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Long noncoding RNA FAM201A involves in radioresistance of non-small-cell lung cancer by enhancing EGFR expression via miR-370

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Abstract. – OBJECTIVE: The aberrant expression of long noncoding RNAs (lncRNAs) is involved in the molecular regulation of non-small cell lung cancer (NSCLC). This study aims to investigate the biological interaction of lnc-FAM201A and its downstream factors and their impacts on the radiotherapy response of NSCLC.

PATIENTS AND METHODS: Quantitative Polymerase Chain Reaction (qPCR) was used to determine the expression of FAM201A in NSCLC tissues. The Chi-square tests explored the association between FAM201A level and the poor clinicopathological characteristics (including radioresistance) of NSCLC. Univariate and multivariate Cox proportional hazards regression analyses were used to evaluate various prognostic factors for overall survival (OS). The effect of FAM201A on OS was tested by the log-rank test. A549/SK-MES-1 cell lines transfected with short hairpin RNA (shRNA) were used to verify the promoting effects of FAM201A on radiotherapy resistance *in vitro* and *in vivo*. Cell apoptosis (analyzed by flow cytometry), cell proliferation (determined by Cell Counting Kit-8), and mice xenograft models were performed to confirm the results. The downstream targets of FAM201A were predicted by bioinformatics tools. Additionally, the Dual-luciferase reporter assay, qPCR, and Western blotting were performed to confirm their interaction.

RESULTS: FAM201A was significantly upregulated in tissues obtained from NSCLC patients resistant to radiotherapy. Increased FAM201A expression was strongly associated with radioresistance and inferior survival in NSCLC, as demonstrated by clinical data. The silence of FAM201A could inhibit cell proliferation and further cell apoptosis of NSCLC cells under X-ray irradiation both *in vitro* and *in vivo*. Moreover, by competitively targeting miR-370, FAM201A elevated the epidermal growth factor receptor (EGFR) and the hypoxia-inducible factor 1alpha (HIF-1α) levels. After FAM201A knockdown, EGFR and HIF-1α were repressed with enhanced radiosensitivity.

CONCLUSIONS: The interference of FAM201A impairs its suppression of miR-370, resulting in the upregulation of EGFR and HIF-1α and enhancement of radiosensitivity in NSCLC patients. Collectively, our results indicated that this regulatory axis might serve as a potential therapeutic target to increase the sensitivity of radiotherapy in NSCLC patients.

Key Words:
Non-small-cell lung cancer, LncRNA family with sequence similarity 201-member A, MiR-370, Epidermal growth factor receptor, Hypoxia-inducible factor 1alpha.

Introduction

Lung cancer (LC) has the highest incidence among all cancers and is the leading cause of cancer-related deaths globally[1,2]. A relatively small percentage of patients with LC are identified in the early stage of the disease and receive radical excision. The majority of them are often diagnosed late as advanced metastatic non-small cell lung cancer (NSCLC). A multi-disciplinary therapy[3], involving radiation concurrent with chemotherapy, is considered a promising treatment for unresectable NSCLC[4,5].

Extensive irradiation can cause inflammation and necrosis of the tissue cells adjacent to cancer, triggering various radiological complications, while a poor therapeutic effect can cause moderate apoptosis of the tumor cells[6]. Recent novel techniques, including intensity-modulated radia-

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tion therapy and three-dimensional conformal radiation therapy, can provide structure-conformed dose to stationary target regions, thereby boosting treatment accuracy[7]. However, the efficacy of radiotherapy is altered by cell heterogeneity, resistance empowering mutations, and negative immune responses in the microenvironment[8,9]. Clinically, even though the side effects of these therapies lower the quality of life, acceptable doses might also be associated with local-regional failure. Since radiotherapy tolerance in NSCLC patients continues to hamper clinical applications[10,11], a biology-based optimization in therapeutic strategy is urgently required.

In the present work, we found a significant difference in the expression levels of lncRNA family with sequence similarity 201-member A (FAM201A), and between the radiotherapy-sensitive and radiotherapy-resistant NSCLC patients. Moreover, the multivariate analysis revealed that FAM201A was an independent predictor for radioresistance in NSCLC. Our results suggest a plausible role for FAM201A in the prognosis of NSCLC. Moreover, the understanding of the downstream epigenetic regulation of FAM201A may assist in finding a novel therapeutic target for treating NSCLC.

Patients and Methods

Patients, Radiotherapy, Therapy Responses and Tissue Specimens

Totally, 69 NSCLC patients were enrolled between June 2016 and September 2018. All patients received transbronchial lung biopsy followed by radiotherapy. Tissue specimens were firstly used for histopathological diagnosis, then frozen in -80°C.

The inclusion criteria were as follows: lung squamous cell carcinoma confirmed by histology (biopsy), Karnofsky Performance Status scores above 70, completive radiotherapy treatment fulfilled. The exclusion criteria for this study were as follows: clinical stage of M1 (the 7th AJCC TNM staging system) proved by bone scan or magnetic resonance imaging, incomplete follow-up data, other conditions that required medical treatment.

A total dose of 60-70 Gy radiotherapy was implemented to all these patients with 6-7 weeks treatment duration (2 Gy per fraction per day, 5 days per week). An enhanced computed tomography was done before the first-time radiotherapy, while another one was done 3 weeks after the last-time radiotherapy to assess the radiation response. Basing on the previous description about tumor response[12], the radiosensitive group (n=37, including complete response, partial response) and the radioresistant group (n=32, including stable disease, progressive disease) were divided by RECIST1.1. A follow-up was administered to evaluate the overall survival of patients. This investigation was approved by the Affiliated Hospital of Southwest Medical University Ethics Committee. We obtained the consent from each patient before their treatment.

