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ORIGINAL ARTICLE

Overexpression of Long Non-Coding RNA FGF14-AS2 Inhibits Colorectal Cancer Proliferation Via the RERG/Ras/ERK Signaling by Sponging microRNA-1288-3p

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Abstract
Colorectal cancer remains one of most common cancer types with poor prognosis globally. Recent years, numerous studies depicted pivotal roles of lncRNAs in colorectal cancer progression. This study aimed to investigate the role of FGF14-AS2 in colorectal cancer development. FGF14-AS2 was found as a significantly downregulated lncRNA in TCGA dataset. Via RT-qPCR, we confirmed the downregulation of FGF14-AS2 in collected colorectal carcinoma samples. Transfection of plasmid containing full length of FGF14-AS2 repressed cell proliferation and induced elevation of cell apoptosis in colorectal cancer cells. In addition, FGF14-AS2 overexpression inactivated MAPK/ERK signaling in cells. Bioinformatic analysis and subsequent cell-based assays showed that FGF14-AS2 sponging miR-1288-3p, an oncogenic miRNA in colorectal cancer. RERG, the regulator of Ras/ERK pathway, was predicted and verified as target gene of miR-1288. Via downregulation of miR-1288, FGF14-AS2 elevated RERG expression in colorectal cancer cells. Rescue assays indicated that FGF14-AS2 relied on regulation of RERG to control cell proliferation and apoptosis in colorectal cancer. Taken together, the current study demonstrated FGF14-AS2 as a regulator of colorectal cancer development via downregulation of miR-1288-3p and inactivation of Ras/ERK signaling.

Keywords FGF14-AS2 · RERG · Colorectal cancer

Introduction

Colorectal cancer is currently one of prevalent cancer types globally. In United States, there are more than 140,000 new cases according to data of 2019 [1]. In recent years, chemotherapy based on 5-fluorouracil has prolonged overall survival of patients with colorectal cancer [2]. However, due to the complexity of signaling network in colorectal cancer, colorectal cancer cells exhibited strong proliferative ability and resistance to cell apoptosis. It is imperative to discover novel targets and mechanisms for colorectal cancer development.

Long non-coding RNAs (lncRNAs) are single-stranded molecules with more than 200 nucleotides in length [3]. According to competing endogenous RNA (ceRNA) hypothesis, lncRNAs interact with microRNAs (miRNAs) and release mRNA from miRNA binding [4]. Via sponging miRNAs, lncRNAs regulate gene expression to control many physiology processes. Aberrant expression of lncRNAs leads to disruption of signaling network and human diseases including colorectal cancer [5–7]. For example, lncRNA MALAT1 sponged miR-145 and upregulated SOX9 expression to suppress colorectal cancer cell proliferation in vitro and in vivo [8]. FGF14 antisense RNA 2 (FGF14-AS2) was screened out as one of most significantly downregulated lncRNAs in breast cancer compared with adjacent normal tissues by RNA sequencing [9]. Its expression was associated with tumor size, lymph node metastasis status and clinical stage of breast cancer [9]. Its tumor suppressor role and molecular mechanism has been reported in breast cancer [10]. The function of FGF14-AS2 in colorectal cancer has not been studied yet.

MAPK/ERK pathway is the most well-characterized signaling in cells [11]. Dysregulation of MAPK/ERK pathway contributed to uncontrolled cell proliferation and resistance to cell apoptotic signal [12, 13]. Sustained activation of MAPK/ERK pathway is related to downregulation of negative

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regulator of the signaling in cancer cells [14]. RAS Like Estrogen Regulated Growth Inhibitor (RERG) exhibited intrinsic GDP/GTP binding and GTP hydrolysis activity but inhibited MAPK/ERK pathway [15]. Downregulation of RERG is observed in several cancer types including colorectal cancer [16–19].

The current study focused on investigation the role of FGF14-AS2 in colorectal cancer. Our data revealed that downregulation of FGF14-AS2 contributed to cell proliferation and resistance to cell apoptosis in colorectal cancer cells. The study manifested that FGF14-AS2 is a promising biomarker for colorectal cancer.

Materials and Methods

Cell Culture

Human colorectal cancer cell lines HCT116 (KRAS G13D, BRAF mutant), SW480 (KRAS G12V, BRAF wild type), HT29 (KRAS wild type, BRAF V600E) were purchased from ATCC (Pennsylvania, USA). Cells were cultured in DMEM with 10% FBS at 37 °C in an incubator with 5% CO_2.

Patients and Samples

All 62 tumors and matched normal tissues were collected from colorectal cancer patients underwent surgery in the Third Hospital of Jilin University during June 2016 to July 2018. The exclusion criteria were the presence of previous treatment, both locally or systemically. All patients provided informed consent. The protocol was approved by ethical approval board of the Third Hospital of Jilin University.

Cell Proliferation Assay

The proliferation ability of cells was detected with a CCK-8 kit. Briefly, CCK-8 solution was added into culture medium and sustained for 2 h. The 450 nm absorbance of medium containing CCK-8 was detected with a Microplate Reader.

Cell Apoptosis Assay

The percentage of apoptotic cells was determined with the flow cytometry. Briefly, cells were harvested and stained with PI and Annexin V provided by Annexin V-FITC/PI Apoptosis Detection Kit (Invitrogen; Thermo Fisher Scientific, Carlsbad, USA). After incubation, cells were subjected to flow cytometry analysis on a MACSQuant Analyzer 10 (Miltenyi Biotech, Germany). The data were analyzed on the FlowJo software (Becton, Dickinson & Company, Franklin Lake, USA).

