing cells showed significant growth inhibition compared to the control cells, in all three colorectal adenocarcinoma cell lines (all at $P<0.05$). Moreover, the inhibitory effect of SOX2 was most obvious in the HT-29 cells; thus, we selected the HT-29 cells for further experiments.

SOX2 inhibits the mTOR pathway in HT-29 cells. To investigate whether the mTOR pathway is involved in the SOX2-induced inhibition of proliferation of HT-29 cells, we first assessed the phosphorylation status of mTOR (S2448). The results of western blot analysis indicated that SOX2 decreased the phosphorylation of mTOR (S2448) (Fig. 2A and B). We then examined whether the activity of mTORC1 and mTORC2 was inhibited by SOX2. The western blot analysis results revealed that SOX2 not only inhibited the mTORC1-induced phosphorylation of S6K1 (T389), 4EBP1 (T37/46) and S6 (S235/236), but also decreased the mTORC2-induced phosphorylation of Akt at position Ser473 in the HT-29 cells (Fig. 2C and D). These results revealed that SOX2 inhibited the mTOR pathway in the HT-29 cells.

SOX2 downregulates cyclin D1 expression and induces cell cycle arrest at the G0/G1 phase in HT-29 cells. To clarify the mechanism underlying the growth inhibitory effect of SOX2 on HT-29 cells, we investigated the cell cycle progression of HT-29 cells using flow cytometry. At 48 h after transfection, 48.6% of the SOX2-overexpressing cells were in the G0/G1 phase, while a much lower proportion of the control cells (34.3%) was in this phase. Similarly, at 72 h after transfection, 54.9% of the SOX2-overexpressing cells and 45.6% of the control cells were

