



# GAS5 regulates viability and apoptosis in TGF- $\beta$ 1-stimulated bronchial epithelial cells by regulating miR-217/HDAC4 axis

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## Abstract

**Background** Asthma is a serious respiratory disease that affects the physical and mental health of children. Airway epithelial apoptosis concomitantly mediated by transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) is a crucial component of asthma pathogenesis. LncRNA growth Arrest Specific 5 (GAS5), microRNA-217 (miR-217) and Histone deacetylase 4 (HDAC4) shown a close relationship with TGF- $\beta$ 1-induced injury of airway epithelial. However, the mechanism underlying TGF- $\beta$ 1-induced injury of airway epithelial in asthma still needs to be investigated.

**Objective** We aimed to investigate the effect and underlying mechanism of GAS5/miR-217/HDAC4 axis in TGF- $\beta$ 1-stimulated bronchial epithelial cells.

**Methods** The levels of were detected by quantitative real-time polymerase chain reaction (RT-qPCR). All protein levels were determined by western blot. Cell viability and apoptosis rate were assessed by Methyl thiazolyl tetrazolium (MTT) and Flow cytometry, respectively. The targeting relationship between miR-217 and GAS5 or HDAC4 was examined with dual-luciferase reporter assay.

**Results** TGF- $\beta$ 1, GAS5, HDAC4 were up-regulated, while miR-217 was down-regulated in bronchial mucosal tissues of asthmatic children and TGF- $\beta$ 1-treated BEAS-2B cells. TGF- $\beta$ 1 could reduce cell viability and induce apoptosis, while these effects could be reversed by downregulation of GAS5 or HDAC4. Mechanically, GAS5 acted as a sponge for miR-217 to regulate the expression of HDAC4. Furthermore, overexpression of HDAC4 rescued the effects of GAS5 knockdown on viability and apoptosis of TGF- $\beta$ 1-induced BEAS-2B cells. GAS5 knockdown induced cell viability and hampered cell apoptosis in TGF- $\beta$ 1-stimulated BEAS-2B cells by regulating the miR-217/HDAC4 axis.

**Conclusions** The lncRNA GAS5/miR-217/HDAC4 axis played an important role in regulating TGF- $\beta$ 1-induced bronchial epithelial cells injury, thus contributing to asthma.

**Keywords** Asthma · TGF- $\beta$ 1 · GAS5 · MiR-217 · HDAC4

## Abbreviations

GAS5 Growth arrest-specific transcript 5  
HDAC4 Histone deacetylase 4  
TGF GAS5 in transforming growth factor

## Introduction

Asthma is a common chronic lung disorder with inflammation in the airways, which affects people from childhood to adulthood (Fahy 2015). In general, childhood onset asthma and adult onset asthma differ with respect to sex ratios, phenotypes, and potentially also for genetic risk factors (Bush and Menzies-Gow 2009; Larsen 2001; Dijk et al. 2013). Current research suggests that childhood asthma is mainly caused by allergy and dysregulation of epithelial barrier function genes, while adult asthma is more lung-centered and environment-determined (Pividori et al. 2019). Childhood asthma is mainly diagnosed in preschool children, is responsible for a heavy burden of ill health, including premature death. It is characterized by chronic airway inflammation in which patients experience wheezing, shortness of

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breath, a sense of urgency in the chest and coughing (Aysola et al. 2008). Accumulating evidence suggested that airway epithelial apoptosis and epithelial mesenchymal transition (EMT) are two crucial components of asthma pathogenesis. The apoptosis of airway epithelial cells affect epithelial integrity (Song and Shi 2018) and thereby exposed airway and lung to excess pathogens or environmental allergens (Hackett et al. 2009; Charbonneau et al. 2016), which ultimately lead to airway remodeling and airway hyper-responsiveness (AHR) (Yang et al. 2017). Thus, protecting airway epithelial cells from injury might improve the occurrence and development of childhood asthma.

Transforming growth factor (TGF)- $\beta$ 1, a profibrotic cytokine, was upregulated in bronchial tissue samples of patients with asthma (Vignola et al. 1997) and bronchoalveolar lavage fluid (Redington et al. 1998), which has reported to be associated with the pathogenesis of asthma. For example, Gagliardo et al. (2013) suggested that TGF- $\beta$ 1 promotes eosinophil and neutrophil adhesion to epithelial cells in the mechanisms leading to the inflammatory and restructuring processes in asthma. Besides, Zhong et al. (2019) indicated that TGF- $\beta$ 1/Smad pathway was inhibited by the mesenchymal stem cells derived from induced pluripotent stem cells, and thereby preventing chronic allergic airway inflammation. Besides, TGF- $\beta$ 1 could regulate both of airway epithelial apoptosis and EMT of airway epithelial cells (Pu et al. 2020). Therefore, exploring the molecular mechanism of TGF- $\beta$ 1 induced apoptosis of airway epithelial cells may provide a new therapeutic target for the treatment of childhood asthma.

