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miR-25 modulates NSCLC cell radio-sensitivity through directly inhibiting BTG2 expression

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ABSTRACT

A large proportion of the NSCLC patients were insensitive to radiotherapy, but the exact mechanism is still unclear. This study explored the role of miR-25 in regulating sensitivity of NSCLC cells to ionizing radiation (IR) and its downstream targets. Based on measurement in tumor samples from NSCLC patients, this study found that miR-25 expression is upregulated in both NSCLC and radio-resistant NSCLC patients compared the healthy and radio-sensitive controls. In addition, BTG expression was found negatively correlated with miR-25a expression in the both tissues and cells. By applying luciferase reporter assay, we verified two putative binding sites between miR-25 and BTG2. Therefore, BTG2 is a directly target of miR-25 in NSCLC cancer. By applying loss-and-gain function analysis in NSCLC cell lines, we demonstrated that miR-25-BTG2 axis could directly regulated BTG2 expression and affect radiotherapy sensitivity of NSCLC cells.

1. Introduction

Lung cancer is a malignant tumor and is one of the leading causes of cancer related death [1]. Non-small cell lung cancer (NSCLC) is the most common type and accounts for about 80%–85% of all lung cancer cases. The five year overall survival rate of NSCLC cases is lower than 15% [2]. Radiation therapy is one of the main treatments of NSCLC. However, a large proportion of the patients were insensitive to radiotherapy, which significantly limits the therapeutic effects [3]. Although it is generally recognized that radio-resistance is related to tumor heterogeneity due to different cell origins and molecular/genetic pathogenesis, the exact mechanism is still unclear [4].

The B-cell translocation gene 2 (BTG2) is a member of the APRO (antiproliferative), which is generally considered as a tumor suppressor gene in various cancers [5]. Several recent studies found that exposure to DNA-damaging agents such as ionizing radiation (IR) and chemical substances induces BTG2 expression via p53 pathway and results in cell cycle arrest [6–8]. In fact, BTG2 is a pan-cell cycle modulator and acts as a major effector of p53-induced proliferation arrest at either G1 or G2 cell cycle [9,10]. Besides, BTG2 can also enhance the activity of pro-apoptotic stimuli [11]. Therefore, it is highly possible that BTG2 is a functional mediator in radiotherapy sensitivity. As a putative tumor suppressor gene, BTG2 down-regulation was observed in NSCLC cases [12] and was related to increased growth, proliferation, and invasiveness of NSCLC cells [13]. However, how its expression is regulated in lung cancer is still unclear.

MicroRNAs (miRNAs) is a group a conservative, small and non-coding RNAs inhibiting translation of or degrading target mRNAs by binding to the complementary sequences in the 3′ untranslated region (3′-UTR) [14]. Recently, the relationship between microRNAs and BTG2 expression was reported [5]. One recent study demonstrated that miR-21 could bind with BTG2 and downregulate its expression in NSCLC cells [13]. Due to the complex regulative network of miRNAs, it is unclear whether other miRNAs are involved in regulation of BTG2.

This study firstly confirmed BTG2 is a novel target of miR-25 and has two binding sites with miR-25 in the 3′-UTR. The miR-25-BTG2 axis could directly regulated BTG2 expression and affect radiotherapy sensitivity of NSCLC.

2. Methods

2.1. Human specimen collection

From 2013 to 2014, 60 NSCLC patients (22 radio-sensitive and 38 radio-resistant) who received surgical resection and 32 pulmonary

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bulla cases (cancer free, as healthy control) who received video-assisted thoracic surgery at the Civil Aviation General Hospital were recruited. Inform content was obtained from every patient before the surgery. Lung cancer tissue was collected immediately after resection and was stored in liquid nitrogen before further use. Blood sample (5 ml) were collected from every participants.

2.2. qRT-PCR to quality the expression of miR-25 and BTG2

Total RNAs were extracted from tissue and cells samples by using Trizol and from blood by using TRIzol LS Reagent reagent (Invitrogen) respectively, according to the manufacturer's instructions. The integrity of total RNA samples were examined by using gel electrophoresis and the purity and concentration were determined by using a UV–visible spectrophotometer (Evolution 260 Bio, Thermo). TaqMan MicroRNA Reverse Transcription Kit was used to synthesize miRNA specific cDNA. Then mature miR-25 expression was quantified by using Taqman miRNA Assays (Applied Biosystems) according to recommended protocol. For BTG2 mRNA expression analysis, first strand cDNA was synthesized by using RevertAid first strand cDNA synthesis kit (Fermentas) according to the manufacturer's instructions. Its expression at mRNA level was detected by using the following primers : F: 5′-CATCATCAGCAGGGTGGC-3′, and R: 5′-CCCAATGCGGTAGGACAC-3′) by using Syber Green PCR mastermix (Applied Biosystems). U6 served as an endogenous control.

2.3. Cell culture

HEK293T, normal human bronchial epithelial cell line BEAS-2B and NSCLC cancer cells, including H226, H1299, A549 and U1810 was obtained from the American Type Culture Collection (ATCC). The cells were cultured in DMEM (HEK293T) or RPMI 1640 (other cell lines) culture medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 μg/mL streptomycin and 100 U/mL penicillin in an incubator with a humidified atmosphere and 5% CO_2 at 37 °C.

2.4. Ionizing radiation (IR) (γ-ray)

24 h before exposure to irradiation, cells were plated in 25 cm^2 polystyrene flasks to generate a subconfluent monolayer. Then, the cell monolayer were irradiated by using a Gamma Cell 40 Exactor (Nordion International) at a dose rate of 2.4 Gy/min.