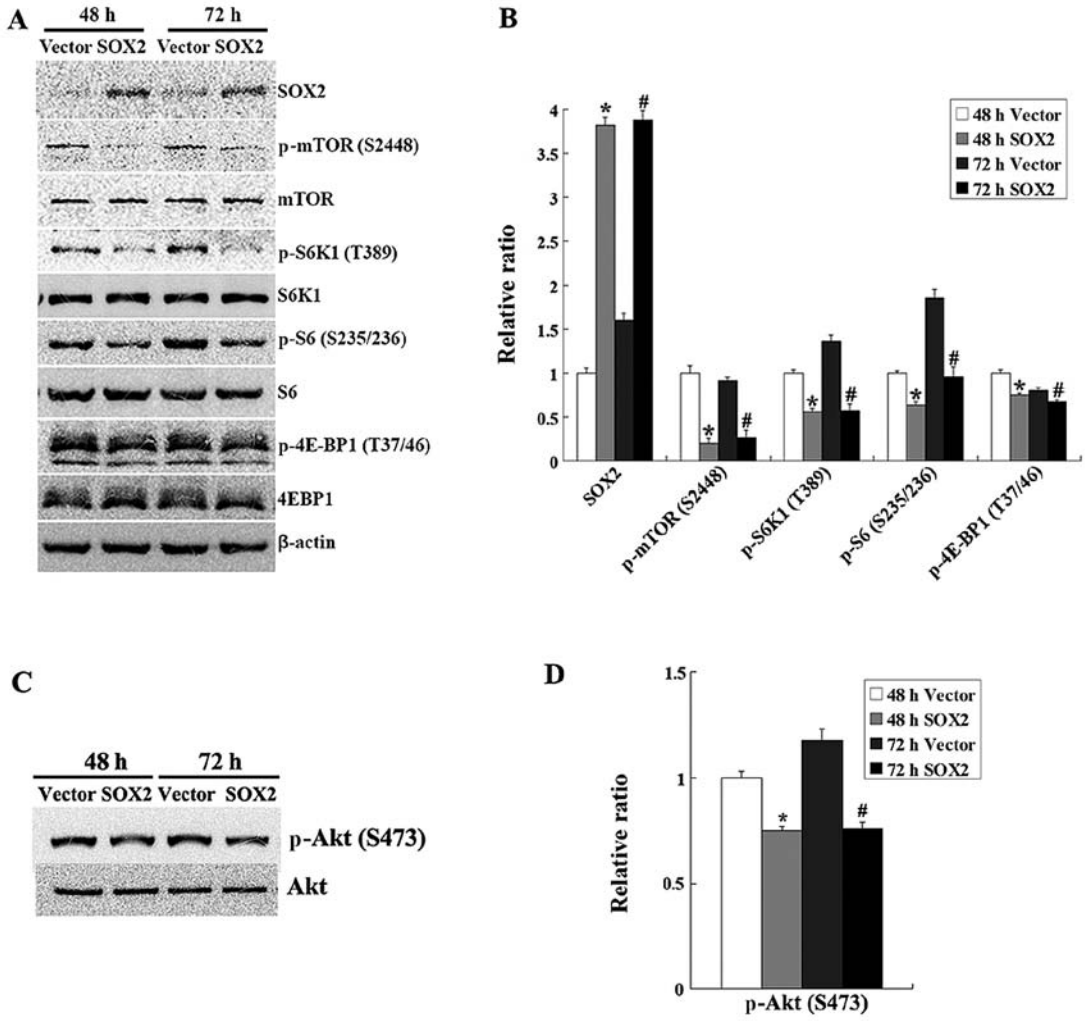

Figure 2. SOX2 inhibits the mTOR signaling pathway in HT-29 cells. (A) HT-29 cells were transfected with PCMV-HA-SOX2 or PCMV-HA. At 48 and 72 h after transfection, the cells were harvested and western blot analysis was performed to determine the levels of mTOR, phospho-mTOR (p-mTOR; S2448), S6K1, phospho-S6K1 (p-S6K1; T389), S6, phospho-S6 (p-S6; S235/236), 4E-BP1, phospho-4E-BP1 (p-4E-BP1; T37/46), Akt and phospho-Akt (p-Akt; S473). Data are representative of three independent experiments. (B) Relative ratio of data in (A). The bar graph represents the mean ± SD of the density (n=3, *P<0.05 vs. control cells at 48 h; $^{\#}$P<0.05 vs. control cells at 72 h). (C) The level of phospho-Akt (S473) was determined by western blot analysis. (D) Relative ratio of data in (C). The bar graph represents the mean ± SD of the density (n=3, *P<0.05 vs. control cells at 48 h; $^{\#}$P<0.05 vs. control cells at 72 h).

in the G0/G1 phase. The SOX2-overexpressing cells showed lower proportions of cells in the S and G2/M phase compared to the control cells (Fig. 3A and B).

Furthermore, the results from qRT-PCR analysis revealed that the mRNA expression level of cyclin D1 (a key factor for the transition of the G0/G1-S phase and whose expression is controlled by mTOR) was significantly decreased in the SOX2-overexpressing cells (Fig. 3C and D). The results of western blot analysis also demonstrated that the protein expression level of cyclin D1 was significantly decreased in the SOX2-overexpressing cells compared to the control cells (Fig. 3E and F). All these results indicate that SOX2 inhibits the proliferation of HT-29 cells by downregulating cyclin D1 and arresting the cells at the G0/G1 phase.