Long non-coding RNAs (lncRNAs) are RNA molecules with a length of more than 200 nucleotides (Li et al. 2018). Increasing studies have indicated that many lncRNAs are abnormal expressed in asthma patients and can regulate cell biological behaviors, for instance, TUG1 was up-regulated in asthma rat and promoted the proliferation and migration of airway smooth muscle cells (ASMCs) (Lin et al. 2019). In addition, Hu et al. analyzed the expression patterns of lncRNAs in BEAS-2B cells induced by TGF- $\beta$ 1 through lncRNA microarray, revealing the important role of TGF- $\beta$ 1-stimulated differentially expressed lncRNA in oncogenic transformation. LncRNA growth arrest-specific transcript 5 (GAS5), a tumor suppressor, has been shown to regulate various biological functions of tumor cells, such as cell proliferation, apoptosis and migration (Wang et al. 2020; Lyu et al. 2019). However, GAS5 was found to be elevated by pro-inflammatory factors in ASMCs and airway epithelial cells (Keenan et al. 2015). We wondered whether GAS5 mediated TGF- $\beta$ 1-induced apoptosis of bronchial epithelial cells in childhood asthma.

Multiple miRNAs have been reported to be abnormally expressed in airway epithelial cells in asthmatic patients, such as miR-744 was down-regulated in bronchial epithelial

cells of children with severe asthma, and its overexpression could retard cell proliferation by targeting TGF- $\beta$ 1 (Huang et al. 2019). As a tumor-suppressing miRNA, miR-217 has recently been revealed to be down-regulated in TGF- $\beta$ 1-stimulated ASMCs, and its elevation distinctly inhibited TGF- $\beta$ 1-induced ASMCs proliferation and migration (Gao et al. 2018). Histone deacetylase 4 (HDAC4) is a member of the class IIa HDAC proteins (Haberland et al. 2009), and Pan et al. (2018) revealed that HDAC4 was facilitated in TGF- $\beta$ 1-induced ASMCs. However, the expression and association of miR-217 and HDAC4 in TGF- $\beta$ 1-induced airway epithelial cells remain unrevealed.

It is reported that lncRNAs could act as competitive endogenous RNAs (ceRNAs) for microRNAs (miRNAs) to competitively adsorb miRNAs to regulate mRNAs expression transcription and translation, thus playing a biological role in the development of multiple diseases, including asthma. However, whether GAS5 regulate miR-217 expression as ceRNAs, indirectly affects the levels of HDAC4 in bronchial epithelial cells is still unknown. In this study, we explored the expression and mechanism underlying GAS5/miR-217/HDAC4 in TGF- $\beta$ 1-induced bronchial epithelial cells.

## Materials and methods

### Acquisition of bronchial mucosal tissues

Bronchial mucosa tissues from 35 asthmatic children were collected at Heping Hospital Affiliated to Changzhi Medical College, and the bronchial mucosal tissues in 35 non-asthmatic children with bronchiectasis were used as the control. All participants signed informed consent forms and our study was permitted by the Ethics Committee of Heping Hospital Affiliated to Changzhi Medical College.

### Cell culture and TGF- $\beta$ 1 treatment

Human bronchial epithelial cell line BEAS-2B was obtained from Shanghai Institutes for Biological Sciences (Shanghai, China). BEAS-2B cells were isolated from normal human bronchial epithelium, which was obtained from autopsy of non-cancerous individuals, could be used as a cell model to investigate the apoptosis of airway epithelial cell in asthma (Pu et al. 2020). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Hyclone, South Logan, UT, USA) containing 10% fetal bovine serum (FBS, Hyclone) at 37 °C under 5% CO<sub>2</sub>. For TGF- $\beta$ 1 stimulation, cells were starved in serum-free for 24 h, and then treated with different concentrations of TGF- $\beta$ 1 (0, 1, 5, 10 ng/mL) (Sigma-Aldrich, St. Louis, MO, USA) for 48 h.

## Transfection

The miR-217 mimics (miR-217) and control (miR-NC), miR-217 inhibitor (anti-miR-217) and control (anti-miR-NC), small interfering RNAs against GAS5 (si-GAS5) and HDAC4 (si-HDAC4) and the negative control si-NC, HDAC4 overexpressed plasmid (HDAC4) and its negative control Vector were purchased from RiboBio (Guangzhou, China). These synthetic oligonucleotides and plasmids were transfected into BEAS-2B cells by using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA).

