Knockdown of *RAI14* suppresses the progression of gastric cancer

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Background: Retinoic acid induced 14 (*RAI14*), also known as *NORPEG*, is reported as being deregulated in non-small-cell lung cancer, together with having involvement in its cell proliferation as a super enhancer related gene.

Purpose: The objective of this study was to investigate the role of *RAI14* in the progression and metastasis of gastric cancer and explore the associated mechanism.

Materials and methods: GEPIA database was used to analyze the expression of *RAI14* in gastric cancer. MNK45 and AGS cells were transfected with siRNA-*RAI14* to block the expression of *RAI14*. Cell Counting Kit 8 and colony formation assays were performed to measure cell proliferation. Cell migration and invasion capacities was examined by transwell assay. Apoptosis rate was detected using flow cytometry, and the protein levels of apoptosis-related proteins was determined using Western blot assay. Reverse-transcription PCR assay was used to detect the expressions of *RAB31*.

Results: Gene expression profiling interactive analysis revealed that *RAI14* was substantially upregulated in gastric cancer and higher expression of *RAI14* was associated with worse prognosis. We also observed that the knockdown of *RAI14* by siRNA-*RAI14* transfection suppressed growth capacity of MKN45 and AGS cells. Also, *RAI14* knockdown inhibited migration and invasion of MKN45 and AGS cells in vitro. Moreover, *RAI14* knockdown was observed to accelerate cell apoptosis via downregulation of Bcl-2 and upregulation of Bax in MKN45 and AGS cells. Furthermore, downregulation of *RAI14* inhibited the activation of Akt pathway, and activation of Akt by IGF-1 could restore the reduced proliferation induced by *RAI14* knockdown. In addition, we found that *RAI14* had a positive correlation with the *RAB31* in gastric cancer by GEPIA reverse-transcription PCR and Western blot assays, and the reduced proliferation caused by *RAI14* knockdown was restored by *RAB31*.

Conclusion: *RAI14* knockdown inhibited proliferation, migration and invasion and promoted apoptosis by downregulating the Akt pathway in gastric cancer cells, and *RAB31* might be a downstream target gene of *RAI14*, providing a novel sight into the molecular mechanism of *RAI14* and a potential target for gastric cancer treatment.

Keywords: *RAI14*, gastric cancer, progression, Akt pathway, *RAB31*

Introduction

Gastric cancer is one of the major malignancies, causing many cancer-related deaths in the world, with approximately 951,000 new cases, leading to approximately 723,000 deaths each year.^{1,2} Although great progress has been made in the clinical treatment and diagnosis of gastric cancer in the past decade, the 5-year survival rate of patients following the radical operation is only between 30% and 50%.^{3,4} Accordingly, identification of the novel molecular target, which is able to repress the progression and metastasis of gastric cancer, is expected to facilitate the development of new cancer therapy strategies.

Retinoic acid induced 14 (*RAI14*), known as *NORPEG* as well, is an actin-binding protein initially identified in the liver.⁵ Previous studies identify that *RAI14* is expressed in a great deal of mammalian tissues or cells, but is primarily expressed in retina, placenta and

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testes and highly expressed in spermatozoa.⁶⁻⁹ *RAI14* is observed to be a regulatory protein at the ectoplasmic specialization, in addition to being confirmed to be involved in maintaining mouse spermatid polarity and cell adhesion through the regulation of the F-actin dynamics.^{9,10} Recent studies highlight that *RAI14* is a super enhancer (SE)-related gene that is upregulated in non-small-cell lung cancer (NSCLC) cell A549 and a portion of tumor tissues (43.66%, 32/71), and functions as a potential biomarker for the patients with lung adenocarcinoma.¹¹ SE is a large cis-regulatory element enhancing the expression of crucial genes that could define cell identity, in addition to playing a pivotal role in the process of development and cancer.¹²⁻¹⁴ SEs are reported to be enriched at oncogenes in cancer cells, which are generated and activated through chromosomal rearrangements, focal amplification, and upregulation of transcription factors.^{11,14,15} As confirmed by multiple research works, the upregulation of SE is involved in the tumor pathogenesis.¹⁶ Besides that, SEs in cancer cells are more susceptible to perturbation as compared with the typical enhancer, which leads to a more pronounced impairment of the expression of SEs-related genes.¹⁷⁻¹⁹ As these studies suggest, it is speculated that *RAI14* might be associated with the tumor progression, but there are few investigations dealing with this point, in particular in gastric cancer.