Significant negative correlation between the expression of SOX2 and tumor size and phospho-S6 (S235/236), phospho-Akt (S473) and cyclin D1 levels in colorectal adenocarcinoma tissues. To further determine whether SOX2 affects tumor cell proliferation through the mTOR pathway, we compared the expression of SOX2, phospho-S6 (S235/236), phospho-Akt (S473) and cyclin D1 by the immunohistochemical analysis of 67 colorectal adenocarcinoma tissues. The results revealed that SOX2 was only detected in 15 sample tissues (22.4%), in which SOX2 was mainly localized in the nuclei of the cells (Fig. 4A). The expression of SOX2 significantly correlated with a small tumor size (P<0.05) (Table I). By contrast, no correlation was observed between SOX2 expression and basal clinicopathological characteristics, such as gender, age, histological grade, tumor stage and lymph node metastasis (P>0.05) (Table I). Immunohistochemical staining revealed that phospho-S6 (S235/236), phospho-Akt (S473) and cyclin D1 expression was detected in 49, 47 and 54 sample tissues, respectively; expression was observed in the cytoplasm of the tumor cells. SOX2 levels showed a significant negative correlation with the phospho-S6 (S235/236), phospho-Akt (S473) and cyclin D1 expression in colorectal adenocarcinoma tissues (P<0.05) (Fig. 4A and B).