## Quantitative real-time polymerase chain reaction (RT-qPCR)

Bronchial mucosa tissues and BEAS-2B cells were collected, and the RNA was extracted by Trizol (Invitrogen). PrimeScript<sup>TM</sup> RT reagent Kit with gDNA Eraser was used to synthesize complementary DNA (cDNA). For GAS5 and HDAC4, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) acted as the internal control, and for miR-217, its expression was normalized to U6. Their primer sequences were as below: GAS5, F 5'-CTTCTGGGC TCAAGTGATCCT-3', R 5'-TTGTGCCATGAGACTCCA TCAG-3'. miR-217, F 5'-TACTGCATCAGGAAGTGA TTGG-3', R 5'-CAGTGCCTGTCGTGGAGT-3'. HDAC4, F 5'-CCCATCATTGCAATAGCAGG-3', R 5'-GTTCAA ACTTCTGCTCCTGA-3'. U6, F 5'-ACCCTGAGAAAT ACCCTCACAT-3', R 5'-GACGACTGAGCCCCTGAT G-3'. GAPDH, F 5'-CCTGCACCACCAACTGCTTA-3', R 5'-GGCCATCCACAGTCTTCTGAG-3'. Finally, RT-qPCR was performed using SYBR Select Master Mix (Applied Biosystems, Foster City, CA, USA).

## Western blot assay

After proteins from bronchial mucosa tissues and BEAS-2B cells were dissociated by RIPA buffer (Sigma-Aldrich), proteins were then separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred into polyvinylidene difluoride (PVDF, Beyotime, Shanghai, China) membranes. Next, the membranes were blocked in 5% skim milk for 2 h, and incubated with primary antibodies against HDAC4 (2 µg/mL, Thermo Fisher Scientific, Waltham, MA, USA), B-cell lymphoma-2-associated X (Bax, 1 µg/mL, Thermo Fisher Scientific), Cleaved PARP (1:1000, Thermo Fisher Scientific), or GAPDH (1:2000, Beyotime) overnight at 4 °C. Following the day, the protein samples were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody

(1:3000, Beyotime) for 2 h. The protein bands were visualized using a BeyoECL Plus Kit (Beyotime).

## Cell viability and apoptosis assay

Cell viability and apoptosis were respectively determined by Methyl thiazolyl tetrazolium (MTT) assay and Flow cytometry assay. For cell viability detection, the transfected or TGF-β1-treated BEAS-2B cells were seeded into the 96-well plates for 48 h. Next, 10 µL of MTT (Beyotime) was reacted with the cells for 3 h. Finally, the optical density (OD) value at 490 nm was analyzed by a spectrophotometer (Thermo Fisher Scientific). For cell apoptosis detection, the Annexin V fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis detection kit (Vazyme, Nanjing, China) was used. Briefly, the treated BEAS-2B cells were harvested and stained with 5 µL FITC and 5 µL PI for 20 min at 37 °C. Then the rate of cell apoptosis was checked by a flow cytometry (BD Biosciences, Franklin Lake, NJ, USA).

## Dual-luciferase reporter assay

The full-length of GAS5 and 3'untranslated region (3'UTR) of HDAC4 containing the wild-type (wt) or mutant (mut) binding sites of miR-217 were introduced into the pmir-GLO vectors (Promega, Fitchburg, WI, USA) to form dual-luciferase reporter vectors GAS5 wt, GAS5 mut, HDAC4-3'UTR-wt and HDAC4-3'UTR-mut, respectively. Then, these reporter vectors were co-transfected into BEAS-2B cells with miR-217 or miR-NC, respectively. The luciferase activity was estimated by a dual-luciferase reporter assay kit (Vazyme).