RNA Extraction and Quantitative Polymerase Chain Reaction (qPCR)

According to the manufacturer's protocol, either lung cancer tissues or cell lines were homogenized for total RNAs extraction using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA). All extracted RNAs were qualified (A260: A280 ratio $\geq$ 2.0) and quantificationally measured by NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). cDNA synthesis was performed by PrimeScript™ RT kit (TaKaRa Biotechnology, Dalian, China), which is complemented with gDNA Eraser for putative target RNAs following the manufacturer's instruction. The quantitative test of target RNA in tissues and cells was done using TB Green™ Premix Ex Taq™ (Tli RNaseH Plus; TaKaRa Biotechnology, Dalian, China) in accordance with the protocol on the Roche LightCyber 480 System (Roche Molecular Systems, Mannheim, Germany). The target RNA expression was calculated utilizing the $2^{-\Delta\Delta}$Ct method in a relative way (GAPDH as the endogenous control). All primer sequences and oligonucleotides used for transfection (Invitrogen, Waltham, MA, USA) were presented in the Supplemental Table I.

Cell Lines and Culture

The NSCLC cell line A549 and SK-MES-1 were purchased from the Chinese Academy of Sciences (Shanghai Institute of Cell Biology, Shanghai, China). The cell lines were cultured in DMEM medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% (v/v) fetal bovine serum (Gibco, Waltham, MA, USA) in a circumstance of 5 % CO_2 at 37°C.

Cell Transfection

The short hairpin RNAs (shRNAs) for FAM201A knockdown were constructed (Invitrogen, Carlsbad, CA, USA) and expressed using pGFP271-puro-RNAi expression vector (Ad-

Supplemental Table I. The sequences of primers for RT-PCR.

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
FAM201A	TCTCTGATGGGAGCCTCTTTA	CAAGCCACAGACGGAGAAA
miR-370	TAGCCTGCTGGGGTGGAA	TATGGTTTTGACGACTGTGTGAT
FAM201A shRNA	GTACCTCGATCTTTCGTCCATTTA CTTCAAGAGAGTAAATGGACGAA AGATCTTTTTGGAAA	AGCTTTTCCAAAAAGATCTTTCGTCC ATTTACTCTCTTGAAGTAAATGGAC GAAAGATCGAG
shRNA control	GTACCTCGCCTTATTTCTATCTTA CGTCAAGAGCGTAAGATAGAAAT AAGGCTTTTTGGAAA	AGCTTTTCCAAAAAGCCTTATTTCTA TCTTACGCTCTTGACGTAAGATAGA AATAAGGCGAG
β-actin	TGACGTTGACATCCGTAAAGACC	CTCAGGAGGAGCAATGATCTTGA
GAPDH	CCCTTCATTGACCTCAACTACA	ATGACAAGCTTCCCGTTCTC
Negative control	UUCUCCGAACGUGUCACGUUU	

dgene, Watertown, MA, USA). The lung cancer cells were seeded in six-well plates with 1×10^6 per well, and were transfected with RNAi expression vector overnight, using Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA) in accordance with the protocol. The efficacy of the transfection must be verified by PCR.

Cells Irradiation

The cells with a concentration of 5×10^3 /well were cultured for 48 h. Then, an exposure to radiation with a gradient dose (0, 2, 4, 6, and 8 Gy) was made using a 6-MV X-ray linear accelerator (ELEKTA, Beijing, China). The cells were placed in the incubator, and the samples were collected at the indicated time points (0, 1, 12, and 24 h).

Cell Proliferation Assay

The viability of the irradiated cells was assessed by Cell Counting Kit-8 (CCK-8; MedChem, Lexington, MA, USA) following the manufacturer's instructions. The absorbance representing each viability was measured by a spectrophotometer at 450 nm.

Apoptosis Assay

The apoptosis analysis of the transfected GC cells (after 48 h culture) was performed Utilizing Annexin V Apoptosis Detection Kit (eBiosciences, Waltham, MA, USA), the apoptosis rate of the irradiated cells was tested after every dose. The stained cells were analyzed using BD FACS AriaII Flow Cytometry (BD Biosciences, Franklin, NJ, USA).

Mice Model Experiments

5-week-old nude mice, purchased from the Laboratory Animal Center of Southwest Medical University, were injected subcutaneously with FAM201A shRNA and the control vector-transfected A549/SK-MES-1 cells, respectively at the concentration of 5×10^5/ml (100 ul). The mice (n=5 per group) were kept under specific pathogen-free conditions with an atmosphere of 12 h light/dark cycle. After the injection, a rest of two weeks allowed the growth for tumor nudes. A treatment of X-ray at 10 Gy was performed to each mouse. The tumor sizes were recorded every week. Six weeks after inoculation, the tumor nodes were resected for weight assessments following the sacrifice of mice. All these animal experiments were approved by the Animal Care and Use Committee of the Affiliated Hospital of Southwest Medical University, following the Institutional Guide for the Care and Use of Laboratory Animals.