Cell Transfection

Full length of FGF14-AS2 was inserted into pcDNA3.1 expression plasmid. miR-1288-3p mimic, miR-1288-3p inhibitor and miR-NC were synthesized by RiboBio (Guangzhou, China). RERG siRNA and control siRNA were synthesized by Genepharma (Suzhou, China). Empty vector, pcDNA3.1-FGF14-AS2, miR-1288-3p mimic, miR-1288-3p inhibitor and miR-NC was transfected into cells by Lipofectamine 3000 reagent (Invitrogen). RERG siRNA and control siRNA was transfected into cells by Lipofectamine RNAiMAX reagent (Invitrogen). The cells were cultured for 3 days then subjected to validation of transfection efficiency.

Western Blotting

p-ERK1/2 and ERK1/2 antibodies were bought from CST (Beverly, USA). GAPDH antibody was obtained from Sigma-Aldrich (Germany). RERG antibody was bought from Abcam (Cambridge, UK). HRP-conjugated anti-mouse and anti-rabbit antibodies were products of Novus Biologicals (Missouri, USA). Proteins were separated from cells by RIPA lysis buffer. Lysates were separated by SDS-PAGE gels and transferred to PVDF membranes. After incubation with primary and secondary antibody, membranes were developed with an ECL western blotting substrate (Thermo Fisher Scientific). The bands were quantified with Image J software (NIH, USA).

RT-qPCR

RNA was extracted from cells with TRIzol reagent (Invitrogen). RT-qPCR was performed with SYBR Premix Ex Taq II (Takara, Tokyo, Japan) on an ABI 7500 StepOnePlus system (Applied Biosystems, CA, USA). GAPDH and U6 were internal controls for lncRNA/mRNA and miRNA with the $2^{-\Delta\Delta Ct}$ method. The primer sequences were listed as follow: FGF14-AS2-F:5'-CCGTCCAAAGCAACTGGAAC-3′; FGF14-AS2-R:5'-TAGCAAACGTTAGGGCACCA-3′; RERG-F:5'-CTTGTGCTTTTTACGAGTGCTC-3′; RERG-R:5'-TAATGGCTTGCTTGACATGCG-3′; GAPDH-F:5'-CTGGGCTACACTGAGCACC-3′; GAPDH-R:5'-AAGTGGTCGTTGAGGGCAATG-3′; miR-1288-3p-F:5'-TCGGCAGGTGGACTGCCCTGAT-3′; miR-1288-3p-R:5'-CTCAACTGGTGTCGTGGA-3′; U6-F:5'-CCGCCCGCCGCCAGGCCCC-3′; U6-R:5'-ATATGGAACGCTTCACGAATT-3′.

Bioinformatic Analysis

The expression of FGF14-AS2 in colorectal tumors and normal tissues from TCGA data were analyzed with GEPIA software (http://gepia.cancer-pku.cn/). UCSC Xena software was

used to analyze the association between FGF14-AS2 expression and mutation status of KRAS and BRAF in TCGA-COAD dataset (https://xena.ucsc.edu/). Several frequent mutations were selected for the study including A146T, G12A, G12C, G12D, G12S, G12V and G13D of KRAS, and V600E of BRAF. GSE41657 dataset (25 colorectal tumors and 12 normal tissues) were analyzed with GEO2R software provided by GEO database (https://www.ncbi.nlm.nih.gov/gds/?term=). Full length of FGF14-AS2 was placed into miRDB software (http://mirdb.org/) to predict interaction with miRNAs. The targets of miR-1288-3p was predicted with TargetScan software (http://www.targetscan.org/vert_72/). The association between FGF14-AS2 and RERG expression in TCGA data was studied with GEPIA software.

Dual Luciferase Reporter Assay

FGF14-AS2, RERG 3'UTR or their mutant forms was ligated into pmirGLO luciferase plasmid. pmirGLO plasmid with or without insertion was co-transfected with miR-NC or miR-1288-3p mimic into cells with Lipofectamine 3000. After 48 h, the luciferase activity of each group was detected with a Dual Luciferase Reporter System (Promega Corps, Madison, USA). The firefly luciferase was normalized to renilla luciferase.

Data Analysis

The data were analyzed by Graphpad Prism 6. The results were presented as mean ± SD. Groups were compared with Student's t test or one-way ANOVA followed by Tukey's test. The association between FGF14-AS2 and RERG was analyzed by Pearson correlation analysis. *P* value less than 0.05 was statistically significant.

Results

FGF14-AS2 Was Lowly Expressed in Colorectal Cancer

To investigate colorectal cancer related lncRNAs, we reviewed list of differentially expressed lncRNAs reported by Chen et al [20]. FGF14-AS2 was one of most significantly downregulated lncRNA across several dataset. In TCGA database, our analysis indicated that FGF14-AS2 was more than 10-fold decrease in colorectal cancer tissues compared with normal tissues (Fig. 1a). It is well-characterized that the development of colorectal cancer is driven by oncogenic gene mutations (especially KRAS and BRAF) and subsequent overactivation of MAPK/ERK signaling [21]. We therefore examined whether the expression of FGF14-AS2 was associated with oncogene mutations. In TCGA-COAD dataset, we found that FGF14-AS2 expression was not increased or decreased in tumors with common KRAS mutations (A146T, G12A, G12C, G12D, G12S, G12V and G13D) compared with those without KRAS mutations (Fig. 1b). Similarly, analysis showed that FGF14-AS2 expression was not altered in tumors with BRAF mutation (V600E) compared with their counterpart (Fig. 1c). In a previously published microarray