Herein, we demonstrate that *RAI14* was upregulated in gastric cancer associated with the patient's prognosis, and *RAI14* knockdown by siRNA interference reduced proliferation and migration, promoted apoptosis through inhibiting the activation of Akt signaling pathway in gastric cancer.

Materials and methods

Cell culture and transfection

The human gastric cancer cell lines MKN45 and AGS were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, People's Republic of China). Cells were cultured in DMEM (Hyclone, Thermo Fisher Scientific, Waltham, MA, USA) medium that contained 10% FBS (Gibco, Thermo Fisher Scientific) at 37°C with 5% CO₂. Cells were treated with IGF (50 ng/mL, R&D Systems Inc., Minneapolis, MN, USA) for 24 hours to activate Akt signaling pathway, or treated with LY294002 (3 μM; MedChemExpress, Monmouth Junction, NJ, USA) for 24 hours to inhibit the phosphorylation of Akt.

The siRNA-*RAI14* sequence was obtained from Oligobio (Beijing, People's Republic of China). Cells were transfected with siRNA-*RAI14* (*RAI14* knockdown, *RAI14*-KD) or siRNA negative control (NC) using Lipofectamine 2000 (Invitrogen, Thermo Fisher Scientific) in accordance with the manufacturer's protocol. Cells without any treatment constituted the blank group. Cells were transfected with

pcDNA3.1-*RAI14* (*RAI14* overexpression, *RAI14*-OV) to upregulate the expression of *RAI14*, and pcDNA3.1 was used as the NC by Lipofectamine 2000.

Western blot

Western blot assay was carried out for the purpose of examining the relative expression of *RAI14* and other related proteins according to the protocol. RIPA Lysis Buffer (CW BIO, Shanghai, People's Republic of China) was performed to lyse cells for protein extraction. The protein concentration was examined by BCA Protein Assay Kit (Beyotime, Jiangsu, People's Republic of China) and 20 μg of protein of each sample was separated by 10% SDS-PAGE gel. Thereafter, proteins were transferred to polyvinylidene fluoride membrane, and incubated with the primary antibodies in blocking solution at 4°C overnight. Subsequent to that, the membrane was incubated with the secondary antibody for 1 hour. An enhanced chemiluminescence kit was performed for the signal development. The primary antibodies were as follows: anti-*RAI14* (Proteintech Group Inc., Rosemont, IL, USA), anti-Bcl-2 (Abcam, Cambridge, UK), anti-Bax (Abcam), anti-Akt (Abcam), anti-p-Akt (Abcam), anti-Cyclin D1 (Abcam), and anti-GAPDH (Abcam). The secondary antibodies were obtained from Proteintech Group Inc.

Cell proliferation and colony formation assay

For CCK8 assay, approximately 1×10³ cells per well were seeded into 96-well plates. Then 10 μL of CCK8 reagent (Solarbio Science & Technology, Beijing, People's Republic of China) was added into each well, followed by incubating for 90 minutes at 37°C. The OD value of excitation light was measured every 24 hours using enzyme standard instrument with 450 nm. In regard to the colony formation assay, almost 500 cells were seeded into 10 cm dish containing 5 mL medium, and cultured at 37°C with 5% CO₂. Following the formation of sufficiently large clones, cells were stained with 0.1% crystal violet. The image was recorded and the number of colonies were counted as well.

Cell migration assay

Transwell chamber was used to assess cell migration and invasion. For invasion assay, chambers coated with Matrigel were used for invasion assay. Cells were trypsinized and resuspended in serum-free culture medium, and almost 1×10⁴ cells were transferred to the upper chamber, and complete medium was added to the lower chamber. Subsequent to incubation for 24 hours, the invasive cells were fixed with 4% paraformaldehyde, followed by staining with 0.1% crystal violet for 5 minutes. The invasive cells were imaged and counted

under the microscope. With respect to the migration assay, a method similar to the invasion assay employed; other than that, Matrigel was absent and the number of cells was 5×10^3 .