Dual-Luciferase Reporter Analysis

The wild-type target lncRNA or the one containing a mutant miRNA-binding area were constructed (Invitrogen, Carlsbad, CA, USA). Both of these lncRNAs were cloned with a Luciferase gene in the pGL3 vector (Promega, Madison, WI, USA). The synthetic vectors, the Renilla luciferase reporter vector, and miRNA mimic were co-transfected into the cells using the Lipofectamine 2000 kit (Thermo Fisher Scientific, Waltham, MA, USA) following the protocol provided by the manufacturer, 48 h later cells were seeded into 96-well plates. The Luciferase activity of Renilla plasmid (as the endogenic control, Promega, Madison, WI, USA) and target gene was assessed via Dual-Luciferase Reporter Assay Kit (Promega, Madison, WI, USA).

Western Blot Assay

A RIPA buffer containing protease inhibitor (Beyotime, Shanghai, China) was used for proteins exaction from cells and tissues. The

protein concentrations were measured using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). The electrophoresis on SDS-PAGE gel (Thermo Fisher Scientific, Waltham, MA, USA) separated the kinds of proteins in each sample, followed by a transfer to polyvinylidene difluoride membranes (PVDF) membranes (Thermo Fisher Scientific, Waltham, MA, USA). The membranes were incubated with the primary antibodies (Anti-EGFR, 1:2500; anti-HIF-1α, 1:2000; β-actin, 1:5000; Abcam, San Francisco, CA, USA) at 4°C overnight after incubation with PBS (5% dry milk) at room temperature for 1 h. Then, the membrane with blotting was incubated with a secondary antibody conjugated with HRP (1:5000 dilution, Santa Cruz Biotechnology, Santa Cruz, CA, USA). An ECLTM chemiluminescence detection system (Pierce, Waltham, MA, USA) was used to compare the protein levels reflected by the blotting.

Statistical Analysis

All experiments were conducted independently in triplicate. The data are presented as mean ± standard deviation (SD). The comparison within the groups was performed using the independent Student's *t*-test. Univariate and multivariate analyses of the prognostic factors for overall survival using Cox proportional hazards regression model. GraphPad Prism software (San Diego, CA, USA) was used for statistical analysis. The binding site prediction between the target miRNA and FAM201A were performed by StarBase 2.0 (http://starbase.sysu.edu.cn/starbase2/index.php). p-value < 0.05 was considered statistically significant.

Results

FAM201A Elevation is Associated to Resistance to Radiotherapy and Poor Prognosis in NSCLC Patients

Quantitative PCR was performed to evaluate the ectopic FAM201A expression in the tissues derived from the corresponding radiosensitive (n = 37) and radioresistant (n = 32) patients with NSCLC. We found that FAM201A was highly expressed in the tissues from radioresistant patients, compared with that from the radiosensitive ones (Figure 1A). Moreover, investigating the relationships between FAM201A expression and the clinicopathological characteristics (Table I), we observed that an increased FAM201A level was strongly associated with N stage (p = 0.039) and radioresistance (p = 0.005). Next, all NSCLC patients were divided into a low expression group (n = 34) and a high expression group (n = 35), based on the median value of lncRNA FAM201A (Figure 1B). The Kaplan-Meir survival curve analysis showed that, compared to a lower expression of FAM201A, a higher expression is significantly associated with shorter overall survival in NSCLC patients (p < 0.05, Figure 1C). In univariate and multivariate Cox proportional hazards regression analysis, an elevated FAM201A expression was identified as an independent predictor for poor overall survival (Table II and III). Our results showed the

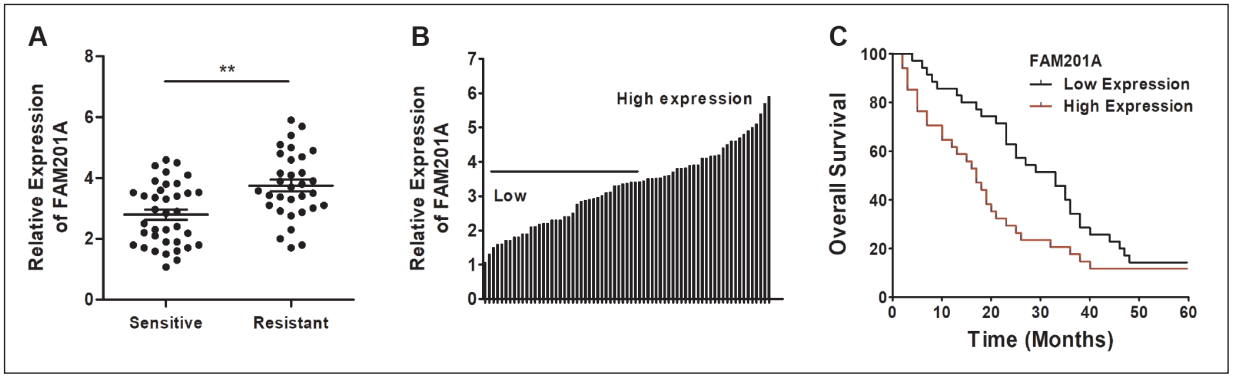

Figure 1. FAM201A elevation is associated with resistance to radiotherapy and poor prognosis in NSCLC patients. ***A,*** Evaluation of lncRNA FAM201A expression in NSCLC tissues from patients with different response to radiotherapy RT-PCR. ***B,*** Division of lncRNA FAM201A expression by median value (high expression group n = 34; low expression group n = 35). ***C,*** Overall survival analysis of NSCLC patients with different lncRNA FAM201A level. ** p < 0.01 compared to the control group.

Table I. Association of LINC00675 with clinicopathological characteristics of ESCC patients.