## Statistical analysis

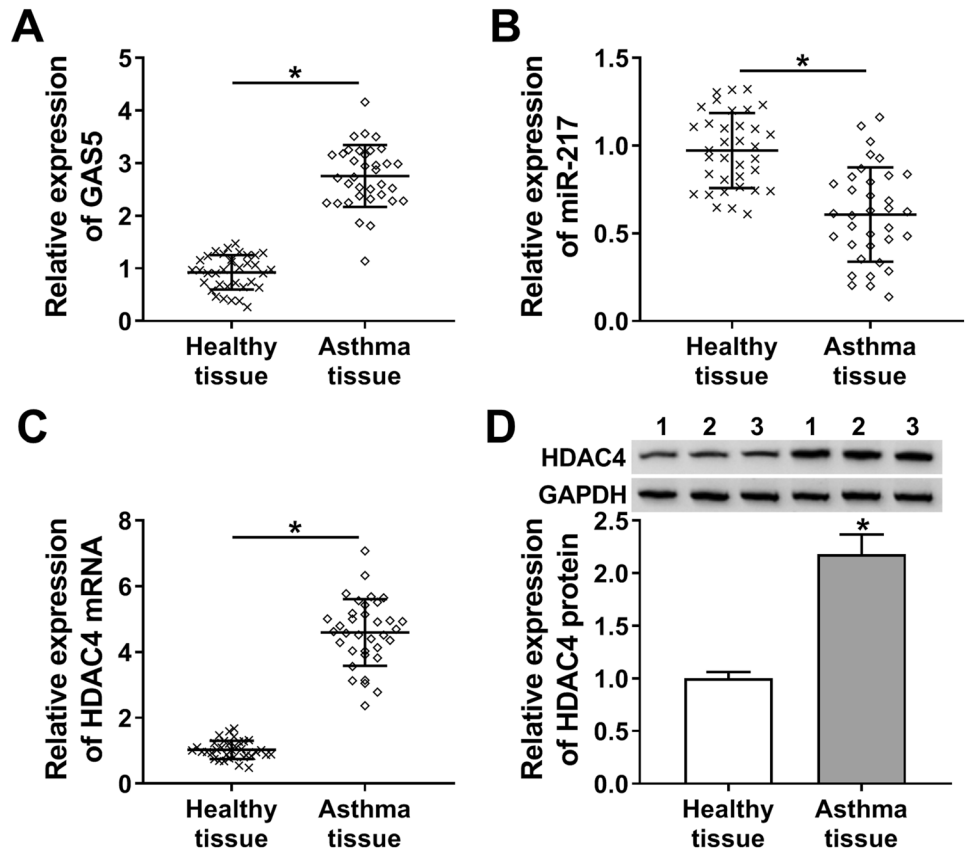
Data were generated at least in triplicate and were displayed as means ± standard deviation (SD). Statistical analysis was conducted using GraphPad Prism 7. To compare the difference between two groups or multi-groups, Student's *t*-test and one-way analysis of variance (ANOVA) were utilized, respectively. The difference was considered significant when *P* value was less than 0.05.

## Results

### GAS5 and HDAC4 were up-regulated, and miR-217 was down-regulated in bronchial mucosa tissues of asthmatic children

We first examined the expression levels of GAS5, miR-217 and HDAC4 in bronchial mucosal tissues of asthmatic children by RT-qPCR. The results showed that GAS5 (Fig. 1a)

**Fig. 1** GAS5 and HDAC4 were up-regulated, and miR-217 was down-regulated in bronchial mucosa tissues of asthmatic children. **a–c** The levels of GAS5, miR-217 and HDAC4 in bronchial mucosal tissues of asthmatic children and healthy children were detected by RT-qPCR. **d** Western blot assay was used to measure the protein expression of HDAC4 in bronchial mucosal tissues of asthmatic children and healthy children. \* $P < 0.05$



and HDAC4 (Fig. 1c) were obviously increased in bronchial mucosal tissues of asthmatic children than that bronchial mucosal tissues in healthy children, while miR-217 was drastically decreased (Fig. 1b). Meanwhile, the protein level of HDAC4 was detected by western blot. As shown in Fig. 1d, HDAC4 protein expression was also up-regulated in bronchial mucosal tissues of asthmatic children.