2.5. Western blot analysis of BTG2 expression

Patient tumor tissues and cells were lysed to extract protein for western blot analysis. Protein concentration was measured by BCA protein assay (Pierce, Thermo Scientific). GAPDH served as a loading control. Protein samples were separated on 10% SDS PAGE gel and then transferred onto nitrocellulose membranes. The membranes were blocked with 5% nonfat dry milk in TBST and probed with primary antibodies (anti-BTG2, Abcam, ab58219, 2 μg/ml; anti-GAPDH, Abcam, ab125247, 0.5 μg/ml) overnight at 4 °C and then with secondary antibody (Anti-Mouse IgG (HRP), Abcam, ab97046, 0.2 μg/ml) for 2 h at room temperature. The signals were visualized by using ECL Western Blotting Substrate (Thermo Scientific Pierce).

2.6. Cell transfection

Chemically synthesized AntagomiR-25 and miR-25 mimics were purchased from Ribo Life Science (China). BTG2 Wide Type (Wt) CMV Expression Vector and the empty control were purchased from Affymetrix. BTG2 mutant (Mut) Expression Vector was generated by using the QuikChange Multi Site-Directed Mutagenesis kit (Stratagene). BTG2 siRNA and siRNA control were purchased from Santa Cruz. Plamid, antagomiR and miR mimics transfection were done by using lipofectamine 2000 (Invitrogen) and siRNA transfection is done by using Oligofectamine (Invitrogen) according to recommended manual. Generally, U1810 cells were transfected with antago-miR-NC (400 nM), antago-miR-25 (400 nM), BTG2 Wt Expression Vector, BTG2 Expression Vector control and a combination of antago-miR-25 (400 nM) and BTG2 siRNA (200 nM) respectively. H226 cells were transfected with miR-25 mimics (100 nM), miR-NC mimics (100 nM), BTG2 siRNA (200 nM), siRNA control (200 nM) and a combination of miR-25 mimics (100 nM) and BTG2 Mut Expression Vector respectively. HEK293T cells were transfected with miR-25 mimics (100 nM).

2.7. MTT assay of cell viability

Cells after different treatments were plated at 5×10^3 cells/well in 96-well plates. Cells were cultured for 48 h and then cell viability was measured by MTT (Sigma–Aldrich) assay according to recommended protocol. Absorbance at 490 nm of the solution was read by using a spectrophotometric plate reader. Each test was performed with three repeats.

2.8. Flow cytometry analysis of apoptosis

U1810 Cells were treated with Antago-miR-25, Antago-miR-NC, co-treated with IR and Antago-miR-25 or co-treated with IR and Antago-miR-NC. H226 cells were treated with miR-25 mimics, miR-NC mimics, co-treated with IR and miR-25 mimics or co-treated with IR and miR-NC mimics. 48 h after treatment, cells were plated in six-well plates at 4×10^5 cells/well. Cell apoptosis was detected by using Fluorescein Active Caspase 3 Staining Kit (Abcam, ab65613). The proportion of apoptotic cells was measured by using a flow cytometer (FACSCalibur, BD Biosciences).

2.9. Luciferase reporter assay

To predict binding sites between miR-25 and BTG2, searching was performed in PICTAR and MIRDB. Considering two putative binding sites identified, four pairs of DNA oligonucleotides containing the BTG2 3′-UTR wild type (Wt) and/or mutant (Mut) sequence corresponding to the putative miR-25 binding sites and with the flanking SacI and SalI restriction enzyme digestion sites were synthesized (Sequence details were given in supplementary material. 1). The DNA oligonucleotide and the pmirGLO Dual-Luciferase miRNA Target Expression Vector (Promega) were used to build the luciferase report vectors. The recombinant luciferase report vectors were designated as Luc-BTG2-Wt-1 + Wt-2, Luc-BTG2-Mut-1 + Wt-2, Luc-BTG2-Wt-1 + Mut-2 and Luc-BTG2-Mut-1 + Mut-2 respectively. The insertion was confirmed by sequencing. The HEK293T cells were co-transfected with 300 ng reporter plasmids and miR mimics-NC or miR-25 mimics respectively. After transfection for 24 h, the cells were lysed and the fluorescence activity was detected by GloMax 20/20 Luminometer. The firefly luciferase activity was normalized to the renilla luciferase activity.

2.10. Statistical analysis

Data were presented as mean ± SD. Group comparison was performed by Mann–Whitney U test. MiR-25 expression change before and after radiotherapy was based on Wilcoxon signed-rank two-related-samples test. p value <0.05 was considered as

significant difference. *, **, and *** donates significance at 0.05, 0.01 and 0.001 level respectively.

3. Results

3.1. miR-25 is upregulated, while BTG2 is downregulated in both NSCLC and radio-resistant NSCLC patients

miR-25 and BTG2 expression in NSCLC patients were quantified by using qRT-PCR. Based on tissue and serum samples of 60 NSCLC patients, we observed that miR-25 expression was significantly upregualted in cancer cases than in healthy controls (Fig. 1A and B). Interestingly, miR-25 expression was also related to radiotherapy sensitivity. Its expression was significantly higher in both tumor and serum of radio-resistant patients than in radio-sensitive counterparts (Fig. 1D and E). We further explored the expression of BTG2 transcript in both healthy controls and NSCLC patients. The results showed that BTG2 expression is significantly lower in cancer cases compared with normal control (Fig. 1C). In addition, its expression was even lower in radio-resistant NSCLC patients than in radio-sensitive patients (Fig. 1F).