We further used qRT-PCR and western blot analysis to examine the expression of SOX2 and the downstrem targets

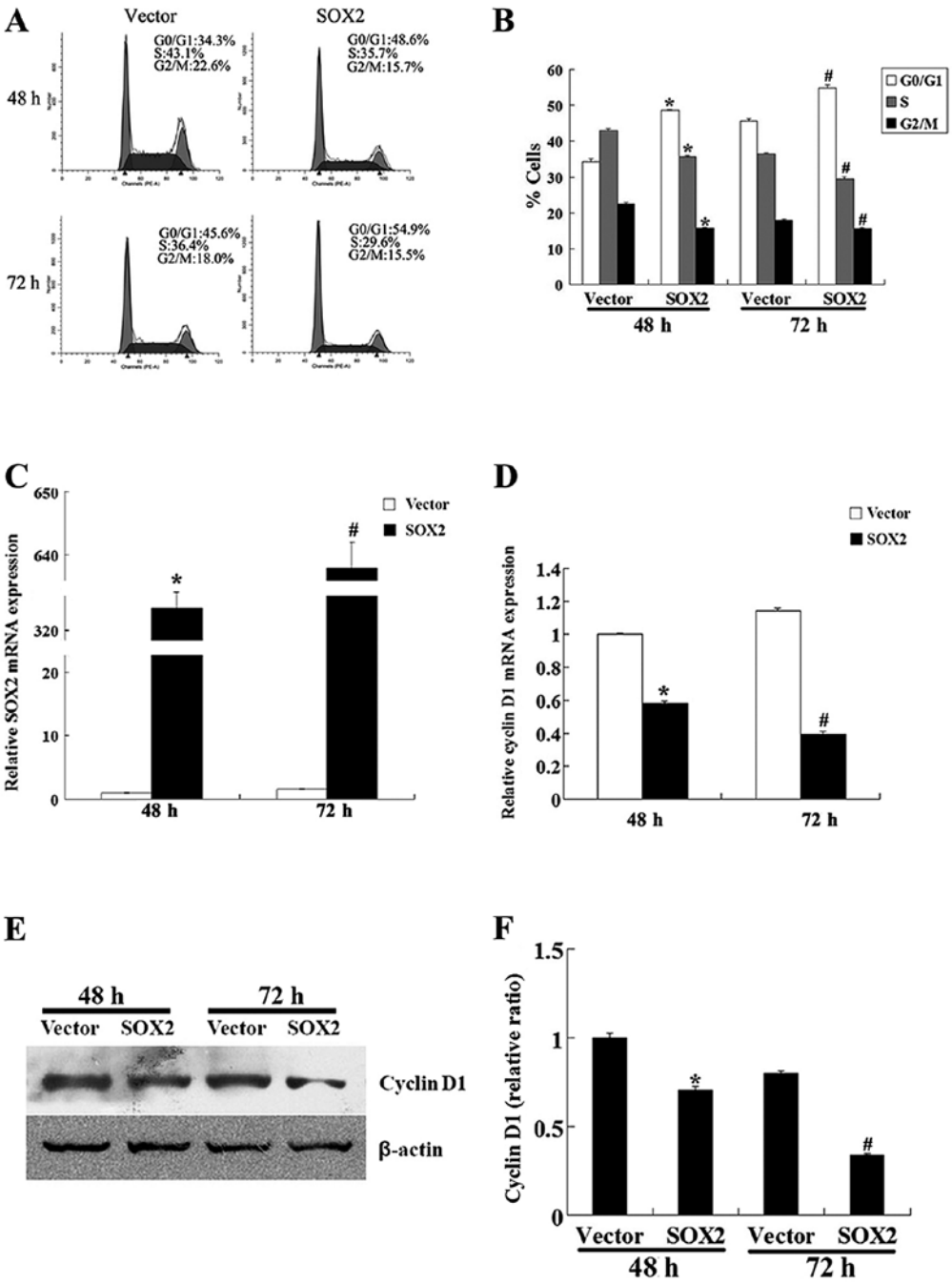

Figure 3. SOX2 downregulates cyclin D1 expression and induces cell cycle arrest at the G0/G1 phase in HT-29 cells. (A) Cell cycle analysis at 48 and 72 h after transfection with PCMV-HA-SOX2 or PCMV-HA. The percentages of cells in each phase are shown in each panel. (B) Cells in the G0/G1, S and G2/M phase of the cell cycle (n=3, *P<0.05 vs. control cells at 48 h; #P<0.05 vs. control cells at 72 h). (C) qRT-PCR analysis showing the relative mRNA expression level of SOX2 at 48 and 72 h after transfection. (D) qRT-PCR analysis showing the relative mRNA expression level of the cyclin D1 at 48 and 72 h after transfection. (E) Western blot analysis showing the protein expression level of cyclin D1 at 48 and 72 h after transfection. (F) Relative ratio of data in (E). The bar graph represents the mean ± SD of the density of each band normalized to β-actin (n=3, *P<0.05 vs. control cells at 48 h; #P<0.05 vs. control cells at 72 h).

of mTOR in colorectal adenocarcinoma tissues. The qRT-PCR results indicated that in another 34 colorectal adenocarcinoma tissues, the mRNA expression levels of SOX2 and cyclin D1 showed a significant negative correlation (r=-0.533, P<0.05) (Fig. 4C). Similarly, the results of western blot analysis revealed a significant negative correlation between SOX2 expression levels and phospho-S6 (S235/236), phospho-Akt (S473) and cyclin D1 expression in several tumor tissues; this correlation was consistent with that observed by immunohistochemical analysis (Fig. 4D and E). Taken together, these results demonstrate that SOX2 levels negatively correlate with mTOR signaling in human colorectal adenocarcinoma.

Discussion

SOX2, a high mobility group box transcription factor, plays a critical role in tumor cell proliferation. In gastric cancer, SOX2 has been shown to suppress cell proliferation by inducing cell-cycle arrestment (13). In lung squamous cell carcinoma, SOX2 has been shown to promote cell proliferation by affecting key