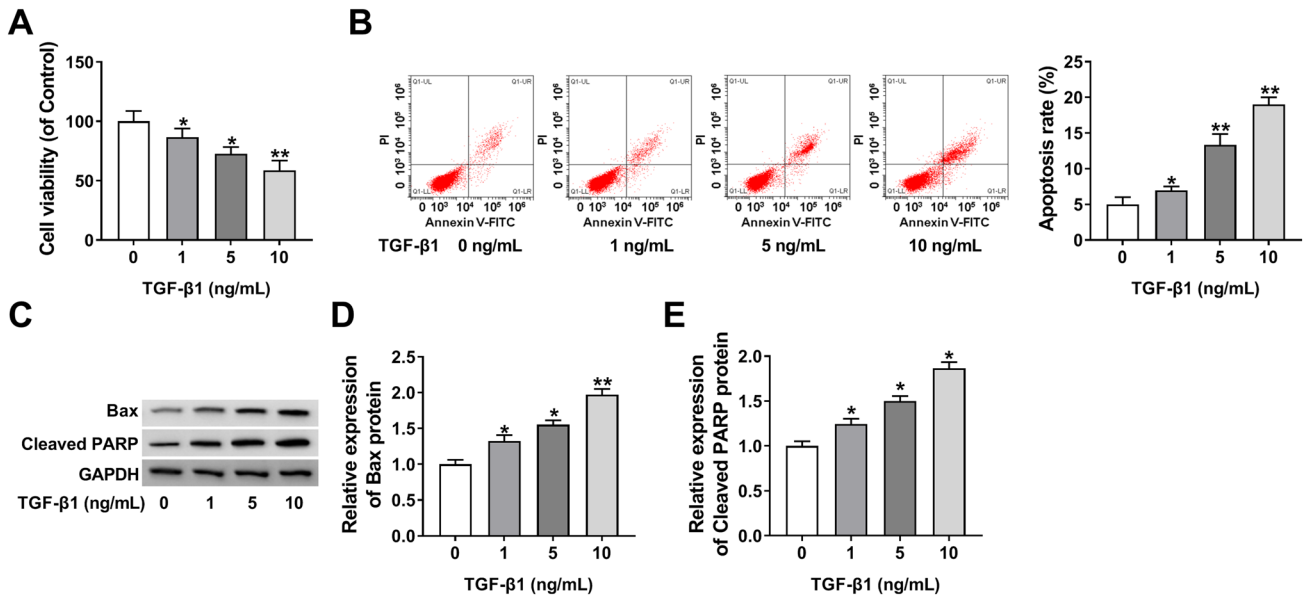
### TGF- $\beta$ 1 reduced cell viability and promoted cell apoptosis in BEAS-2B cells

The expression of TGF- $\beta$ 1 in bronchial mucosal tissues of asthmatic children was also detected. As shown in Supplementary Fig. 1, TGF- $\beta$ 1 expression in bronchial mucosal tissues was obviously enhanced compared to healthy bronchial mucosal tissues. Besides, treatment of TGF- $\beta$ 1 also elevated the protein level of Smad, suggested that the TGF- $\beta$ 1/Smad axis was activated by TGF- $\beta$ 1 (Supplementary Fig. 2). Then the effect of TGF- $\beta$ 1 on human bronchial epithelial cells BEAS-2B was evaluated. Cells cultured in serum-free medium were treated with different concentrations of TGF- $\beta$ 1 (0, 1, 5, 10 ng/mL) for 48 h, and cell viability and apoptosis were analyzed. The data showed that TGF- $\beta$ 1 markedly impaired cell viability in a dose-dependent manner (Fig. 2a). Additionally, we examined the effect of TGF- $\beta$ 1 on cell apoptosis. Flow cytometry results showed that TGF- $\beta$ 1

enhanced BEAS-2B cells apoptosis in a dose-dependent manner (Fig. 2b). Besides, the expression of pro-apoptotic proteins Bax and Cleaved PARP was detected by western blot. As shown in Fig. 2c–e, the levels of Bax and Cleaved PARP were drastically raised in TGF- $\beta$ 1-treated BEAS-2B cells in a dose-dependent manner. These findings indicated that TGF- $\beta$ 1 inhibited cell viability and promoted apoptosis in BEAS-2B cells.

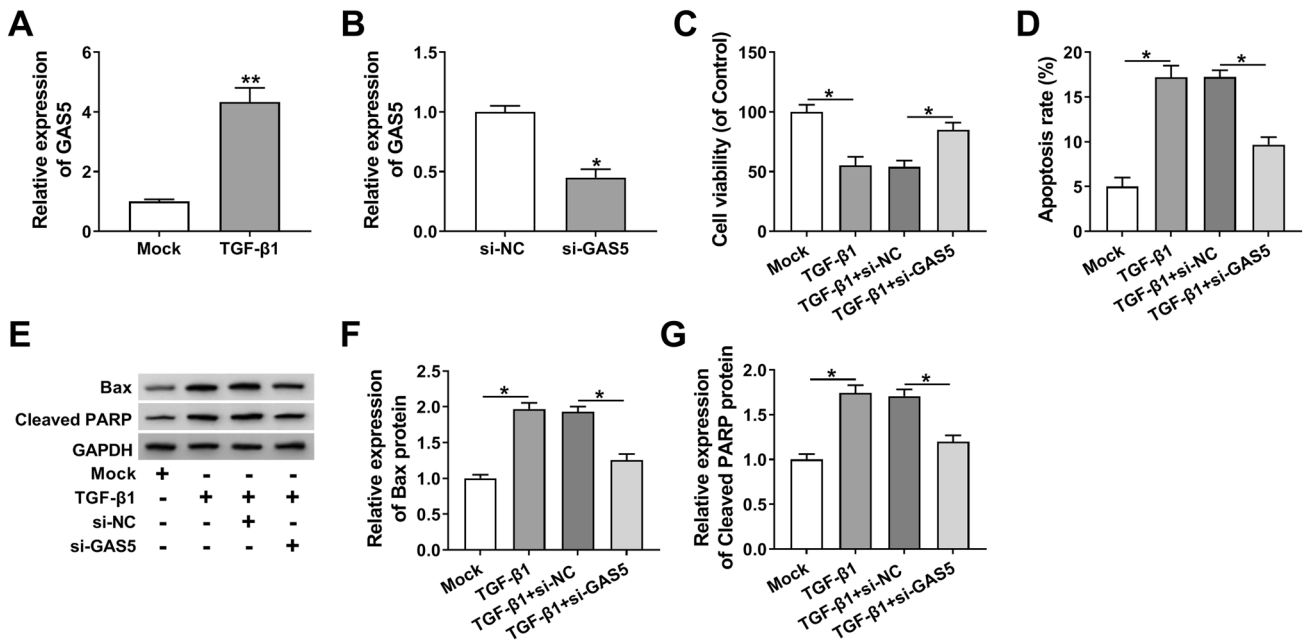
### Knockdown of GAS5 induced cell viability and inhibited cell apoptosis in TGF- $\beta$ 1-induced BEAS-2B cells