3.2. miR-25 modulates sensitivity to radiation induced apoptosis

To explore the association between miR-25 and sensitivity to radiation therapy, we firstly assessed sensitivity to IR of five cell lines with different level of miR-25 expression. Compared with BEAS-2B, H226 and U1810 had the lowest and highest miR-25 expression respectively (Fig. 2A). After treatment with IR and normalized to BEAS-2B, cell proliferation of the four NSCLC cell lines were positively related to miR-25 expression. U1810 and H226 had the highest and lowest proliferation rate respectively (Fig. 2B). Interestingly, BTG2 expression in the four NSCLC cell lines was negatively correlated with miR-25a expression. U1810 and H226 had the lowest and highest BTG2 expression respectively (Fig. 2C). To further study the function of miR-25 in IR sensitivity of NSCLC cells, U1810 with miR-25 knockdown and H226 with miR-25 overexpression were generated by transfection of antagomiR-25 and miR-25 mimics respectively (Fig. 2D1 and D2). MiR-25 knockdown could significantly increase sensitivity of U1810 cells to IR (Fig. 2E1), while miR-25 overexpression presented the opposite effect in H226 cells (Fig. 2E2). Then, cell apoptosis induced under these treatments was assessed by detecting caspase 3 activation. miR-25 knockdown significantly increased apoptosis of U1810 cells induced by IR ((Fig. 2G1 and F1), while miR-25 overexpression significantly rescued apoptosis of H226 cells induced by IR (Fig. 2G2 and F2).

3.3. miR-25 directly targets BTG2 and suppresses its expression

Since the regulative role of miR-25 on NSCLC cells' sensitivity to IR and inverse expression between miR-25 and BTG2 in the cells were identified, we further explored whether miR-25 could regulate BTG2 expression. Through searching and comparison in bioinformatics databases, we identified two putative binding sites between miR-25 and BTG2 (Fig. 3A). To further verify the bindings, luciferase report vectors carrying wide type and mutant sequence of the predicted binding sites (Fig. 3A) were constructed. HEK293T cells were transfected with miR-25 mimics for overexpression (Fig. 3B). The relative luciferase activity of Luc-BTG2-Mut-1 + Mut-2 was mostly inhibited by miR-25. Mutation at any one of the two putative binding sites could partly abrogate the inhibitive effect, while mutation at both sites could fully abrogate the inhibition (Fig. 3C), suggesting miR-25 can effectively bind to these two sites. Furthermore, Knockdown of endogenous miR-25 in U1810 and overexpression of miR-25 in H226 cells led to increased and decreased BTG2 protein expression respectively (Fig. 3D and E). In five randomly selected NSCLC patients, it was also observed that BTG2 protein expression was inversely correlated with miR-25 expression in cancer tissues (Fig. 3F and G). These results thus verified the two miR-25-BTG2 interactions and confirmed miR-25 can directly suppress BTG2 expression.