Cell apoptosis assay

Flow cytometry was carried out for the detection of the cell apoptosis using Annexin V-FITC-PI Apoptosis Detection Kit (4A Biotech Co., Beijing, People's Republic of China). Cells were resuspended in $1 \times$ binding buffer at a density of $1-5 \times 10^6$ cells/mL. The $100 \mu\text{L}$ cell suspensions were incubated with $5 \mu\text{L}$ of Annexin V-FITC for 5 minutes in the dark, followed by the addition of $10 \mu\text{L}$ of PI and $400 \mu\text{L}$ of PBS. The samples were analyzed using FACSCalibur instrument and analyzed by BD FACSDiva software.

Quantitative Reverse-Transcription PCR (RT-qPCR)

Total RNA was extracted using Trizol (PuFei, Shanghai, People's Republic of China) and reverse transcribed to cDNA by using the SuperRT cDNA Synthesis kit (CWbio). The expression of mRNAs was detected using SYBR Master Mixture (Takara Bio Inc., Kusatsu, People's Republic of China). Primers were as followed: RAI14 upstream: 5'-GTGGATGTGACAGCCCAAGA-3', downstream: 5'-TTTCCCAGAGCTGTCGACAC-3'; RAB31 upstream: 5'-TTGACCACAACATCAGCCCT-3', downstream: 5'-ACATCCCCTCCATGTGCATT-3'. Analysis of the relative expression of target gene was performed by the comparative Ct value.

Statistical analysis

The data were represented from three independent experiments and expressed as mean $\pm$ SD. The comparisons between the two groups were analyzed using Student's *t*-test. SPSS

18.0 software was used for analyzing the data in the present study. $P < 0.05$ was considered statistically significant.

Results

Expression of *RAI14* is upregulated in gastric cancer and associated with the poor prognosis

Gene expression profiling interactive analysis (GEPIA) is a website for a deep understanding of gene functions through excavation of the RNA sequencing data from TCGA and GTEx databases.²⁰ The expression level of *RAI14* in gastric cancer tissue was substantially higher in comparison with that of the normal tissue by GEPIA, which suggested that *RAI14* is upregulated in gastric cancer (Figure 1A). Further prognostic analysis revealed that the overall survival (OS) of gastric cancer patients with higher *RAI14* expression level was significantly worse as compared with that of the patient with lower expression level, indicating that *RAI14* expression level had correlation with the prognosis of gastric cancer patients ($P=0.0001$, Figure 1B). Survival analysis results from Kaplan–Meier Plotter (<http://kmplot.com/analysis/index.php?p=service&cancer=gastric>) shed light on the fact that patients with low expression of *RAI14* had better prognosis in comparison with the patients with high expression ($P=2.9 \times 10^{-12}$, Figure 1C). Based on these results, *RAI14* might be involved in gastric cancer and correlated with the prognosis of gastric cancer patients.

RAI14 knockdown reduces growth capacity of gastric cancer in vitro

To investigate the role of *RAI14* in gastric cancer, we utilized the gastric cancer cell lines MKN45 and AGS for transfecting with *RAI14* siRNA-1# and siRNA-2# or siRNA NC in order to block the expression of *RAI14*. The expression of *RAI14* mRNA was significantly inhibited by siRNA14-2#

Figure 1 (Continued)

Figure 1 RAI14 downregulation is associated with prognosis of gastric cancer patients and reduces proliferation and viability of gastric cancer cells.

Notes: (A) The expression of RAI14 mRNA in gastric cancer tissues (red box) and normal tissues (black box) from GEPIA. (B) The survival curve of gastric cancer patients with different level of RAI14 mRNA from GEPIA. (C) The survival curve of gastric cancer patients from KM plotter analysis. (D) The expression of RAI14 mRNA in MNK45 and AGS cells by RT-PCR assay. (E, F) Expression of RAI14 protein in MNK45 (E) and AGS (F) cells with or without siRNA-RAI14. (G, H) Cell proliferation curve of MNK45 (G) and AGS (H) cells with or without siRNA-RAI14 from CCK8 assay. (I, J) Colony formation ability of MNK45 (I) and AGS (J) cells with or without siRNA-RAI14. Blank: cells without any treatment; NC: negative control, cells transfected with siRNA negative control; RAI14-KD: cells transfected with siRNA-RAI14, RAI14 knockdown. * $P < 0.05$. Results are from three repeated experiments.