Variables	Total N	FAM201A expression		*p*-value
		Low n = 34	High n = 35	
Gender				0.315
Male	47	24	23	
Female	22	10	12	
Age				0.398
≤60	34	15	19	
>60	35	19	16	
Pathology				0.356
Squamous carcinoma	45	24	21	
Adenocarcinoma	24	10	14	
T stage				0.277
T1-2	43	19	24	
T3-4	26	15	11	
N stage				0.039*
N0-1	28	18	10	
N2-3	41	16	25	
TNM stage				0.722
II	15	8	7	
III	54	26	28	
Radiation response				0.005*
Resistant	32	10	22	
Sensitive	37	24	13	

Notes: *$p < 0.05$ represents the statistical difference.

influence of FAM201A level in the efficacy of radiotherapy and prognosis in NSCLC patients.

FAM201A Contributes to Radioresistance of NSCLC In Vitro

To understand in-depth the role of FAM201A on the regulation of the radiosensitivity of NSCLC, we performed irradiation on NSCLC cell lines; A549 (human lung adenocarcinoma) and SK-MES-1 (human squamous cell carcinoma) were stably transfected with FAM201A shRNA (sh-FAM201A). The efficacy of the knockdown was evaluated by quantitative PCR. The expression levels of FAM201A in A549 and SK-MES-1 were significantly decreased after transfection, compared to those cells transfected with control vector (Figure 2A and 2B, $p < 0.05$). Moreover, the cells under varying dose of irradiation were

Table II. Univariate analyses of prognostic factors for overall survival (no. = 93).

Variables	*p*-value	Hazard ratio	95% confidence interval
Gender	0.412	1.124	0.540-2.131
Age	0.923	0.976	0.599-1.577
Pathology	0.813	0.916	0.524-1.556
T stage	0.280	0.704	0.456-1.184
N stage	0.020*	0.578	0.356-0.965
TNM stage	0.354	0.698	0.424-1.317
FAM201A expression	0.003*	0.534	0.311-0.976

Notes: *$p < 0.05$ represents the statistical difference.

Table III. Multivariate analyses of prognostic factors for overall survival (N = 93).

Variables	*p*-value	Hazard ratio	95% confidence interval
N stage	0.286	0.746	0.412-1.246
FAM201A expression	0.035*	0.490	0.342-0.826

Notes: *$p < 0.05$ represents the statistical difference.