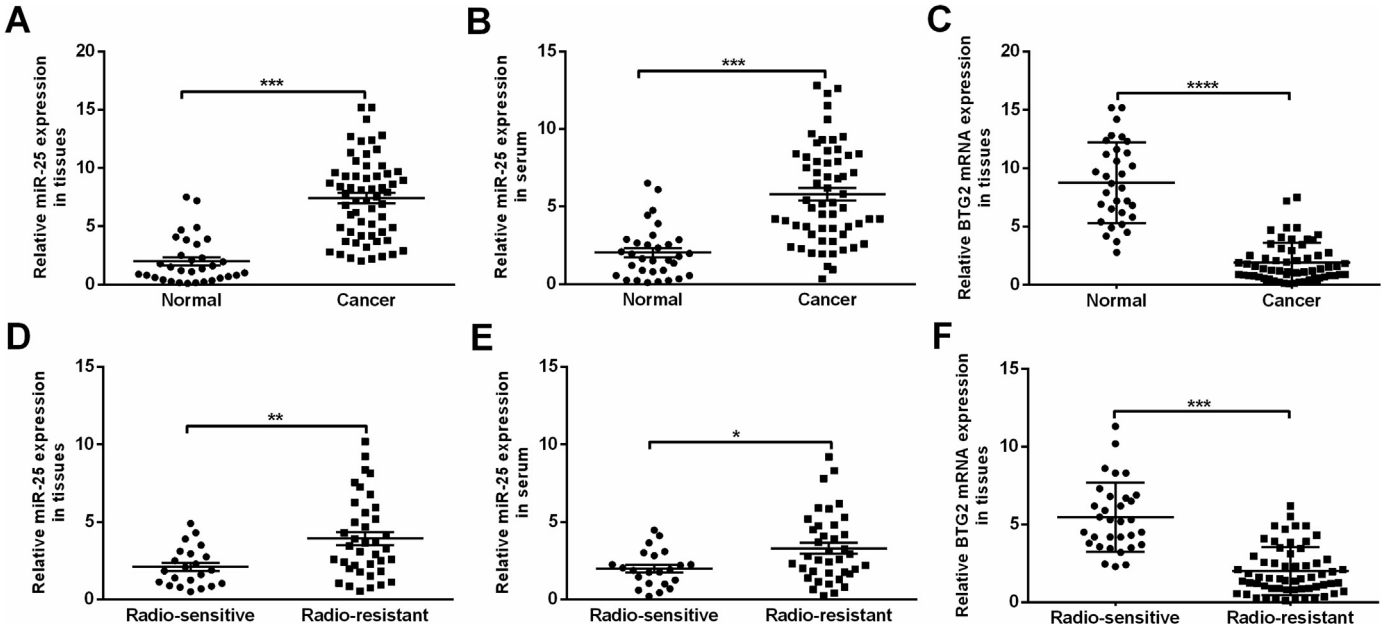

Fig. 1. Up-regulation of miR-25 while down-regulation of BTG2 in both NSCLC and radio-resistant NSCLC patients using qRT-PCR analysis. (A and B) Compared with normal healthy group (n = 32), miR-25 in tissues (A) and serum (B) was significantly down-regulated in human NSCLC patients (n = 60). (C) mRNA level of BTG2 in human NSCLC samples was decreased in contrast with healthy control. (D and E) miR-25 expression was lower in both tissues (D) and serum (E) in radio-sensitive patients (n = 22) than in radio-resistant patients (N = 38). (F) BTG2 expression at mRNA level was down-regulated in radio-sensitive tumor tissues compared with radio-resistant tissues. Values are the average of triple determinations with the S.D. indicated by error bars. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.001.