Abbreviations: GEPIA, gene expression profiling interactive analysis; RT-PCR, reverse-transcription PCR; STAD, stomach adenocarcinoma.

both in MNK45 and AGS cells ($P < 0.05$, Figure 1D), and siRAI14-2# was used for subsequent experiments. Western blot was also carried out and the expression level of RAI14 protein was assessed. As evident from Figure 1E and F, RAI14 siRNA transfected cells showed a significantly decreased level of RAI14 protein as compared with the NC or blank group ($P < 0.05$). CCK8 assay was conducted to examine the effect of RAI14 knockdown on cell proliferation in MNK45 and AGS cells. Our data showed that,

following the culture for 48 hours, the proliferation ability of the siRNA-RAI14 interfered cell was clearly attenuated as compared with the NC or blank group ($P < 0.05$, Figure 1G and H). Moreover, the colony formation assay demonstrated that the colony formation ability was significantly decreased in RAI14 knockdown cells as compared with the NC or blank group ($P < 0.05$, Figure 1I and J). Taken together, as these results indicated, RAI14 knockdown significantly inhibited growth capacity of gastric cancer cells in vitro.

RAI14 knockdown inhibits cell migration and invasion of gastric cancer

In order to investigate whether *RAI14* affected tumor metastasis, a Transwell assay was adopted. As presented in Figure 2A and B, the migratory ability was both significantly decreased in *RAI14* deficiency MKN45 and AGS cells as compared with the NC or

blank group ($P < 0.01$). Besides that, Transwell invasion assays revealed that *RAI14* knockdown inhibited MKN45 and AGS cells invasion (Figure 2C and D). In parallel, the roles of *RAI14* overexpression in MKN45 and AGS cells were also investigated. *RAI14* overexpression increased the migration and invasion of gastric cancer cells in vitro (Figure S1A and B). Together, these

Figure 2 (Continued)

Figure 2 RAI14 knockdown inhibits cell migration and invasion, promotes apoptosis of gastric cancer cells.

Notes: (A, B) Cell migration of MKN45 (A) and AGS (B) cells with or without siRNA-RAI14 was assessed using Transwell assay. (C, D) Cell migration of MKN45 (C) and AGS (D) cells with or without siRNA-RAI14 was assessed. (E) Cell apoptosis of MKN45 (left) and AGS (right) cells with or without siRNA-RAI14 was evaluated using flow cytometry assay. (F) Expression of apoptosis-related proteins (Bcl-2, Bax, active Caspase3 and 9) in MKN45 and AGS cells with or without siRNA-RAI14. * $P < 0.05$, ** $P < 0.01$. Results are from three repeated experiments.

Abbreviations: KD, knockdown; NC, normal control.

data suggested an inhibition effect of *RAI14* knockdown on migration and invasion of gastric cancer cell in vitro.

RAI14 knockdown promotes cell apoptosis of gastric cancer

One of the significant hallmarks of tumor cells is dysregulated apoptosis, and flow cytometry was performed to examine the apoptosis rate in *RAI14* knockdown cells. We observed that *RAI14* knockdown significantly enhanced the apoptosis rate as compared with the NC or blank group both in MKN45 and AGS cells ($P < 0.05$, Figure 2E). Furthermore, *RAI14* silencing downregulated the expression of anti-apoptotic protein Bcl-2 and upregulated the expression of pro-apoptotic protein Bax, active Caspase3 and active Caspase9 in MKN45 and AGS cells ($P < 0.05$, Figure 2F). Meanwhile, *RAI14* overexpression was observed to reduce apoptosis rate in MKN45 and AGS cells (Figure S1C), and upregulate the expression of Bcl-2 and downregulate the expression of Bax, active Caspase3 and active Caspase9 in gastric cancer cells ($P < 0.05$, Figure S1D). Taken together, these results indicated that silencing *RAI14* could accelerate apoptosis in gastric cancer cells via regulating Bcl-2/Bax axis and Caspase cascade.

RAI14 knockdown inhibits the activation of the Akt pathway in gastric cancer

In order to further explore the related mechanisms of *RAI14* affecting the progression of gastric cancer, we detected changes