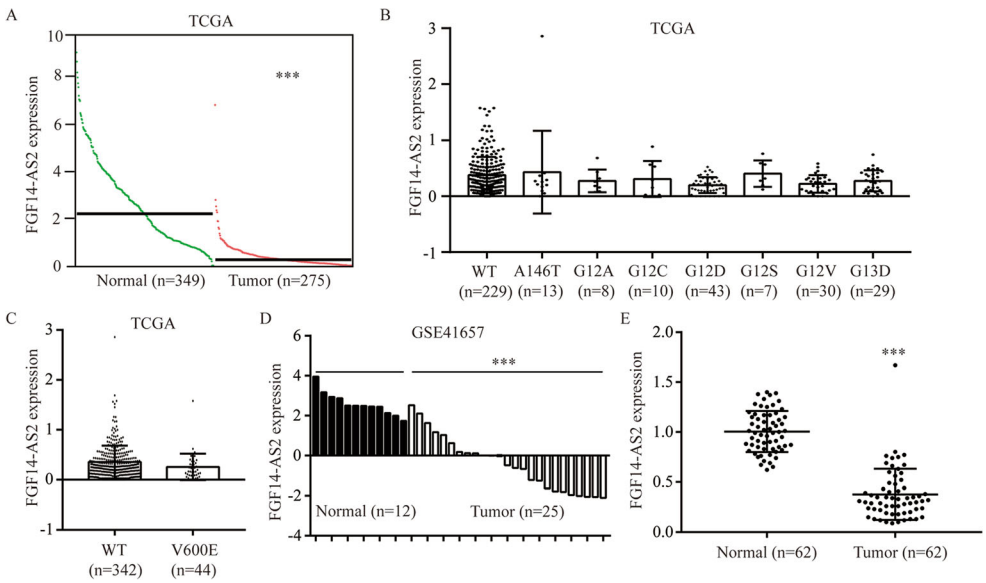

Fig. 1 FGF14-AS2 was a downregulated lncRNA in colorectal cancer. **a** GEPIA software was used to analyze the expression of FGF14-AS2 in TCGA data (275 colorectal tumors and 349 normal colorectal tissues). **b** The expression of FGF14-AS2 in colorectal tumors with wild-type (WT) KRAS, tumors with KRAS mutations (A146T or G12A or G12C or G12D or G12S or G12V or G13D) was analyzed using TCGA-COAD dataset. **c** The expression of FGF14-AS2 in colorectal tumors with WT BRAF, tumors with BRAF mutations (V600E) was analyzed using TCGA-COAD dataset. **d** Expression of FGF14-AS2 in GSE41657 dataset (25 colorectal tumors and 12 normal colorectal tissues) was analyzed. **e** FGF14-AS2 expression in 62 pairs of colorectal tumors and matched normal tissues was determined by RT-qPCR. ***, $p < 0.001$

dataset (GSE41657), FGF14-AS2 was also decreased in colorectal tumors (Fig. 1d). In our collected specimens (62 pairs of tumors and normal tissues), RT-qPCR further showed a decreased expression of FGF14-AS2 in colorectal tumors (Fig. 1e). The data suggested a potential role of FGF14-AS2 in colorectal cancer.

Overexpression of FGF14-AS2 Inhibited Colorectal Cancer Cell Proliferation and Promoted Cell Apoptosis

We analyzed FGF14-AS2 expression in a panel of colorectal cancer cell lines and normal colorectal tissues. It was found that FGF14-AS2 was decreased in colorectal cancer cells (HCT116, SW480, HT29) compared with normal colorectal tissues ($n = 6$) (Fig. 2a). We then transfected full length of FGF14-AS2 into colorectal cancer cells. Transfection of FGF14-AS2 induced 7-fold increase of FGF14-AS2 expression in HCT116 cells (Fig. 2b). Overexpression of FGF14-AS2 reduced cell proliferation rate of HCT116 cells in a CCK-8 assay (Fig. 2c). Additionally, via flow cytometry analysis, it was further revealed that FGF14-AS2 overexpression dramatically elevated percentage of apoptotic cells (Fig. 2d). Consistent with our observation in HCT116 cells, transfection of FGF14-AS2 increased FGF14-AS2 expression in SW480 cells which induced cell proliferation inhibition and cell apoptosis (Fig. 2e-g). The data indicated that FGF14-AS2 repressed colorectal cell proliferation and contributed to colorectal cancer cell apoptosis.

FGF14-AS2 Inactivated MAPK/ERK Pathway

Sustained activation of MAPK/ERK pathway facilitated colorectal cancer cell proliferation and resistance to cell apoptosis [11]. Western blotting showed that FGF14-AS2 overexpression decreased p-ERK1/2 expression and did not change total ERK1/2 expression in HCT116 and SW480 cells (Fig. 3a-b), indicating FGF14-AS2 regulated MAPK/ERK pathway in colorectal cancer.