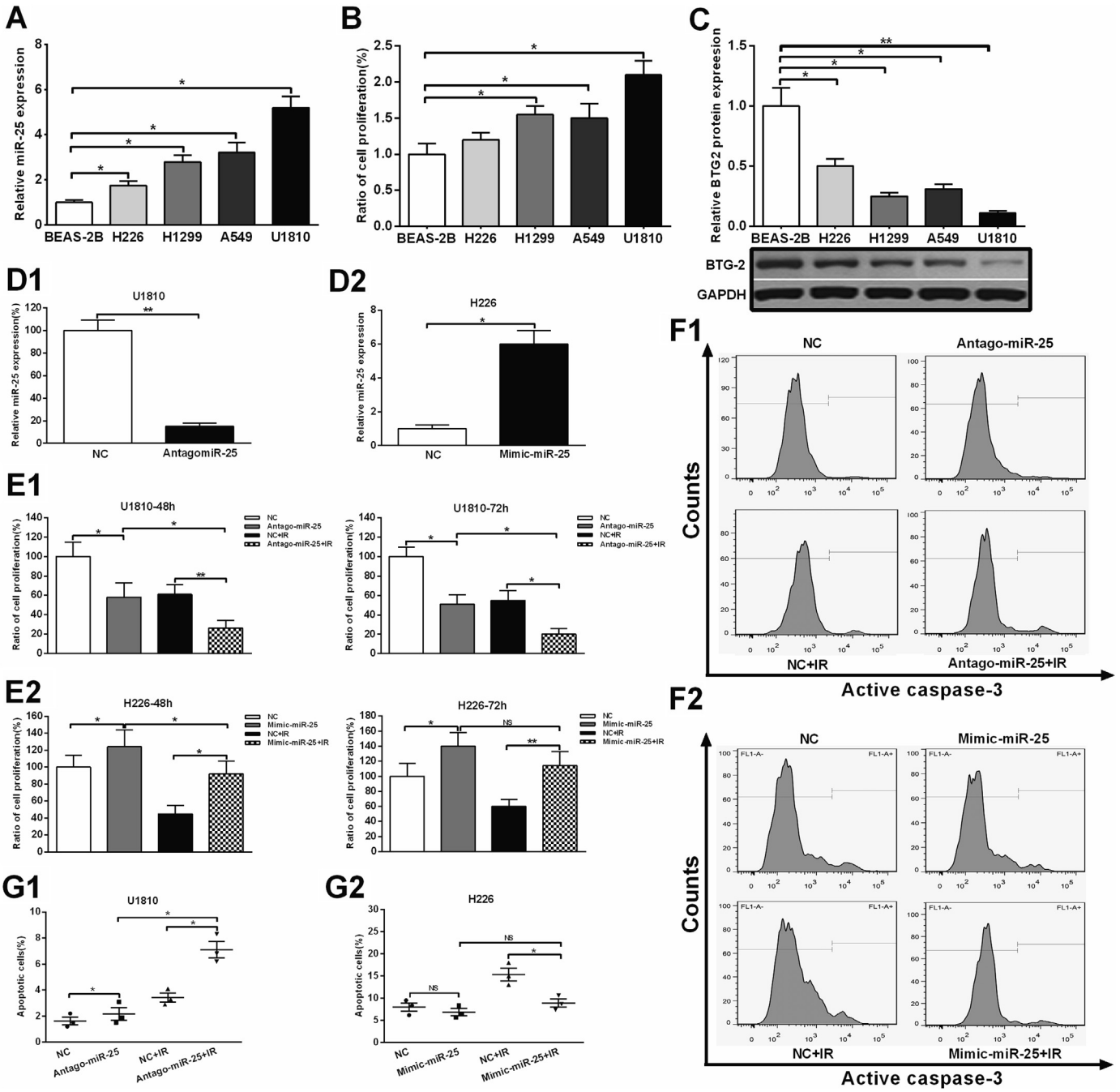

Fig. 2. Expression level of miR-25 modulates sensitivity of radiation induced apoptosis. (A) Relative expression of miR-25 in four NSCLC cell lines (H226, H1299, A549 and U1810) and a normal human bronchial epithelial cell line (BEAS-2B) by qRT–PCR methods, U6 served as an endogenous control. (B) MTT analysis of cell viability of four NSCLC cell lines normalized to BEAS-2B 48 h post IR. (C) BTG2 protein levels in four different cell lines were assessed by western blot analysis. The GAPDH served as an internal control. (D1 and D2) Transfection (48 h) of miR-25 antagomir in U1810 cell line (D1) and mimics in H226 (D2) caused significantly decreased and increased expression of miR-25 respectively. (E1 and E2) Cell viability of U-1810 cells transfected with miRNA-25 antagomiR (E1) and H226 with miR-25 mimics (E2) was analyzed at 48 h post IR by MTT. (F1 and F2) Representative images of flow cytometry analysis of apoptotic cells by active caspase-3 staining 48 h post IR. U-1810 treated with miRNA-25 antagomiR (F1) and H226 with miR-25 mimics (F2). (G1and G2) Histograms served as the quantification of apoptotic cells by active caspase-3 in F1 and F2 respectively. Representative results are showed as mean ± S.D from three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{NS}P>0.05$.

3.4. miR-25 modulates sensitivity to radiotherapy through if not all, inhibiting BTG2 expression

We further explored the role of BTG2 in sensitivity of NSCLC cells to radiation. U1810 with BTG2 overexpression and H226 cells with BTG2 knockdown was generated (Fig. 4A1 and A2). BTG2 overexpression significantly increased radio-sensitivity of U1810 cells (Fig. 4B1), while BTG2 knockdown promoted redio-resistance of H226 cells (Fig. 4B2). BTG2 knockdown could significantly rescue decreased radio-resistance due to miR-25 knockdown in U1810 cells (Fig. 4C1). Overexpression of mutant BTG2 (without miR-25 binding site) significantly alleviate increased radio-resistance due to miR-25 overexpression (Fig. 4C2). Therefore, these results suggest that miR-25 modulates