in the Akt pathway following the *RAI14* knockdown in MKN45 cells. The Akt pathway is one of the pivotal cellular pathways involved in cell proliferation, differentiation and apoptosis and participates in tumor progression and metastasis. It has been revealed that the Akt pathway is frequently activated in gastric cancer.²¹ As the Western blot results suggest, no significant change was observed in the expression of total Akt in *RAI14* knockdown MKN45 cells as compared with the NC or blank group (Figure 3A). Nevertheless, *RAI14* knockdown significantly reduced the level of the phosphorylated form p-Akt in MKN45 cells ($P < 0.01$, Figure 3A). Meanwhile, the expression of its downstream protein Cyclin D1 was also decreased in the *RAI14* deficiency MKN45 cells ($P < 0.01$, Figure 3A). It was inferred that *RAI14* knockdown could inhibit the activation of Akt signaling pathway in gastric cancer. Besides that, as the Western blot results suggest, the inhibition on p-Akt by *RAI14* knockdown was similar to that of LY294002, an Akt inhibitor, and it was also applied to the inhibition on proliferation (Figure 3B and C). Together with that, on the activation of Akt by IGF-1, a well-known Akt agonist, the reduction in phosphorylated of Akt and cell proliferation induced by *RAI14* knockdown was significantly restored ($P < 0.05$, Figure 3B and C). These results highlighted that Akt pathway was involved the modulation of *RAI14* in the progression of gastric cancer.

RAB31 is a potential target for *RAI14*

The GEPIA revealed that the expression of *RAI14* was correlated with *RAB31* in gastric cancer ($P = 1.1 \times 10^{-55}$, $R = 0.65$,

Figure 3 RAI14 knockdown inhibits the activation of Akt pathway.

Notes: (A) Western blot results of key proteins in Akt pathway in MKN45 cell with or without siRNA-RAI14. (B) Expression of p-Akt in MKN45 cell with siRNA-RAI14, IGF-1 or LY294002. (C) Cell proliferation curve of MKN45 cells with siRNA-RAI14, IGF-1 or LY294002 from CCK8 assay. Results are from three repeated experiments. IGF-1: cells were treated with IGF-1 (50 ng/mL). * $P < 0.05$, ** $P < 0.01$.

Abbreviations: KD, knockdown; NC, normal control.

Figure 4 RAB31 is a potential target gene for RAI14.

Notes: (A) The correlation analysis from GEPIA between expression of RAI14 mRNA and RAB31 mRNA in gastric cancer. (B) Expression of RAB31 mRNA in MKN45 cells with or without siRNA-RAI14. (C) Expression of RAB31 mRNA in MKN45 cells with or without RAB31-overexpression vector. RAB31-OV: cells were transfected with RAB31-overexpression vector. (D) Expression of RAI14, RAB31 and p-Akt proteins in MKN45 cells by Western blot assay. (E) Cell proliferation curve of MKN45 cells with or without RAB31-overexpression vector from CCK8 assay. Results are from three repeated experiments. * $P < 0.05$, ** $P < 0.01$.

Abbreviations: GEPIA, gene expression profiling interactive analysis; KD, knockdown; NC, normal control; OV, overexpression.

Figure 4A). Our data also show that the expression of *RAB31* mRNA was significantly reduced when *RAI14* was knocked down in MKN45 cells ($P < 0.01$, Figure 4B). To further confirm the results, MKN45 cells were transfected with *RAB31* overexpression vector (*RAB31*-OV) (Figure 4C). And *RAB31* overexpression did not have significant effect on the level of *RAI14* protein and p-Akt in MKN45 cells as compared with the blank group, and upregulation of *RAB31* could not restore the decrease in the level of *RAI14* and p-Akt caused by loss of *RAI14* in MKN45 cells (Figure 4D). As shown in Figure 4E, *RAB31* overexpression could significantly restore the decrease in cell proliferation induced by *RAI14* knockdown ($P < 0.05$). These results indicated that *RAB31* might be a downstream target gene of *RAI14* in gastric cancer.

Discussion

RAI14 has been proved as an SE associated gene that is upregulated in lung adenocarcinoma and impacts the cell proliferation.¹¹ To the best of our understanding, no data are available to indicate the relationship between *RAI14* and gastric cancer. In our study, we observed that the expression of *RAI14* was upregulated in gastric cancer and the *RAI14* expression level was correlated with the prognosis of gastric cancer patients, indicating that *RAI14* might be involved in progression of gastric cancer. This possibility has received further support of the findings that *RAI14* knockdown by siRAI14 interference reduced growth capacity, migration and invasion of MKN45 and AGS cells in vitro, meanwhile, upregulation of *RAI14* increased cell migration and invasion, suppressed cell apoptosis in MKN45 and AGS cells. In ovarian cancer, *RAI14* is suggested to increase cell proliferation and shorten the cell cycle as a downstream gene of NR2F2.^{23,24} Yuan et al¹¹ previously reported that *RAI14* is upregulated in lung adenocarcinoma cells and tissues, indicating *RAI14* acts as a SE-associated oncogene in lung adenocarcinoma, while over-expression of *RAI14* has an inhibitory effect on proliferation of human bronchial epithelial cell line BEAS-2B. As the above results suggest, *RAI14* functions as an oncogene in the progression of gastric cancer and *RAI14* deficiency could suppress growth and metastasis of gastric cancer. Nevertheless, the inconsistent role of *RAI14* between tumor cells and normal cells requires further investigation, most likely owing to the diversity between tumor cells and normal cells.