FGF14-AS2 Sponged miR-1288-3p

miRDB database was used to predict potential complementary sequences between FGF14-AS2 and miRNAs. Among 27 predict results, miR-1288-3p was a reported colorectal cancer related miRNA and might bind to FGF14-AS2 in the region near 5′ end (Fig. 4a). RT-qPCR showed that FGF14-AS2 overexpression decreased miR-1288-3p expression in HCT116 and SW480 cells (Fig. 4b). To validate their association, we transfected miR-1288-3p mimic into cells to elevate miR-1288-3p levels (Fig. 4c). Luciferase plasmids containing FGF14-AS2(FGF14-AS2 WT) or its mutant form (FGF14-AS2 Mut) were constructed (Fig. 4d). The dual luciferase reporter assay data showed that miR-1288-3p mimic could repress luciferase activity of FGF14-AS2 WT in HCT116 and SW480 cells (Fig. 4e-f). Thus, FGF14-AS2 sponged miR-1288-3p in colorectal cancer cells.

miR-1288-3p Repressed RERG Expression

TargetScan was used to predict target gene of miR-1288-3p. RERG, a negative regulator of MAPK/ERK pathway, was predicted as a target gene of miR-1288-3p (Fig. 5a). Transfection of miR-1288-3p inhibitor decreased miR-1288-3p expression in HCT116 and SW480 cells (Fig. 5b). Meanwhile, RERG mRNA expression was elevated (Fig.

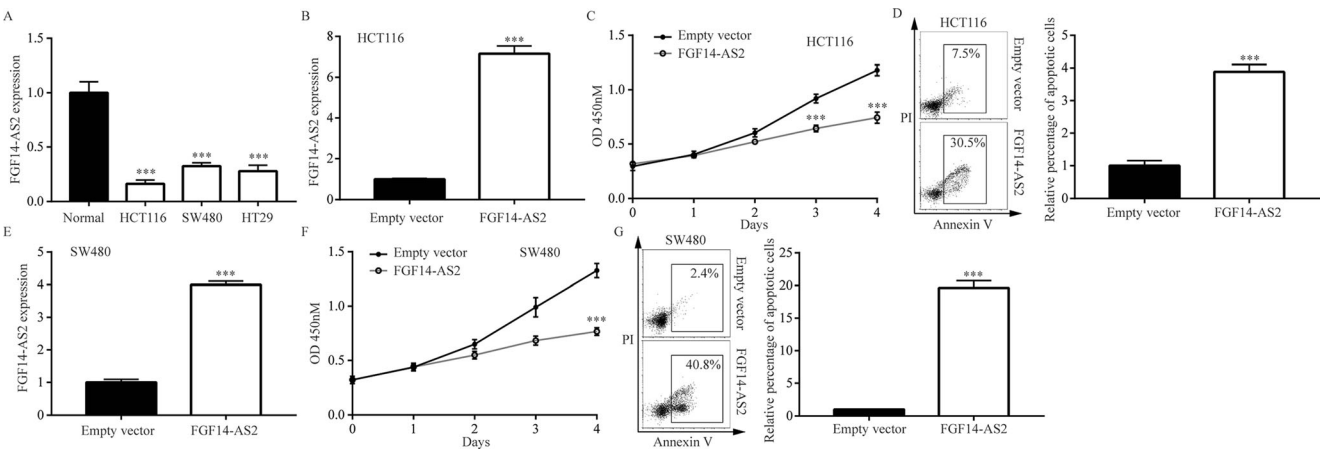

Fig. 2 Upregulation of FGF14-AS2 negative regulated cell proliferation and triggered cell apoptosis. **a** The expression of FGF14-AS2 was detected in normal colorectal tissues ($n = 6$) and colorectal cancer cell lines (HCT116, SW480, HT29) by RT-qPCR. **b** Transfection of FGF14-AS2 elevated FGF14-AS2 expression in HCT116 cells. **c** The CCK-8 assay was used to detect cell proliferation ability in HCT116 cells transfected with empty vector or FGF14-AS2. **d** Flow cytometry was used to detect cell apoptosis status in HCT116 cells transfected with empty vector or FGF14-AS2. **e** Transfection of FGF14-AS2 elevated FGF14-AS2 expression in SW480 cells. **f** The CCK-8 assay was used to detect cell proliferation ability in SW480 cells transfected with empty vector or FGF14-AS2. **g** Flow cytometry was used to detect cell apoptosis status in SW480 cells transfected with empty vector or FGF14-AS2. ***, $p < 0.001$