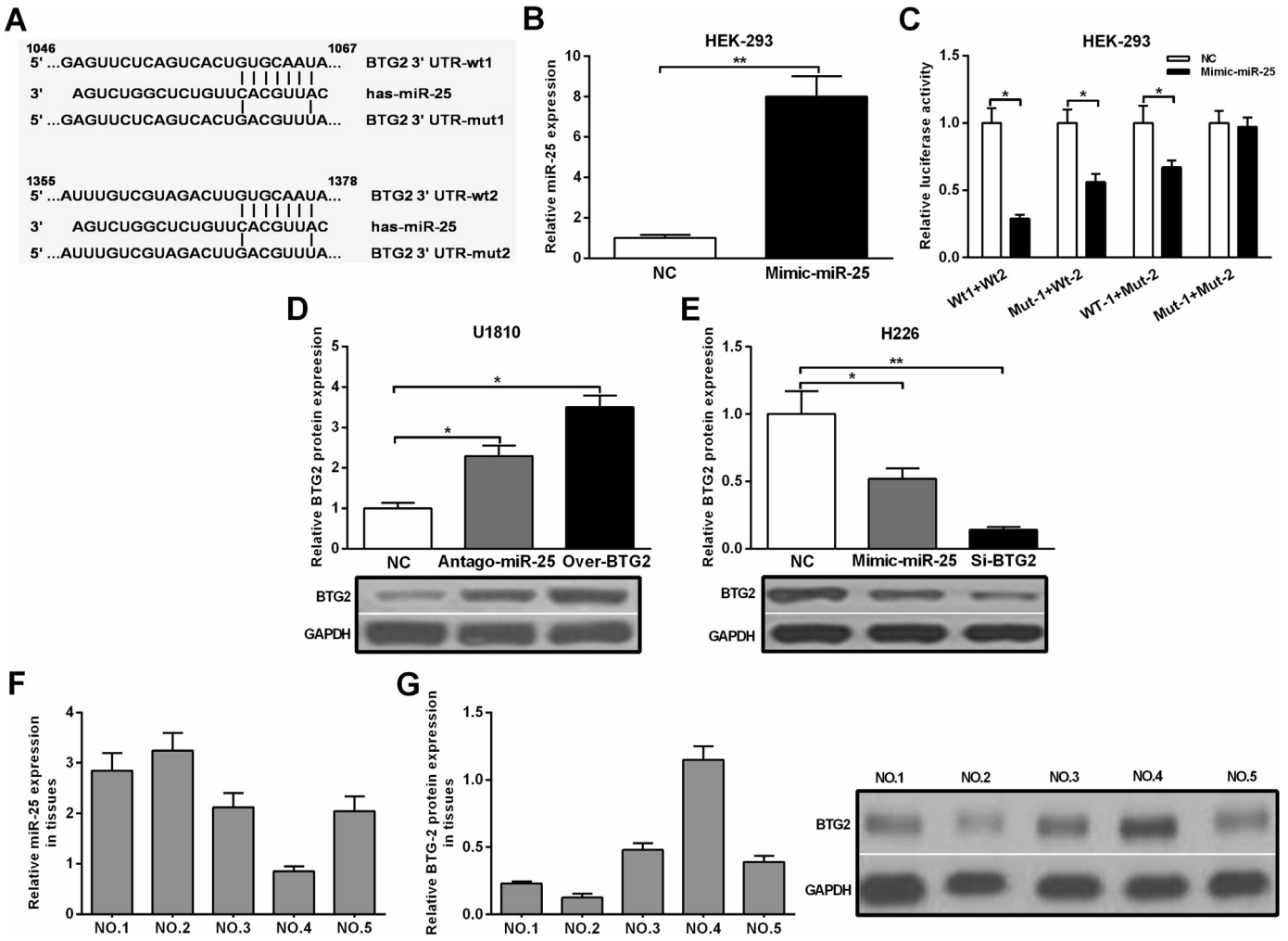

Fig. 3. BTG2 is a direct target of miR-25 which suppressed the expression of BTG2 through binding to its 3′-UTR. (A) Bioinformatics prediction between miR-25 and 3′-UTR of BTG2. The predicted BTG2-wild (wt1 and wt2) and designed BTG2-mutant (mut1 and mut2) binding sequences with miR-25 were showed. (B) Transfecting 100 nM miR-25 mimics resulted in remarkably increased miR-25 in HEK-293T cells. (C) 293T cells were co-transfected with either 100 nM miR-25 mimics or NC oligos and 200 ng plasmid carrying either wt or mut 3′-UTR of BTG2. The relative firefly luciferase activity normalized with Renilla luciferase was measured 48 h after transfection. (D) Knockdown of endogenous miR-25 in U1810 cells caused increased BTG2 protein level. Overexpressing BTG2 served as positive control. (E) In H226 cells with low endogenous miR-25, miR-25 ovexpression led to downregulated protein level of BTG2. siRNA targeting BTG2 served as positive control. (F and G) qRT-PCR analysis of miR-25 (F) and Western blot analysis of BTG2 (G) in cancer tissues from five randomly selected NSCLC patients. Results showed that protein level of BTG2 was inversely correlated with miR-25 expression in NSCLC tissues. Data are showed as mean ± S.D and the result are from three independent experiments. *P < 0.05, **P < 0.01.

sensitivity of NSCLC cells to IR by regulating BTG2 expression directly.

4. Discussion

Increased miR-25 expression was observed in several types of cancer, including prostate, gastric, hepatocellular ovarian and esophageal squamous cell carcinomas [15–17]. In these cancers, miR-25 act as an oncogene by targeting different genes. In gastric cancer, it promotes gastric cancer migration, invasion and proliferation by directly targeting ERBB2 and TOB1 [17]. In esophageal squamous cell carcinoma, miR-25 promotes cell migration and invasion by targeting E-cadherin (CDH1) [16]. In ovarian cancer, miR-25 attenuates cancer cell apoptosis by targeting Bim [18] and promotes cancer cell proliferation and motility by targeting LATS2 [19]. Therefore, miR-25 has a complex regulative network and its targets might be cancer specific. In lung cancer, previous studies observed that miR-25 expression might be associated with tumor progression [20] and it might regulate proliferation of NSCLC cells through targeting CDC42 [21]. However, the exact expression pattern and function of miR-25 in NSCLC cancer, especially in radio-sensitive and radio-resistant NSCLC is still not clear. In the current study, we demonstrated that miR-25 is upregulated in NSCLC, especially in radio-resistant NSCLC patients and can modulate sensitivity of radiation induced apoptosis. Based on the confirmed regulative role of miR-25 in sensitivity to radiotherapy, we further explored its downstream targets in NSCLC.

BTG2 is expressed in various organs and tissues and is considered an early growth response gene. The decreased expression of BTG2 is found in several human cancer tissues [5]. BTG2 usually acts as tumor suppressor due to its anti-proliferation and apoptosis inducing effect [5]. In fact, BTG2 is a pan-cell cycle modulator and acts as a major effector of p53-induced G1 or G2 cell cycle arrest [9,10]. In lung cancer, previous study demonstrated that BTG2 can inhibit cell proliferation and invasion by decreasing expression of cyclin D1, MMP-1, and MMP-2 [12,13]. However, it is highly possible that BTG2 has some unidentified functions due to its wide and strong regulation over cell cycle.