Apoptosis is a pivotal regulatory mechanism for controlling cell growth, the intrinsic mitochondrial pathway is a crucial mechanism to trigger apoptosis. Escaping apoptosis is the key point for the malignant transformation and tumor progression.

Herein, we worked out that *RAI14* knockdown significantly promoted the apoptosis rate of MKN45 and AGS cells in vitro and downregulated the expression of Bcl-2 and upregulated the expression of Bax, active Caspase3 and Caspase9, suggesting that loss of *RAI14* could induce cell apoptosis via regulating Bcl-2/Bax axis and Caspase cascade in gastric cancer.

As everyone understands, Akt pathway is involved in cell proliferation and regulation of apoptosis by impacting the expression level of downstream proteins. The Akt pathway is confirmed to be involved in tumor progression, and has been revealed to be upregulated in gastric cancer.^{24,25} Accordingly, the Akt pathway appears to be a good candidate for evaluating the mechanism of *RAI14* to impair proliferation, migration and apoptosis of gastric cancer cells. We discovered that downregulation of *RAI14* dramatically inhibited the activation of Akt pathway through reducing the phosphorylated form of Akt and its downstream Cyclin D1. Besides that, the reduction in proliferation caused by *RAI14* knockdown was significantly restored by Akt activation stimulated by IGF-1. And the inhibition on p-Akt by *RAI14* knockdown was similar to that of LY294002. Therefore, our data suggest that Akt pathway is involved in the effect of *RAI14* on the progression and growth in gastric cancer. Moreover, the specific way by which *RAI14* affects the Akt pathway should be explored in future research.

RAB31, a member of Rab family, is proved to be upregulated in hepatocellular carcinoma tissues, and upregulation of *RAB31* could accelerate proliferation of multiple cancer cells, including glioblastoma, cervical cancer, hepatocellular carcinoma and breast cancer.²⁶⁻²⁸ Accordingly, knockdown of *RAB31* is expected to inhibit tumor growth through decreasing cell proliferation and promoting apoptosis,^{26,27} suggesting that *RAB31* plays an important role in promoting tumor progression. Herein, the regulatory pattern of *RAI14* in gastric cancer was similar to *RAB31*, and the expression of *RAI14* had a positive correlation with *RAB31*. We also observed that decrease in cell proliferation induced by *RAI14* knockdown was rescued by *RAB31* overexpression. As these results suggested, *RAB31* might be a downstream target gene of *RAI14* and involved in the regulation of *RAI14* on the growth of gastric cancer.

Conclusion

The current study confirmed that *RAI14* was aberrantly upregulated in gastric cancer and associated with prognosis. Herein, we also observed that loss of *RAI14* inhibited cell proliferation, migration, invasion and survival. Moreover, *RAB31* was a downstream target gene of *RAI14* and involved

in the regulation of *RAI14* on the growth of gastric cancer. These data provide proof that *RAI14* is a novel potential target for gastric cancer treatment.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary material

Figure S1 RAI14 overexpression promotes cell migration and invasion, reduces cell apoptosis in gastric cancer cells.

Notes: (A) Cell migration of MNK45 (left) and AGS (right) cells with RAI14 overexpression vector was assessed using Transwell assay. (B) Cell migration of MNK45 (left) and AGS (right) cells with RAI14 overexpression vector was assessed. (C) Cell apoptosis of MNK45 (left) and AGS (right) cells with RAI14 overexpression vector was evaluated using flow cytometry assay. (D) Expression of apoptosis-related proteins (Bcl-2, Bax, active Caspase3 and 9) in MNK45 and AGS cells with RAI14 overexpression vector. * $P < 0.05$. Results are from three repeated experiments.

Abbreviations: KD, knockdown; NC, normal control; OV, overexpression.

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