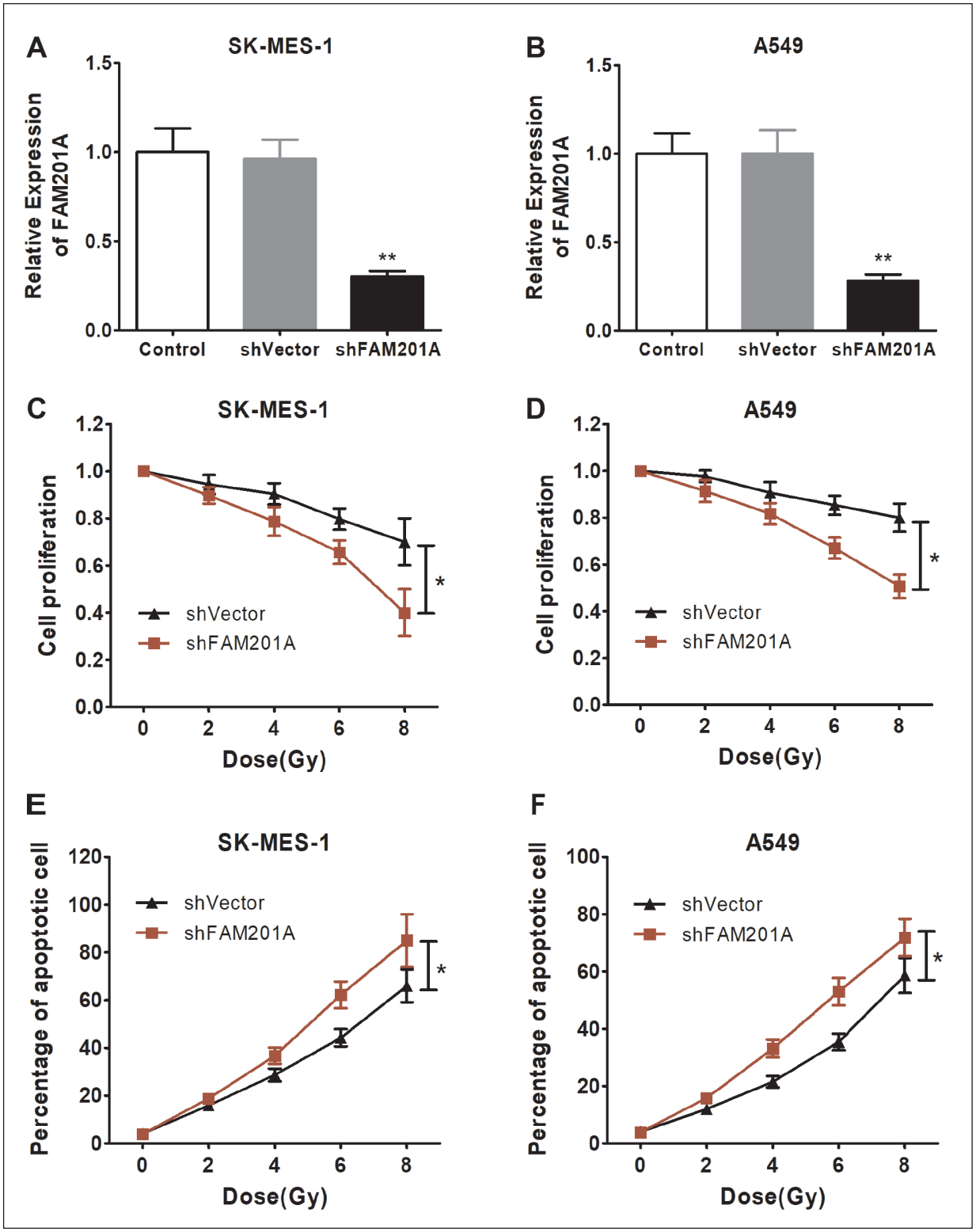

Figure 2. FAM201A contributes to radioresistance of NSCLC in *vitro*. ***(A)*** SK-MES-1 and ***(B)*** A549 were transfected with a synthesized shRNA targeting FAM201A to decrease expression. ***(C)*** Proliferation of SK-MES-1 and ***(D)*** A549 under various dose of irradiation measured by CCK-8 assay. ***(E)*** Apoptotic rate of SK-MES-1 and ***(F)*** A549 transfected with shRNA or controls tested by flow cytometry. * $p < 0.05$, ** $p < 0.01$ compared to the control group.

analyzed by methylthiazol tetrazolium assay (MTT), and the results showed that a decrease in FAM201A expression, both in A549 and SK-MES-1 cell, was associated with a lower cell viability rate, compared to normal FAM201A expression (Figures 2C and 2D, $p < 0.05$). Radiation-induced apoptosis was considered as an indicator for radiosensitivity. Our flow cytometry results showed that the radiation-induced apoptosis was higher in FAM201A knockdown cells (both in A549 and SK-MES-1), compared with the control vector-transfected cells (Figures 2E and 2F, $p < 0.05$). Collectively, our data indicated that FAM201A reduction ameliorates radioresistance of NSCLC *in vitro*.

FAM201A Knockdown Improved the Radiosensitivity of NSCLC In Vivo

A xenograft mice model was adopted and exposed to irradiation to verify the impact of sh-FAM201A on radioresistance *in vivo*. A549 and SK-MES-1 cell lines stably transfected with shRNA were inoculated into nude mice. Compared to the vector control groups, the tumor size and weight were significantly restricted in FAM201A silenced group (Figure 3, $p < 0.05$). This result indicates that a decrease in FAM201A levels in NSCLC can enhance radiosensitivity *in vivo*.

FAM201A Acts as Competing Endogenous RNA to MiR-370, Increasing EGFR Expression

The bioinformatics tools (TargetScan) predicted that FAM201A might target the complementary binding sites of miR-370 (Figure 4A). A series of experiments were performed to confirm this interaction. The Dual-Luciferase Reporter Assay demonstrated that the wildtype FAM201A, rather than the mutant one, inhibited the Luciferase activity of miR-370 in both A549 (Figure 4B, $p < 0.05$) and SK-MES-1 (Figure 4C, $p < 0.05$) cells. In addition, FAM201A silencing upregulated miR-370 expression in NSCLC cells, compared with the vector control (Figures 4D and 4E, $p < 0.05$). These results suggested that miR-370 in NSCLC cells was significantly repressed by direct interaction with FAM201A sequence.

Liu et al[13] have reported the epidermal growth factor receptor (EGFR) as the potential downstream targets of miR-370, and this result has been confirmed by TargetScan. Thus, we studied the role of FAM201A in the expression level of EGFR, and its relevant transcription factor, hypoxia-inducible factor 1alpha (HIF-1α) in NSCLC cells. In A549/SK-MES-1 cells, the expression level of EGFR (Figures 5A and 5B, $p < 0.05$) and HIF-1α (Figures 5C and 5D, $p < 0.05$) was downregulated by a decrease in FAM201A. Under 4 Gy irradiation, the expression of EGFR and HIF-1α in the vector group was higher than that under 0 Gy (Figures 5A, B, C, D, $p < 0.05$). The protein levels obtained by the Western blotting were consistent with the results of PCR (Figures 5E and 5F). Altogether, our results identified EGFR as the downstream effector of FAM201A-miR-370-axis in NSCLC cells.

Discussion

Our study demonstrated the ectopic expression of lncRNA FAM201A in radiotherapy resistant patients with NSCLC. Located on chromosome 9[14], FAM201A gene has no protein-coding capacity, but plays an important role in the pathogenesis of the human diseases. The sequencing of the samples from patients with osteonecrosis of the femoral head revealed a FAM201A-related mechanism in the progression of the disease[15]. Moreover, several SNPs in FAM201A gene are significantly common in both obsessive-compulsive disorder and Tourette's syndrome[16]. However, only a few researches have explored the biological role of lncRNA FAM201A in the development of cancers. An association between FAM201A and radiosensitivity was first identified by Chen et al[17] in esophageal squamous_cell cancer. Consistent with the results of the previous study, our work also showed that increased expression of FAM201A was involved independently in the radioresistance and poor prognosis of NSCLC (Figure 1). Furthermore, FAM201A silencing repressed the tumor cell proliferation and growth under irradiation both *in vitro* and *in vivo* (Figures 2 and 3). Collectively, our results suggest that FAM201A is a promising predictor to analyze the efficacy of radiotherapy and inferior overall survival in NSCLC patients.