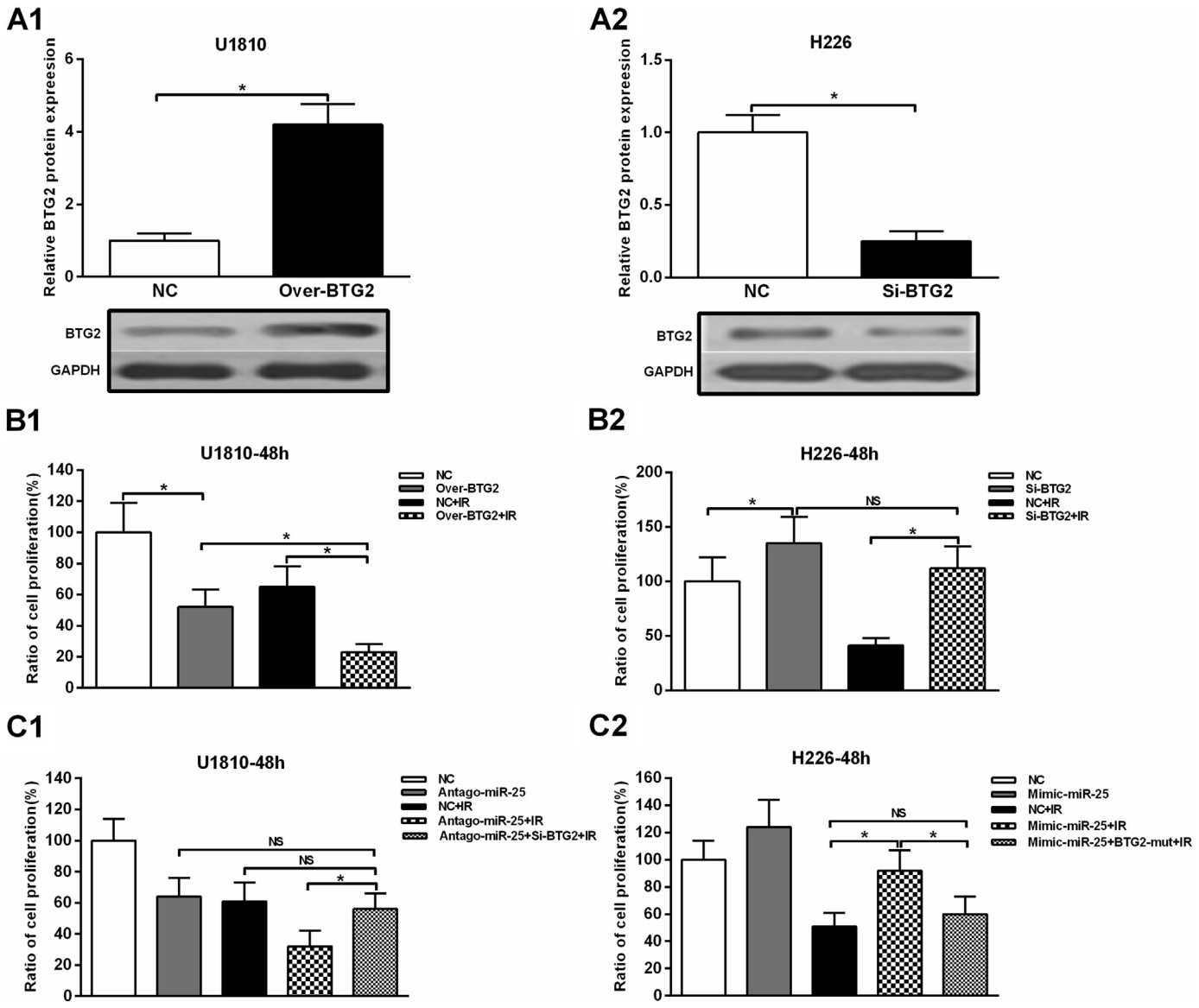

Fig. 4. miR-25 modulates sensitivity of radiation through, if not all, inhibiting BTG2 expression in some part. (A1 and A2) Successful overexpression of BTG2 in U1810 (A1) while knockdown in H226 (A2). (B1 and B2) Cell viability of U-1810 cells with BTG2 overexpression (B1) and H226 with BTG2 knockdown (B2) were analyzed 48 h post IR by MTT. (C1 and C2) Effects of co-transfection of antago-miR-25 and BTG2 siRNA in U1810 (C1) and combined overexpression of miR-25 and BTG2 without binding site with miR-25 in H226 (C2) on response to IR were analyzed by MTT assays. Data are showed as mean ± S.D and the result are from three independent experiments. $^{*}P < 0.05$, $^{***}P < 0.001$, $^{NS}P > 0.05$.

DNA damage is associated with following upregulated p53 expression. Generally, P53 induces DNA repair and initiates cell cycle arrest at G1/S-phase. When the damage could not be repaired, programmed cell death or apoptosis is initiated through p53 pathway. In fact, p53 upregualtion directly leads to increased expression of BTG2 [22] and decreased expression of cyclin D1, a protein promoting DNA damage repair [8]. Several recent studies found that exposure to DNA-damaging agents such as IR and chemical substances induces BTG2 expression via p53 pathway and results in the cell cycle arrest [6–8]. Therefore, it is high possible that BTG2 might be involved in the radiotherapy sensitivity. One recent study found BTG2 overexpression in human breast cancer cell line MCF-7 could increase cell sensitivity to IR by inhibiting DNA repair-related protein expression and enhancing apoptosis induced by radiation [23]. However, whether it influences radiotherapy sensitivity in NSCLC and how its expression is regulated in NSCLC is not clear.

The regulation of miRNA on protein expression is a complex network. A miRNA may target several different genes, while a target gene may also be regulated by several miRNAs. One recent study observed that miR-21 is overexpressed in lung cancer cells and can negatively regulate BTG2 expression [12]. The current study observed inverse expression between miR-25 and BTG2 in NSCLC cancer patients and cell lines. Thus, we further explored the association between miR-25 and BTG2 expression. By using database searching and dual luciferase assay, we verified two putative binding sites between miR-25 and BTG2. Therefore, BTG2 is a direct target of miR-25 in NSCLC cancer. In addition, by applying loss-and-gain function analysis, this study also demonstrated that miR-25-BTG2 axis could regulate radio-sensitivity of NSCLC cancer cells.

In conclusion, this study firstly confirmed BTG2 is a novel target of miR-25 and has two binding sites with miR-25 in the 3′-UTR. The miR-25-BTG2 axis could directly regulated BTG2 expression and affect radiotherapy sensitivity of NSCLC.

Conflict of interest

Authors have no conflict of interest

Appendix. BSupplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2014.12.094.