To explore the effect of GAS5 on cell viability and apoptosis, BEAS-2B cells were treated with 10 ng/mL TGF- $\beta$ 1 for 48 h, and GAS5 expression was then measured by RT-qPCR. The results showed that TGF- $\beta$ 1 enhanced GAS5 expression more than four times compared with the control group (Fig. 3a). The results of RT-qPCR showed that GAS5 expression was decreased in BEAS-2B cells transfected with si-GAS5 than that cells transfected with si-NC (Fig. 3b). MTT assay indicated that GAS5 knockdown reversed the decreased cell viability in TGF- $\beta$ 1-induced BEAS-2B cells (Fig. 3c), and Flow cytometry showed that GAS5 suppression hindered cell apoptosis of TGF- $\beta$ 1-induced BEAS-2B cells (Fig. 3d). Besides, in



**Fig. 2** TGF-β1 reduced cell viability and promoted cell apoptosis in BEAS-2B cells. BEAS-2B cells were stimulated by different concentrations of TGF-β1 (0, 1, 5, 10 ng/mL) for 48 h. **a** Cell viability was analyzed by MTT assay. **b** Cell apoptosis capacity was evaluated

by Flow cytometry. **c–e** The levels of apoptosis-related proteins Bax and Cleaved PARP were detected by western blot assay. \* $P < 0.05$ , \*\* $P < 0.01$



**Fig. 3** Knockdown of GAS5 induced cell viability and inhibited cell apoptosis in TGF-β1-induced BEAS-2B cells. **a** BEAS-2B cells were treated with TGF-β1 (10 ng/mL) or Mock (control), and GAS5 was detected by RT-qPCR. **b** GAS5 expression in BEAS-2B cells transfected with si-GAS5 or si-NC was examined by RT-qPCR. **c–g**

BEAS-2B cells were treated with Mock, TGF-β1, TGF-β1+si-NC and TGF-β1+si-GAS5. **c, d** Cell viability and apoptosis capacity were assessed by MTT assay and Flow cytometry, respectively. **e–g** The levels of Bax and Cleaved PARP were examined by western blot assay. \* $P < 0.05$ , \*\* $P < 0.01$