LncRNAs act as microRNA sponges to regulate the downstream miRNAs[18,19]. Several lncRNAs, including FAM201, demonstrate an endogenous competition to miR-370 in cancer development[20,21]. In the current study, the bioinformatics techniques were used to predict the interaction between FAM201A and miR-370 (Fig-

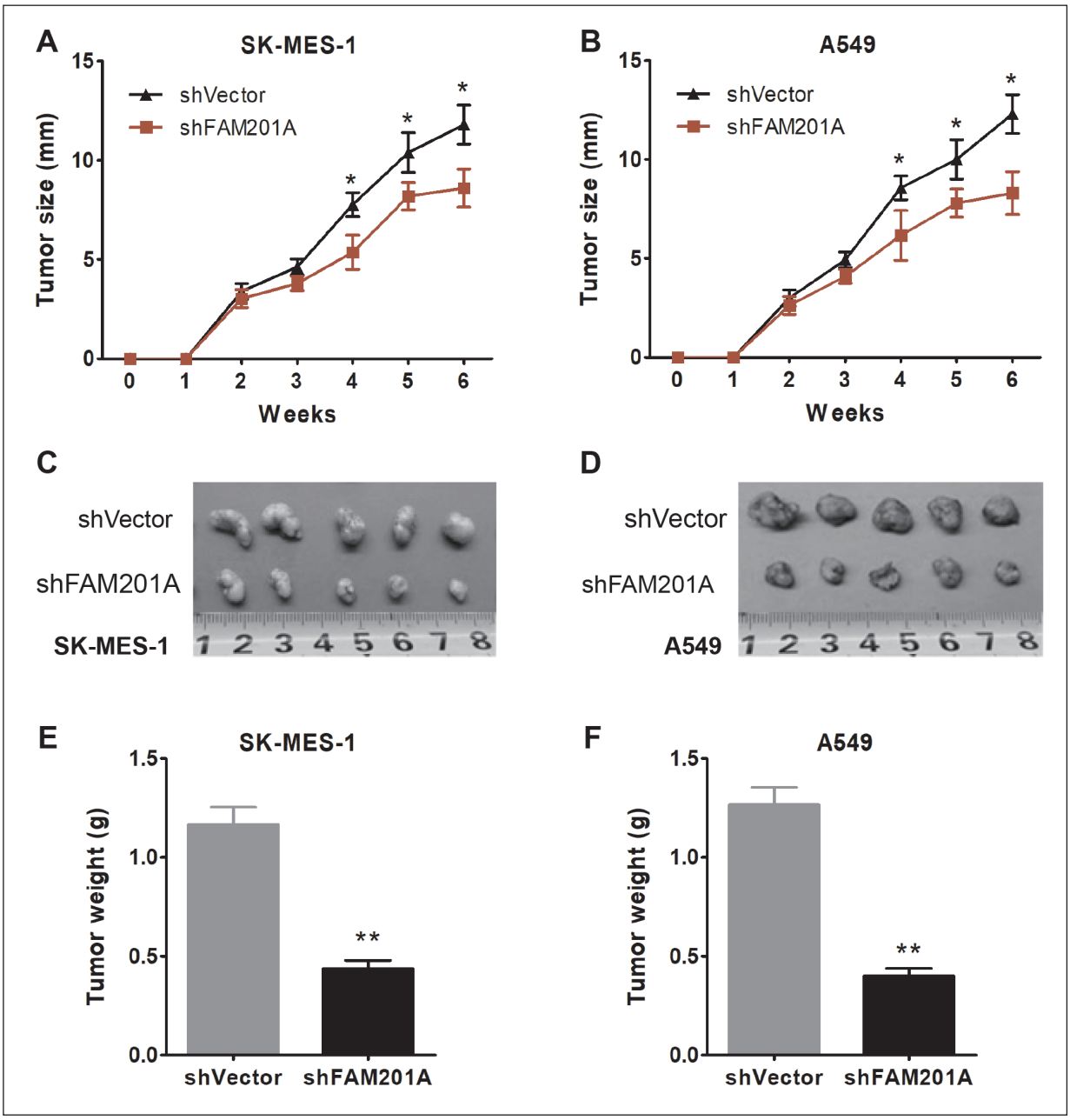

Figure 3. FAM201A Knockdown improved the radiosensitivity of NSCLC *in vivo*. ***A, B,*** Tumor size in mice incubated with FAM201A silencing or control cells (SK-MES-1, left A; A549, right B). ***(C)*** Tumor growth of SK-MES-1 and ***(D)*** A549 *in vivo* showed by macrography. ***E, F,*** Tumor weight of mice with FAM201A knockdown or control using SK-MES-1 and A549. * $p < 0.05$, ** $p < 0.01$ compared to the control group.

ure 4A). The luciferase activity of miR-370 was repressed by the wildtype PVT1-214 but not by the mutant one, verifying the directive binding effect between them (Figures 4B and 4C). Furthermore, our findings revealed that decreased expression of miR-370 in tumor cells could be reversed by FAM201A knockdown (Figures 4D and E). These data suggest that miR-370 is a potential tumor suppressor gene in NSCLC, which is consistent with the findings of some previously published reports on the progression of the inhibiting mechanisms in lung cancer[22,23]. However, in the field of radiosensitivity, our study is trying to explore the vital function of miR-370 in NSCLC.