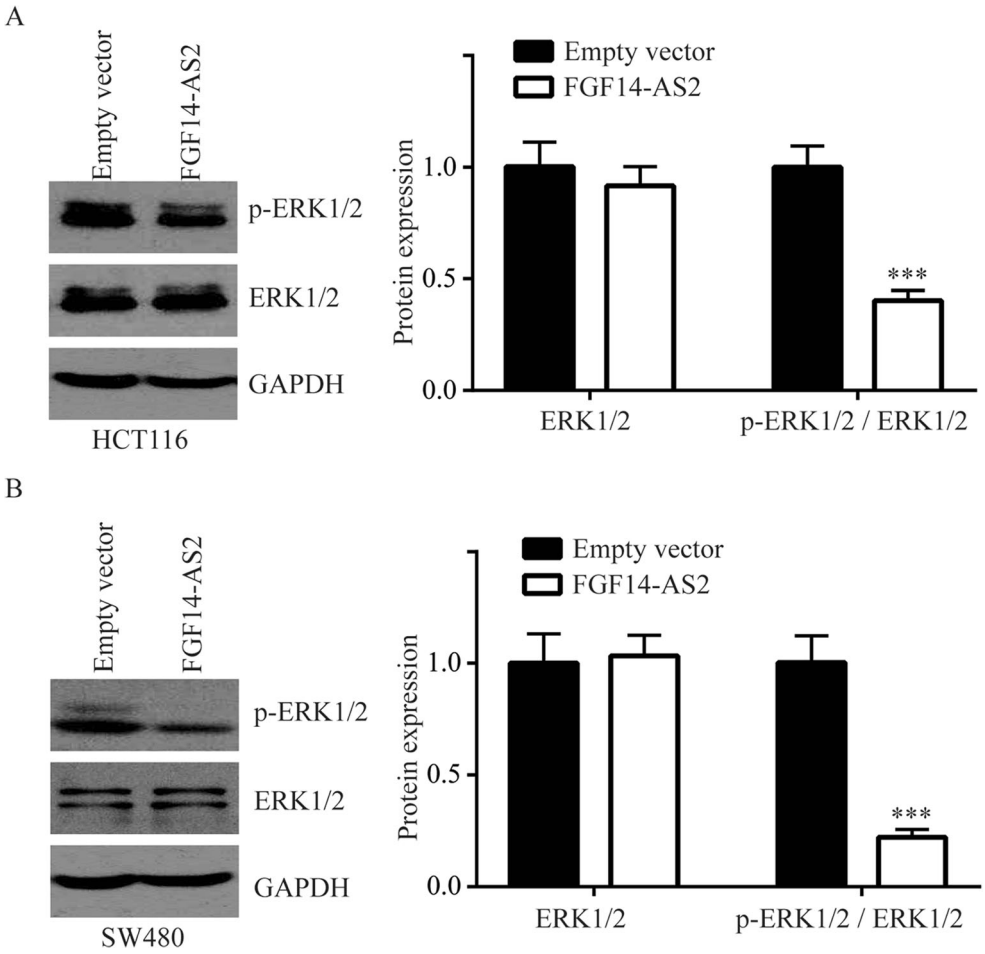

Fig. 3 FGF14-AS2 inactivated MAPK/ERK pathway. **a**-**b** Western blotting detected p-ERK1/2 and total ERK1/2 expression in HCT116 (**a**) and SW480 (**b**) cells transfected with empty vector or FGF14-AS2. ***, $p < 0.001$

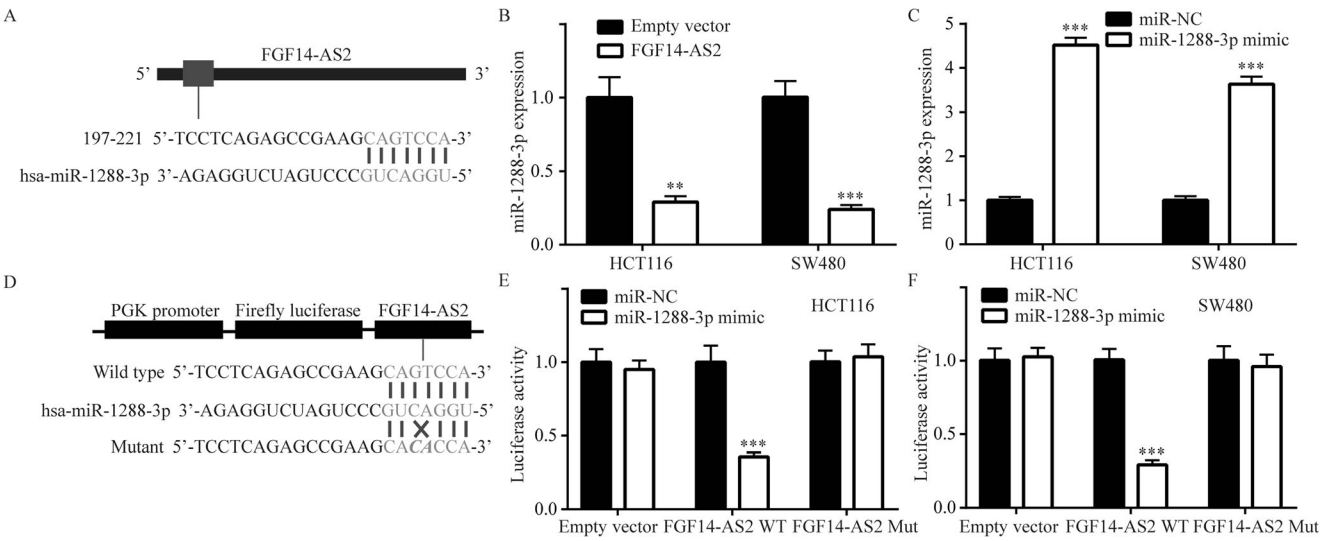

Fig. 4 FGF14-AS2 repressed miR-1288-3p expression. **a** miRDB software indicated that FGF14-AS2 might interact with miR-1288-3p. **b** miR-1288-3p expression was detected in HCT116 and SW480 cells transfected with empty vector or FGF14-AS2 by RT-qPCR. **c** miR-1288-3p expression was detected in HCT116 and SW480 cells transfected with miR-NC or miR-1288-3p mimic by RT-qPCR. **d** FGF14-AS2 WT or FGF14-AS2 Mut was inserted into luciferase plasmid. **e**-**f** Dual luciferase reporter assay was carried out to detect luciferase activity in HCT116 (**e**) and SW480 (**f**) cells transfected with empty vector or FGF14-AS2 WT or FGF14-AS2 Mut. **, $p < 0.01$; ***, $p < 0.001$