References

[1] R. Siegel, J. Ma, Z. Zou, A. Jemal, Cancer statistics, 2014, CA: A Cancer J. Clin. 64 (2014) 9–29.
[2] P. Fidias, S. Novello, Strategies for prolonged therapy in patients with advanced non-small-cell lung cancer, J. Clin. Oncol. 28 (2010) 5116–5123.
[3] C. Le Pechoux, Role of postoperative radiotherapy in resected non-small cell lung cancer: a reassessment based on new data, Oncologist 16 (2011) 672–681.
[4] M. Provencio, A. Sanchez, P. Garrido, F. Valcarcel, New molecular targeted therapies integrated with radiation therapy in lung cancer, Clin. Lung Cancer 11 (2010) 91–97.
[5] B. Mao, Z. Zhang, G. Wang, BTG2: a rising star of tumor suppressors (Review), Int. J. Oncol. 46 (2014) 459–464.
[6] S. Tachiiri, T. Katagiri, T. Tsunoda, N. Oya, M. Hiraoka, Y. Nakamura, Analysis of gene-expression profiles after gamma irradiation of normal human fibroblasts, Int. J. Radiat. Oncol. Biol. Phys. 64 (2006) 272–279.
[7] E. Kis, T. Szatmari, M. Keszei, R. Farkas, O. Esik, K. Lumniczky, A. Falus, G. Safrany, Microarray analysis of radiation response genes in primary human fibroblasts, Int. J. Radiat. Oncol. Biol. Phys. 66 (2006) 1506–1514.
[8] T.Y. Chu, J.T. Yang, T.H. Huang, H.W. Liu, Crosstalk with cancer-associated fibroblasts increases the growth and radiation survival of cervical cancer cells, Radiat. Res. 181 (2014) 540–547.
[9] J.P. Rouault, N. Falette, F. Guehenneux, C. Guillot, R. Rimokh, Q. Wang, C. Berthet, C. Moyret-Lalle, P. Savatier, B. Pain, P. Shaw, R. Berger, J. Samarut, J.P. Magaud, M. Ozturk, C. Samarut, A. Puisieux, Identification of BTG2, an antiproliferative p53-dependent component of the DNA damage cellular response pathway, Nat. Genet. 14 (1996) 482–486.
[10] J.W. Hong, M.S. Ryu, I.K. Lim, Phosphorylation of serine 147 of tis21/BTG2/pc3 by p-Erk1/2 induces Pin-1 binding in cytoplasm and cell death, J. Biol. Chem. 280 (2005) 21256–21263.
[11] Y.B. Lim, T.J. Park, I.K. Lim, B cell translocation gene 2 enhances susceptibility of HeLa cells to doxorubicin-induced oxidative damage, J. Biol. Chem. 283 (2008) 33110–33118.
[12] Q. Sun, M. Hang, X. Guo, W. Shao, G. Zeng, Expression and significance of miRNA-21 and BTG2 in lung cancer, Tumour Biol. 34 (2013) 4017–4026.
[13] S. Wei, C. Hao, X. Li, H. Zhao, J. Chen, Q. Zhou, Effects of BTG2 on proliferation inhibition and anti-invasion in human lung cancer cells, Tumour Biol. 33 (2012) 1223–1230.
[14] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.
[15] Y. Li, W. Tan, T.W. Neo, M.O. Aung, S. Wasser, S.G. Lim, T.M. Tan, Role of the miR-106b-25 microRNA cluster in hepatocellular carcinoma, Cancer Sci. 100 (2009) 1234–1242.
[16] X. Xu, Z. Chen, X. Zhao, J. Wang, D. Ding, Z. Wang, F. Tan, X. Tan, F. Zhou, J. Sun, N. Sun, Y. Gao, K. Shao, N. Li, B. Qiu, J. He, MicroRNA-25 promotes cell migration and invasion in esophageal squamous cell carcinoma, Biochem. Biophys. Res. Commun. 421 (2012) 640–645.
[17] B.S. Li, Q.F. Zuo, Y.L. Zhao, B. Xiao, Y. Zhuang, X.H. Mao, C. Wu, S.M. Yang, H. Zeng, Q.M. Zou, G. Guo, MicroRNA-25 promotes gastric cancer migration, invasion and proliferation by directly targeting transducer of ERBB2, 1 and correlates with poor survival, Oncogene 0 (2014).
[18] H. Zhang, Z. Zuo, X. Lu, L. Wang, H. Wang, Z. Zhu, MiR-25 regulates apoptosis by targeting Bim in human ovarian cancer, Oncol. Reports 27 (2012) 594–598.
[19] S. Feng, W. Pan, Y. Jin, J. Zheng, MiR-25 promotes ovarian cancer proliferation and motility by targeting LATS2, Tumour Biol. 35 (2014) 12339–12344.
[20] F.X. Xu, Y.L. Su, H. Zhang, J.Y. Kong, H. Yu, B.Y. Qian, Prognostic implications for high expression of MiR-25 in lung adenocarcinomas of female non-smokers, Asian Pac. J. Cancer Prev. 15 (2014) 1197–1203.
[21] T. Yang, T. Chen, Y. Li, L. Gao, S. Zhang, T. Wang, M. Chen, Downregulation of miR-25 modulates non-small cell lung cancer cells by targeting CDC42, Tumour Biol. (2014). Epub ahead of print.
[22] Z. Zhang, C. Chen, G. Wang, Z. Yang, J. San, J. Zheng, Q. Li, X. Luo, Q. Hu, Z. Li, D. Wang, Aberrant expression of the p53-inducible antiproliferative gene BTG2 in hepatocellular carcinoma is associated with overexpression of the cell cycle-related proteins, Cell Biochem. Biophys. 61 (2011) 83–91.
[23] X. Hu, L. Xing, Y. Jiao, J. Xu, X. Wang, A. Han, J. Yu, BTG2 overexpression increases the radiosensitivity of breast cancer cells in vitro and in vivo, Oncol. Res. 20 (2013) 457–465.