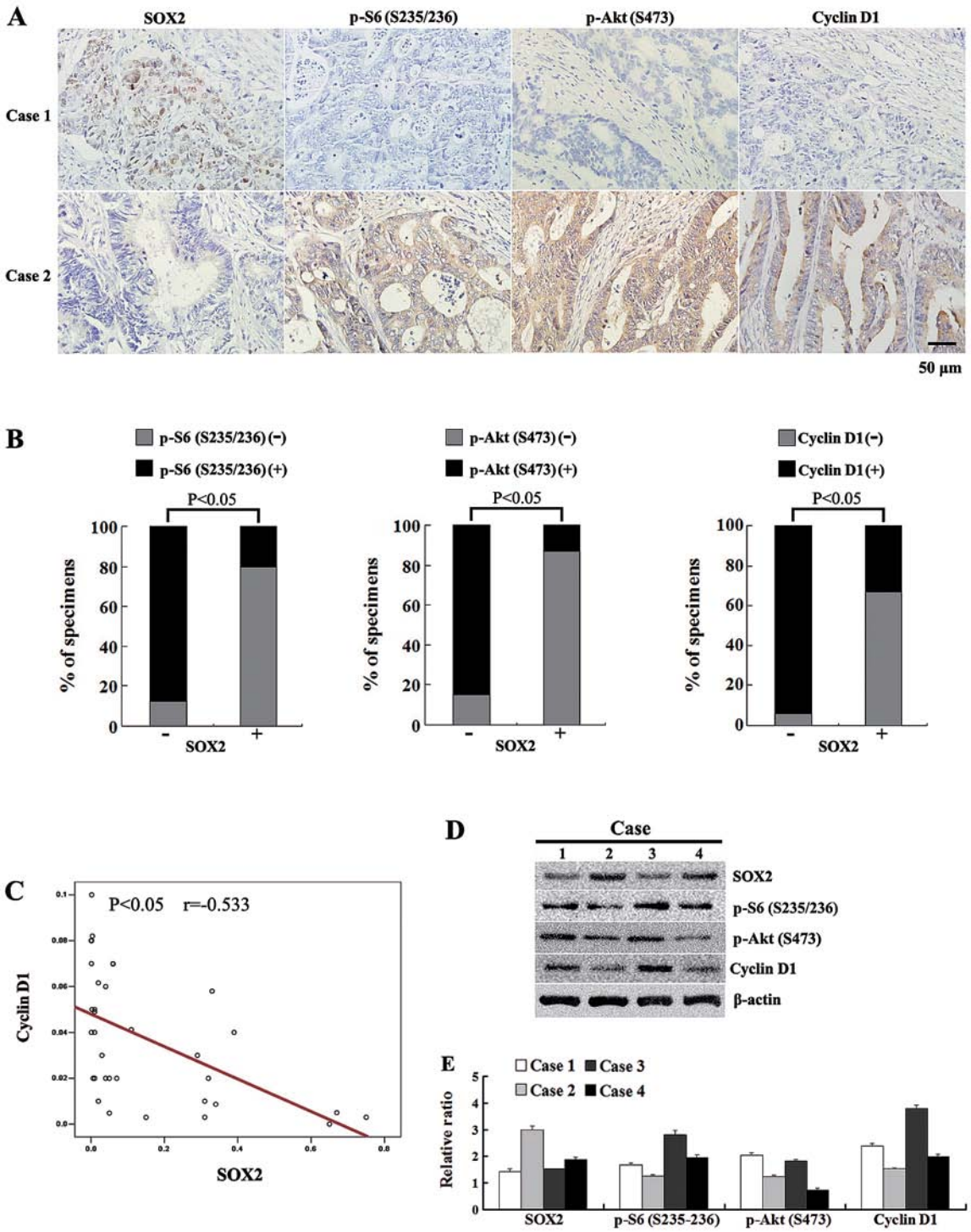

Figure 4. SOX2 expression correlates with mTOR signaling activity in colorectal adenocarcinoma tissues. (A) Immunohistochemical staining of SOX2, phospho-S6 (p-S6; S235/236), phospho-Akt (p-Akt; S473) and cyclin D1 expression in colorectal adenocarcinoma specimens (x400 magnification). Shown are two representative specimens. (B) Immunohistochemistry analysis revealed that SOX2 levels showed a significant negative correlation with the phospho-S6 (S235/236), phospho-Akt (S473) and cyclin D1 expression in colorectal adenocarcinoma tissues (P<0.05). (C) Correlation of SOX2 and cyclin D1 at the mRNA level analyzed using qRT-PCR (r=-0.533, P<0.05). (D and E) The expression levels of SOX2, phospho-S6 (S235/236), phospho-Akt (S473) and cyclin D1 in colorectal adenocarcinoma tissues lysates were determined by western blot analysis. Representative results are shown.

regulators of the cell cycle (10). The contradictory effect of SOX2 in gastric cancer and lung squamous cell carcinoma suggests that SOX2 plays a differential role in adenocarcinomas and squamous cell carcinomas. In the current study, we demonstrate that SOX2 effectively inhibits the proliferation of colorectal adenocarcinoma cells. Our results are consistent with those of a previous study, demonstrating the effects of SOX2 in gastric cancer (13). However, in contrast to our results, another previous study reported that SOX2 promoted the growth of SW620 colorectal cancer cells (23). The reason for these different results may be the different cell lines and experimental methods used, such as reaction temperature and

Table I. Correlation of SOX2 expression with clinicopathological characteristics in patients with colorectal adenocarcinoma.