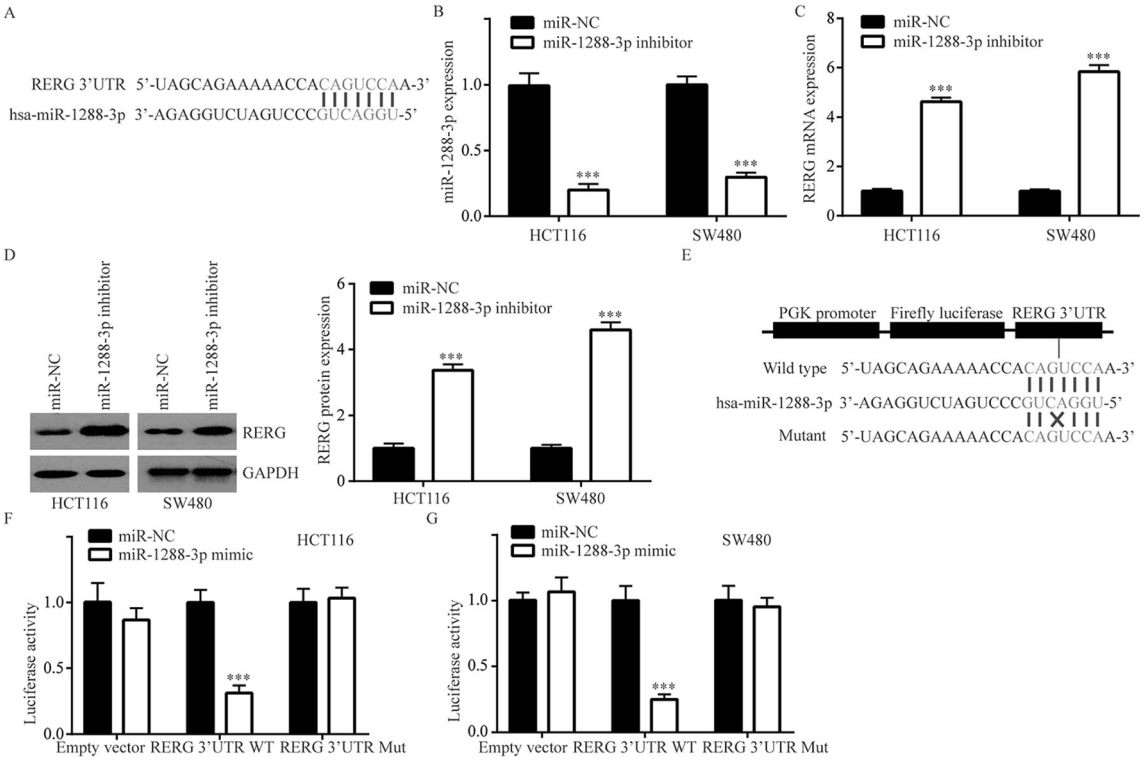

Fig. 5 RERG was repressed by miR-1288-3p. **a** TargetScan software indicated that RERG 3'UTR might interact with miR-1288-3p. **b-c** Expression of miR-1288-3p (**b**) and RERG (**c**) was detected in HCT116 and SW480 cells transfected with miR-NC or miR-1288-3p inhibitor by RT-qPCR. **d** Western blotting was performed to detect RERG protein expression in in HCT116 and SW480 cells transfected with miR-NC or miR-1288-3p inhibitor. **e** RERG 3'UTR WT or RERG 3'UTR Mut was inserted into luciferase plasmid. **f-g** Dual luciferase reporter assay was carried out to detect luciferase activity in HCT116 (**f**) and SW480 (**g**) cells transfected with empty vector or RERG 3'UTR WT or RERG 3'UTR Mut. ***, $p < 0.001$

5c). Western blotting confirmed that miR-1288-3p inhibitor increased RERG protein expression in examined cells (Fig. 5d). We next construct luciferase plasmid containing RERG 3'UTR (RERG 3'UTR WT) or its mutant form (RERG 3'UTR Mut) (Fig. 5e). The dual luciferase reporter assay data showed that miR-1288-3p mimic could repress luciferase activity of RERG 3'UTR WT in HCT116 and SW480 cells (Fig. 5f-g).

FGF14-AS2 Promoted RERG Expression

We retrieved FGF14-AS2 and RERG expression in TCGA data and analyzed the association between their expression. Consistent with our expectation, the expression of FGF14-AS2 was strongly correlated with RERG expression in colorectal cancer samples from TCGA ($R = 0.72$, $p < 0.001$) (Fig. 6a). We also observed downregulation of RERG in collected colorectal tumor samples (Fig. 6b) and a positive correlation between RERG and FGF14-AS2 expression ($R = 0.628$, $p < 0.001$) (Fig. 6c). In HCT116 and SW480 cells, it was observed that FGF14-AS2 overexpression increased RERG mRNA and protein expression (Fig. 6d-e).

FGF14-AS2 Relied on Regulation of RERG to Control Colorectal Cancer Cell Proliferation

RERG specific siRNA was transfected into HCT116 cells to downregulate RERG expression (Fig. 7a). In the rescue experiments, it was found that RERG silencing reversed the cell proliferation inhibition and cell apoptosis induction effect of FGF14-AS2 in HCT116 cells (Fig. 7b-c). Similarly, in SW480 cells, RERG silencing also attenuated the effect of FGF14-AS2 (Fig. 7d-f). Collectively, FGF14-AS2 repressed colorectal cancer cell proliferation and induced cell apoptosis via upregulation of RERG.