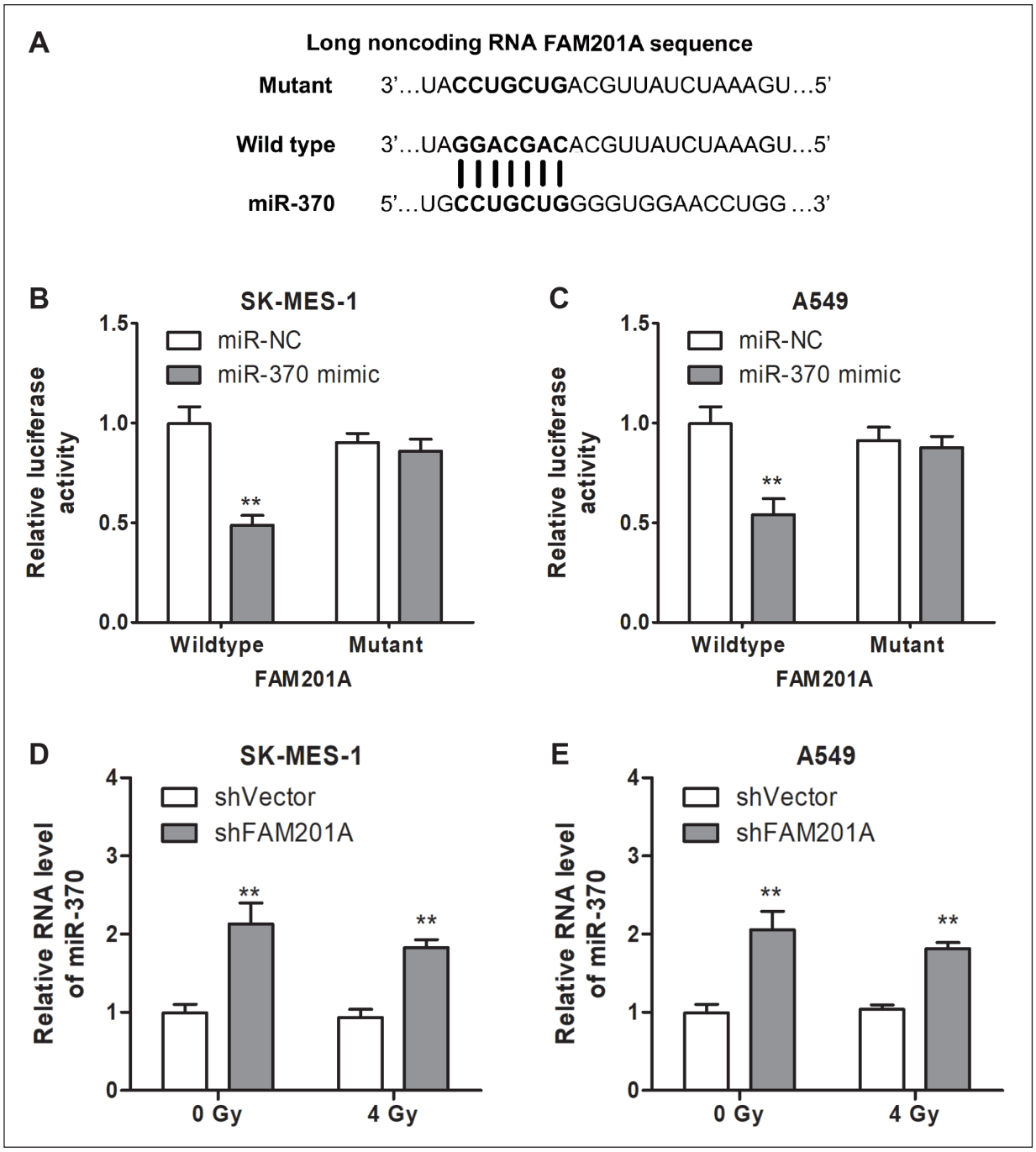

Figure 4. FAM201A acts as competing endogenous RNA to miR-370. ***A,*** The bioinformatics tool reveals the complementary binding sites within FAM201A and miR-370. ***B, C,*** Luciferase reporter assay confirmed the molecular binding between FAM201A and miR-370 in SK-MES-1 and A549. ***D, E,*** qPCR showed the miR-370 expression in SK-MES-1 and A549 transfected with shFAM201A or controls under 0 or 4 Gy irradiation. ** $p < 0.01$ compared to the control group.

A significant proportion of advanced NSCLC patients (40-50%) in the East Asian population possess oncogenic mutations in EGFR gene[24]. Exon 19 deletion and exon 21-point mutation, the most common alterations in EGFR, are widely used as a therapeutic target of EGFR tyrosine kinase inhibitor (EGFR TKI)[25]. However, the role of EGFR in the radiosensitivity of cancer and its definitive molecular mechanisms have not yet been elucidated. Investigators[26,27] origi-