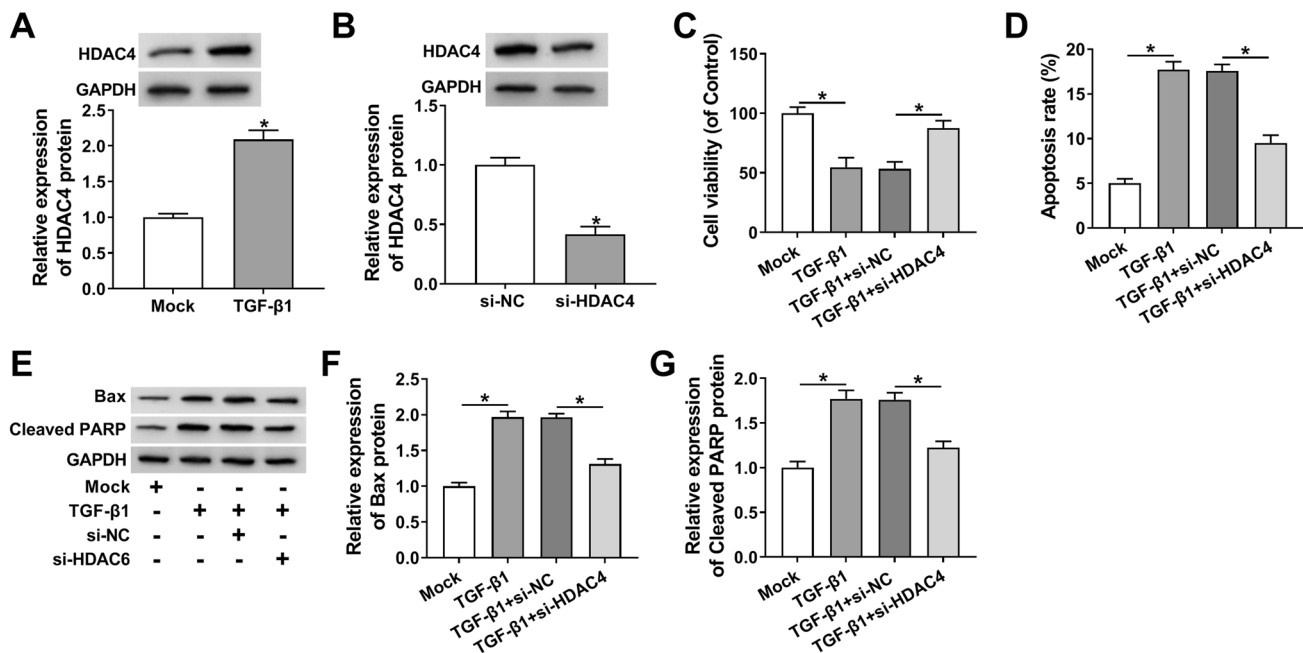
TGF- $\beta$ 1-stimulated BEAS-2B cells with GAS5 knock-down, the levels of Bax and Cleaved PARP were declined (Fig. 3e–g). The above data indicated that GAS5 inhibition could increase cell viability and restrain cell apoptosis in TGF- $\beta$ 1-induced bronchial epithelial cells.

### Repression of HDAC4 alleviated the TGF- $\beta$ 1-induced bronchial epithelial cells injury

We further examined the effect of TGF- $\beta$ 1 on HDAC4 expression and whether HDAC4 participated in the regulation of cell viability and apoptosis in TGF- $\beta$ 1-treated BEAS-2B cells. As shown in Fig. 4a, the protein level of HDAC4 was declined in TGF- $\beta$ 1-treated BEAS-2B cells, and HDAC4 protein level was further decreased in BEAS-2B cells with si-HDAC4 transfection (Fig. 4b). HDAC4 knockdown enhanced cell viability (Fig. 4c) and repressed cell apoptosis (Fig. 4d) in TGF- $\beta$ 1-induced BEAS-2B cells. Furthermore, HDAC4 depletion degraded the protein levels of Bax and Cleaved PARP (Fig. 4e–g). These results supported that HDAC4 knockdown facilitated the viability of TGF- $\beta$ 1-induced BEAS-2B cells and inhibited cell apoptosis.

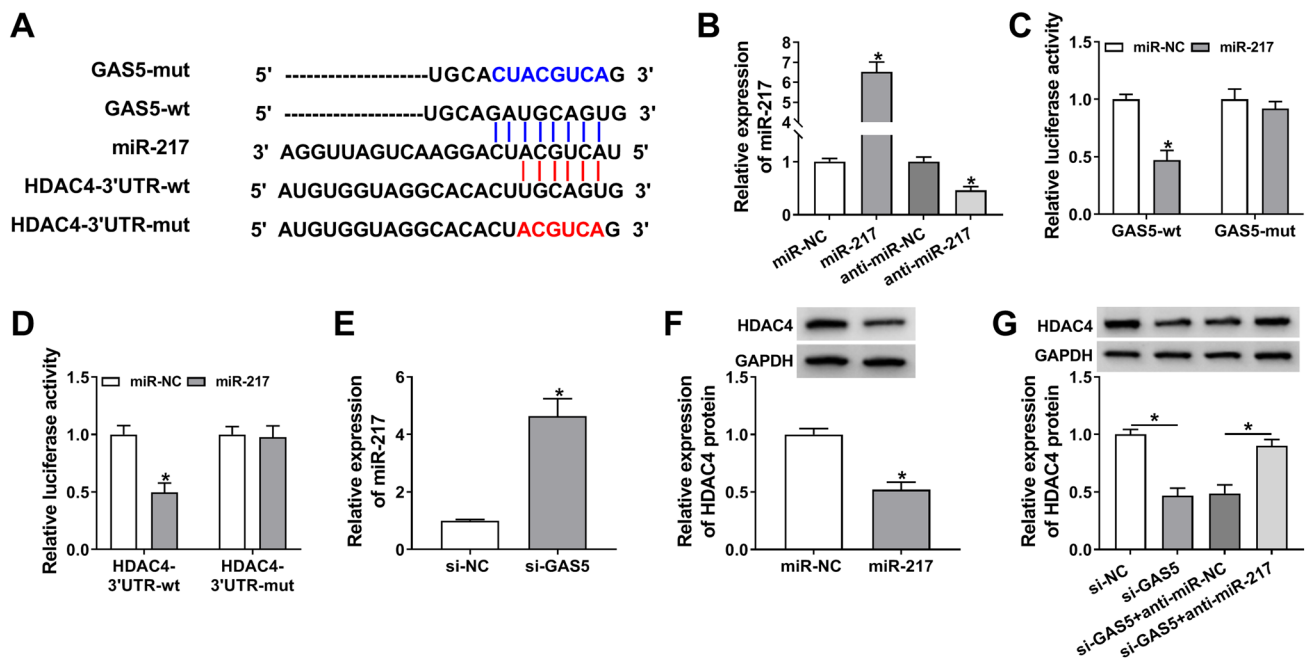
### GAS5 could modulate miR-217-targeted HDAC4 expression by acting as miR-217 sponge