Discussion

FGF14-AS2 is previously reported as a downregulated lncRNA in breast cancer and suppressed cancer cell proliferation and metastasis [10]. The function of FGF14-AS2 has not been studied in other cancer types yet. According to a recently published genome wide analysis, FGF14-AS2 was also a significantly downregulated lncRNA in colorectal cancer [20]. We confirmed that FGF14-AS2 was decreased in colorectal

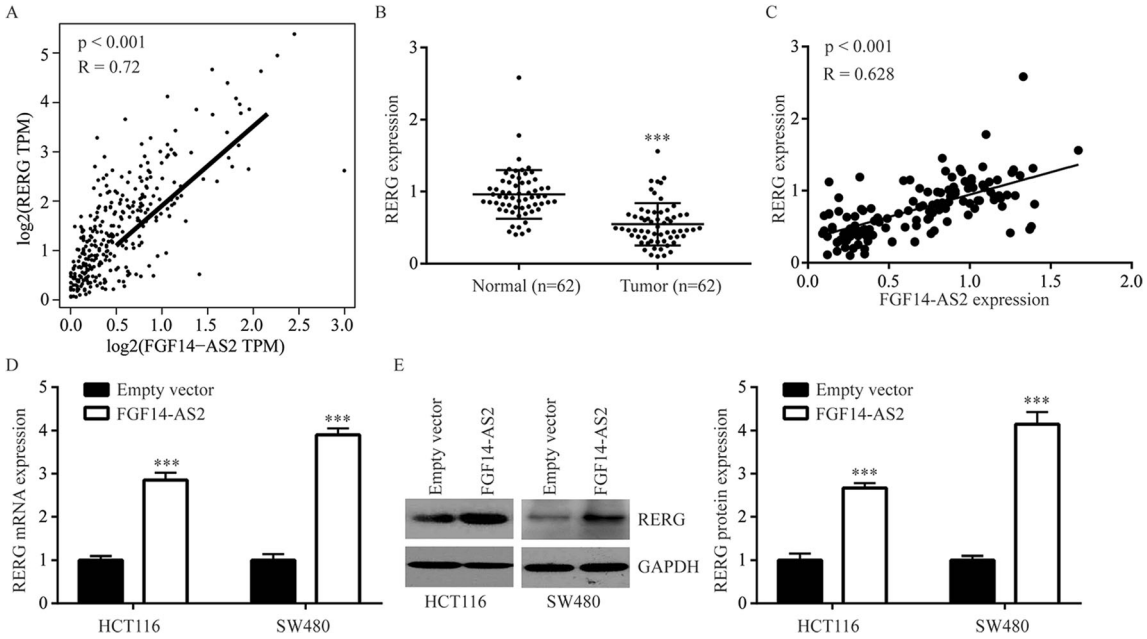

Fig. 6 FGF14-AS2 co-expressed with RERG and promoted RERG expression. **a** Using TCGA data, the association between RERG and FGF14-AS2 expression was analyzed by Pearson correlation analysis. **b** Expression of RERG was detected in 62 pairs of colorectal tumor tissues and normal tissues by RT-qPCR. **c** In samples from patients with colorectal cancer, the association between RERG and FGF14-AS2 expression was analyzed by Pearson correlation analysis. **d** RERG mRNA expression was detected in HCT116 and SW480 cells transfected with empty vector or FGF14-AS2 via RT-qPCR. **e** RERG protein expression was detected in HCT116 and SW480 cells transfected with empty vector or FGF14-AS2 via Western blotting. ***, $p < 0.001$