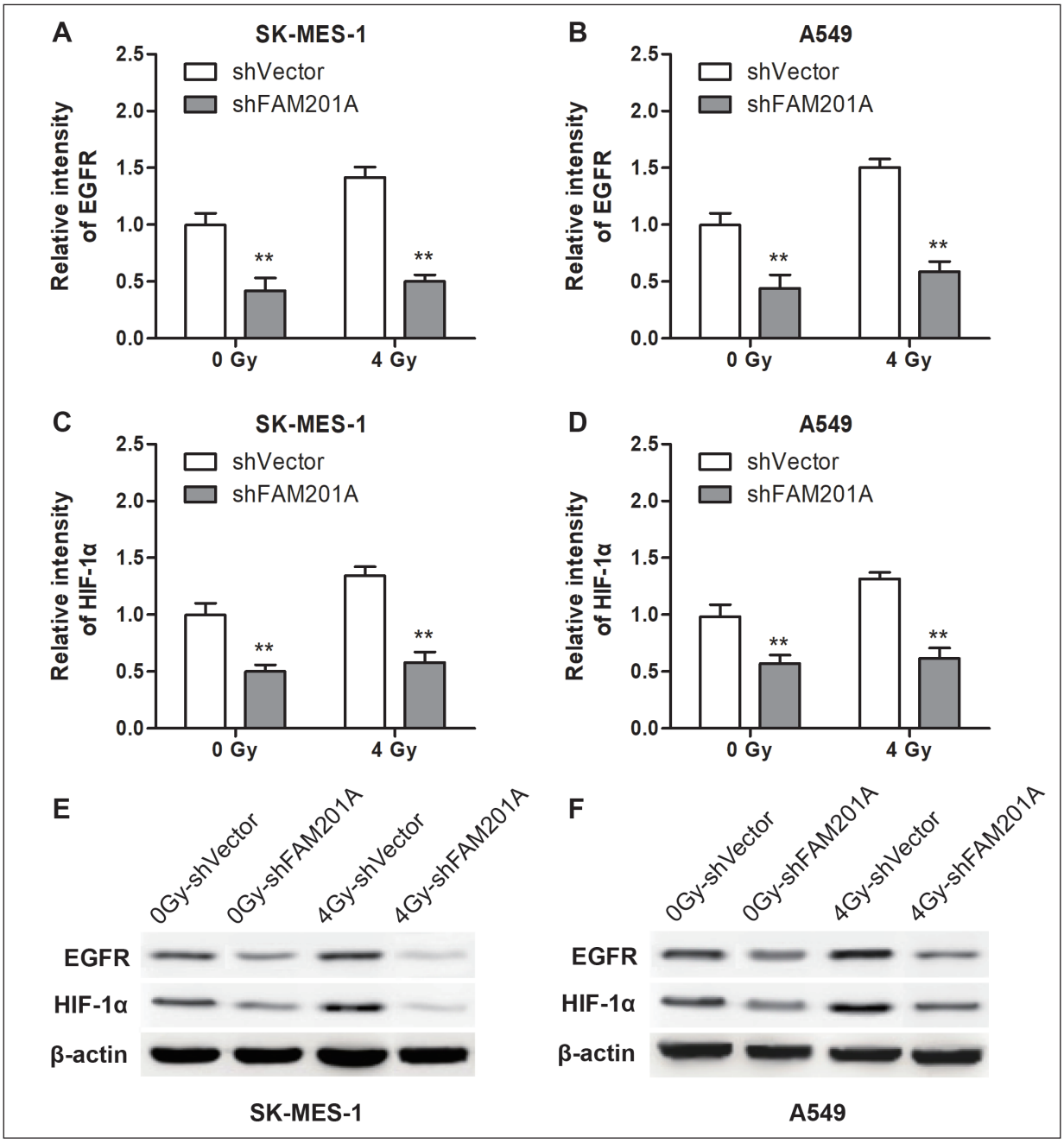

Figure 5. FAM201A inhibits miR-370 resulting in EGFR up-regulation. ***A, B,*** qPCR showed the EGFR level in SK-MES-1 and A549 transfected with shFAM201A or controls under 0 or 4 Gy irradiation. ***C, D,*** qPCR showed the HIF-1α level in SK-MES-1 and A549 transfected with shFAM201A or controls under 0 or 4 Gy irradiation. ***E, F,*** Western blot assay showed the EGFR and HIF-1α protein expression in SK-MES-1 and A549 transfected with shFAM201A or controls under 0 or 4 Gy irradiation. *$p < 0.05$, ** $p < 0.01$ compared to the control group.

nally found the EGFR as a therapeutic switch to radiation sensitivity in cervical cancer through different inducing molecules. Furthermore, both PERK-eIF2α-GRP94 and IRE1α-XBP1-GRP78 are potentially involved in EGFR-induced radioresistance in oropharyngeal carcinoma[28]. EGFR conspires with ZEB1 to enhance the radioresistance which can be reversed by miR-875 in prostate cancer[29]. Recently, the connection between microRNA and EGFR in cancer radiosensitivity is attracting increased attention. In our study, lncRNA FAM201A-miR370 upregulated the EGFR

and HIF-1α expression in radio-resistant NSCLC cell lines (Figure 5C). Our results indicate that hypoxia-related EGFR functions as an effector in radiation modulation. Consistently, Liu et al[30] found that EGFR loss resulted from hypoxia-induced autophagy which can cause cell death and enhance radiosensitivity in NSCLC. Lee et al[31] showed that hypoxia/reoxygenation-induced the activation of Nrf2 and EGFR which can further evoke radioresistance in A549 cell.

Conclusion

We demonstrated that lncRNA FAM201A induces cell proliferation and tumor growth under radiation treatment *in vitro* and *in vivo*. In addition, the interference of FAM201A impairs its suppression of miR-370, resulting in EGFR and HIF-1α upregulation and radiosensitivity enhancement. Our results indicate that this regulatory axis may be a potential therapeutic target to enhance the sensitivity response of NSCLC to radiotherapy.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Disclosure of Financial Arrangements

The research and manuscript preparation are funded by Yue Chen.

Authors' Contribution

Anmin Liu made a contribution to molecular biological assays, data analysis, and manuscript preparation. Yan Zhu assisted in performing the literature research and clinical enrollment. Lei Lei helped Anmin Liu to perform the animal experiment. Shaozhi Fu analyzed the survival data from NSCLC patients. Zhanwen Huang reviewed the paper and gave advice. Yue Chen directed all these researches and reviewed this manuscript. All authors read and approved the final manuscript.

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