Variables	No. of patients	SOX2		P-value
		Negative (-)	Positive (+ ,++, +++)	
Age (years)				0.281
<50	35	29	6	
≥50	32	23	9	
Gender				0.401
Male	43	32	11	
Female	24	20	4	
Tumor size				0.008
<5 cm	25	15	10	
≥5 cm	42	37	5	
Histological grade				0.256
Well/moderate	48	39	9	
Poor	19	13	6	
Tumor grade				0.972
1/2	36	28	8	
3/4	31	24	7	
Lymph node metastasis				0.533
Negative	53	42	11	
Positive	14	10	4	

time; thus, we performd further experiments to confirm our results.

To further determine the effects of SOX2 on HT-29 cell proliferation, we performed western blot analysis. The results showed that the mTOR pathway was inhibited by SOX2 in the HT-29 cells. Generally, the mTOR pathway plays a central role in the regulation of cell growth and proliferation. Hay and Sonenberg (14) suggested that mTOR contributes to cancer development through its effect on cell cycle progression. mTOR mediates the cell cycle progression partly through increasing the translation of cyclin D1 mRNA and other regulators of G0/G1-S phase progression (14,24). In certain cell types, the inhibition of the mTOR pathway induces G0/G1 phase cell cycle arrest, which correlates with the downregulation of cyclin D1 levels (25-28). In our study, flow cytometric analysis revealed that the cell cycle of SOX2-overexpressing cells was arrested at the G0/G1 phase. Cyclin D1, an important regulator of G0/G1-S phase celly cycle progression in many cell types, is often overexpressed in cancer cells and plays a critical role in the development and progression of many types of cancer, including colorectal adenocarcinoma (29). We performed qRT-PCR and western blot analysis, and found that the mRNA and protein expression level of cyclin D1 was significantly reduced in the SOX2-overexpressing cells, which indicated that SOX2 downregulated cyclin D1 expression in the HT-29 cells. Cyclin D1 is also known as a key factor whose expression is controlled by mTOR. The data presented above suggest that SOX2 inhibits the proliferation of colorectal adenocarcinoma cells, possibly by downregulating cyclin D1 expression via the mTOR pathway. On the basis of the aforementioned data, SOX2 may be a potential target for the inhibition of the proliferation of colorectal adenocarcinoma cells.

However, a causal link of SOX2 with mTOR signaling in cancers has not yet been reported. To examine whether SOX2 levels correlate with mTOR signaling in human colorectal adenocarcinoma, we carried out a series of experiments to explore the association of SOX2 and the mTOR pathway in colorectal adenocarcinoma tissues. Immunostaining analysis revealed that SOX2 was largely absent in the colorectal adenocarcinoma tissues, which is consistent with the notion that SOX2 is highly expressed in squamous cell carcinomas, but sparsely expressed in adenocarcinomas (30). Based on the clinicopathological analysis of SOX2, we reported a significant negative correlation between the expression of SOX2 and tumor size. The study by Gontan *et al* (31) also reported that SOX2-overexpressing lungs are reduced in size, which is possibly due to the slight reduction in cell proliferation. Of note, immunostaining and western blot analyses revealed that SOX2 negatively correlated with phospho-S6 (S235/236), phospho-Akt (S473) and cyclin D1 in the colorectal adenocarcinoma tissues, which is in accordance with our results obtained *in vitro*. The qRT-PCR results revealed a significant negative correlation between SOX2 and cyclin D1 at the mRNA level in the colorectal adenocarcinoma tissues. Taken together, these data further suggest that SOX2 inhibits the proliferation of colorectal adenocarcinoma cells through the mTOR pathway.

In conclusion, to our knowledge, our study demonstrates for the first time that SOX2 exerts potential anti-proliferative effects on colorectal adenocarcinoma cells through the mTOR pathway. We also indicate that the inhibition of the mTOR

pathway by the overexpression of SOX2 may contribute to the downregulation of cyclin D1 in colorectal adenocarcinoma cells. These findings confirm the important role of SOX2 in human colorectal adenocarcinoma, and may aid in the understanding of the molecular mechanisms behind the anti-proliferative effects of SOX2 in colorectal adenocarcinoma. Therefore, SOX2 can be considered a potential novel therapeutic agent for the treatment of colorectal adenocarcinoma.

Acknowledgements

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