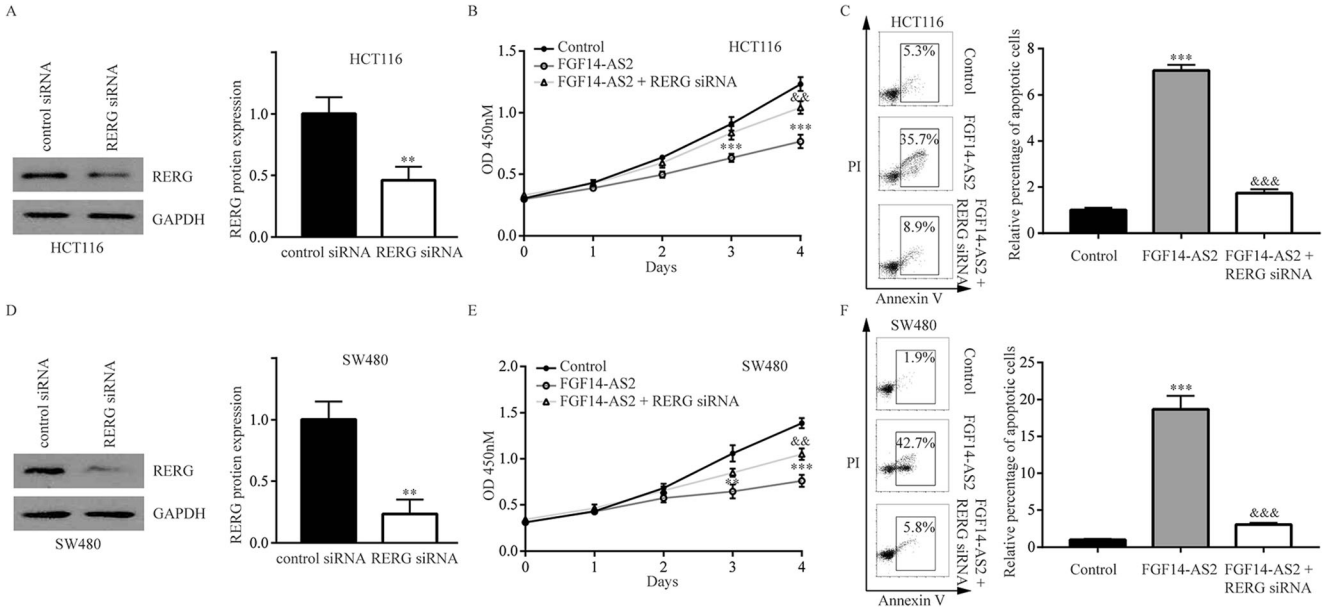

Fig. 7 FGF14-AS2 regulated colorectal cancer cell proliferation and apoptosis via RERG. **a** RERG protein expression was detected in HCT116 cells transfected with control siRNA or RERG siRNA by Western blotting. **b** The CCK-8 assay was used to detect cell proliferation in HCT116 cells transfected with control siRNA + empty vector or FGF14-AS2 + control siRNA or RERG siRNA + FGF14-AS2. **c** The flow cytometry was used to detect cell apoptosis in HCT116 cells transfected with control siRNA + empty vector or FGF14-AS2 + control siRNA or RERG siRNA + FGF14-AS2. **d** RERG protein expression was detected in SW480 cells transfected with control siRNA or RERG siRNA by Western blotting. **e** The CCK-8 assay was used to detect cell proliferation in SW480 cells transfected with control siRNA + empty vector or FGF14-AS2 + control siRNA or RERG siRNA + FGF14-AS2. **f** The flow cytometry was used to detect cell apoptosis in SW480 cells transfected with control siRNA + empty vector or FGF14-AS2 + control siRNA or RERG siRNA + FGF14-AS2. *** vs. control, $p < 0.001$; && vs. FGF14-AS2, $p < 0.01$; &&& vs. FGF14-AS2, $p < 0.001$

cancer in both TCGA data and our collected samples. FGF14-AS2 low expression was associated with advanced tumor stage. No association was found between FGF14-AS2 expression with KRAS or BRAF mutations in colorectal tumors. Functional assays showed that FGF14-AS2 inhibited colorectal cancer cell proliferation and triggered cell apoptosis. The data firstly indicated that FGF14-AS2 might repress cell proliferation via inducing cell apoptosis in cancer cells.

The activity of MAPK/ERK pathway was abnormally activated in colorectal cancer cells via multiple mechanisms. Mutations of KRAS and BRAF genes contributed to activation of MAPK/ERK pathway and dysregulation of several regulators of MAPK/ERK pathway further promoted activation of MAPK/ERK pathway in colorectal cancer [22]. Several lncRNAs were responsible for mediating MAPK/ERK signaling. For example, NNT-AS1 inactivated MAPK/ERK signaling in colorectal cancer and attenuated epithelial-mesenchymal transition (EMT) process [23]. OVAAL mediated pro-survival signal in colorectal cancer via activation of MAPK/ERK signaling [24]. Our data showed that FGF14-AS2 also inactivated MAPK/ERK pathway. RERG is a negative regulator of MAPK/ERK pathway and its downregulation is found in several cancer [15, 18, 25]. RERG expression was strongly associated with FGF14-AS2 in colorectal cancer and normal tissues. Overexpression of FGF14-AS2 increased RERG expression in colorectal cancer cells. miRNAs including miR-382-5p and miR-135 are responsible for decreased expression of RERG in breast cancer and esophageal squamous cell carcinoma [26, 27]. We found that the sequence of RERG 3'UTR and FGF14-AS2 harbored complementary sites to miR-1288-3p. miR-1288-3p promoted cell proliferation in colorectal cancer cell lines SW480 (KRAS G12V and BRAF wild type) and SW48 (KRAS and BRAF wild type) [28]. The mechanism of miR-1288-3p in colorectal cancer has not been defined yet. Our experiments and bioinformatic analysis demonstrated that miR-1288-3p targeted RERG and itself was repressed by FGF14-AS2 in KRAS mutant (G12V or G13D) colorectal cancer cells without BRAF mutation. The data revealed that FGF14-AS2 regulated MAPK/ERK pathway in colorectal cancer cells with oncogenic mutations of RAS signaling. In addition, RERG silencing rescued the biological function of FGF14-AS2 in examined cells. Our results manifested a FGF14-AS2/miR-1288-3p/RERG/MAPK/ERK axis in colorectal cancer cells with oncogenic mutations of RAS signaling. However, the data only supported the critical role of FGF14-AS2 on regulating MAPK/ERK pathway in colorectal cancer cells carrying oncogenic mutations of RAS signaling. As we found there was no association between mutations of Ras signaling and FGF14-AS2 expression, further investigation is needed to explore whether the function of FGF14-AS2 is dependent on KRAS and/or BRAF mutations in colorectal cancer cells.

Conclusion

In conclusion, the work presented here showed that FGF14-AS2 was downregulated in colorectal cancer. The data suggested that FGF14-AS2 sponged miR-1288-3p to inactivate MAPK/ERK signaling. Thus, we believe FGF14-AS2 is a promising biomarker for patients with colorectal cancer.

Availability of Data and Materials All data generated or analyzed during this study are included in this published article.

Author Contributions Ruizhi Hou, Yan Liu, Yanzhuo Su, Zhenbo Shu acquired data and performed data analysis. Ruizhi Hou and Yan Liu designed the study. Yanzhuo Su and Zhenbo Shu wrote the manuscript. Study was supervised by Zhenbo Shu.

Compliance with Ethical Standards

Conflict of Interests All the authors declare that they have no competing interests of any form.

Consent for Publication Not applicable.

Ethical Standards The study was performed in accordance with the Declaration of Helsinki and obtained the approval from ethical approval board of the Third Hospital of Jilin University.

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