Subsequently, bioinformatics software Starbase3.0 showed that there were binding sites between miR-217 and GAS5 as well as HDAC4 (Fig. 5a). To verify these predictions, dual-luciferase reporter assay was performed. In order to carry out the subsequent experiments smoothly, we first tested the overexpression and interference efficiency of miR-217 and anti-miR-217 on miR-217 in BEAS-2B cells by RT-qPCR (Fig. 5b). Then, GAS5 wt, GAS5 mut, HDAC4-3'UTR-wt and HDAC4-3'UTR-mut were co-transfected into BEAS-2B cells with miR-217 or miR-NC, respectively. As displayed in Fig. 5c, miR-217 evidently curbed the luciferase activity of GAS5 wt compared to GAS5 mut. Consistently, the luciferase activity of HDAC4-3'UTR-wt was decreased by miR-217, while the luciferase activity of HDAC4-3'UTR-mut was not significantly changed (Fig. 5d). The results suggested that GAS5 could target miR-217, and HDAC4 was the target of miR-217. Besides, suppression of GAS5 in BEAS-2B cells increased miR-217 expression (Fig. 5e), and miR-217 elevation decreased the protein expression of HDAC4 (Fig. 5f). Meanwhile, we observed that interference with miR-217 could reverse the inhibitory effect of si-GAS5 on HDAC4 protein expression (Fig. 5g). These data



**Fig. 4** Repression of HDAC4 alleviated the TGF- $\beta$ 1-induced bronchial epithelial cells injury. **a** BEAS-2B cells were treated with TGF- $\beta$ 1 or Mock, and HDAC4 expression was detected by western blot. **b** The protein expression of HDAC4 in BEAS-2B cells transfected with si-HDAC4 or si-NC was measured by western blot. **c–g** BEAS-

2B cells were treated with Mock, TGF- $\beta$ 1, TGF- $\beta$ 1 + si-NC and TGF- $\beta$ 1 + si-HDAC4. **c, d** Cell viability and apoptosis were estimated using MTT assay and Flow cytometry, respectively. **e–g** The protein levels of Bax and Cleaved PARP were determined by western blot. \* $P < 0.05$



**Fig. 5** GAS5 could modulate miR-217-targeted HDAC4 expression by acting as miR-217 sponge. **a** Starbase3.0 predicted that there were binding sites between miR-217 and GAS5 as well as miR-217 and HDAC4. **b** The relative expression of miR-217 in BEAS-2B cells transfected with miR-NC, miR-217, anti-miR-NC or anti-miR-217 was determined by RT-qPCR. **c, d** Dual-luciferase reporter assay was used to verify the interactions between miR-217 and GAS5 as

well as miR-217 and HDAC4. **e** The relative expression of miR-217 in BEAS-2B cells transfected with si-GAS5 or si-NC was examined by RT-qPCR. **f** The protein expression of HDAC4 in BEAS-2B cells transfected with miR-NC or miR-217 was detected by western blot. **g** The protein expression of HDAC4 in BEAS-2B cells transfected with si-GAS5, si-NC, si-GAS5 + anti-miR-NC or si-GAS5 + anti-miR-217 was examined by western blot. \* $P < 0.05$

illustrated that GAS5 could regulate HDAC4 expression by sponging miR-217.

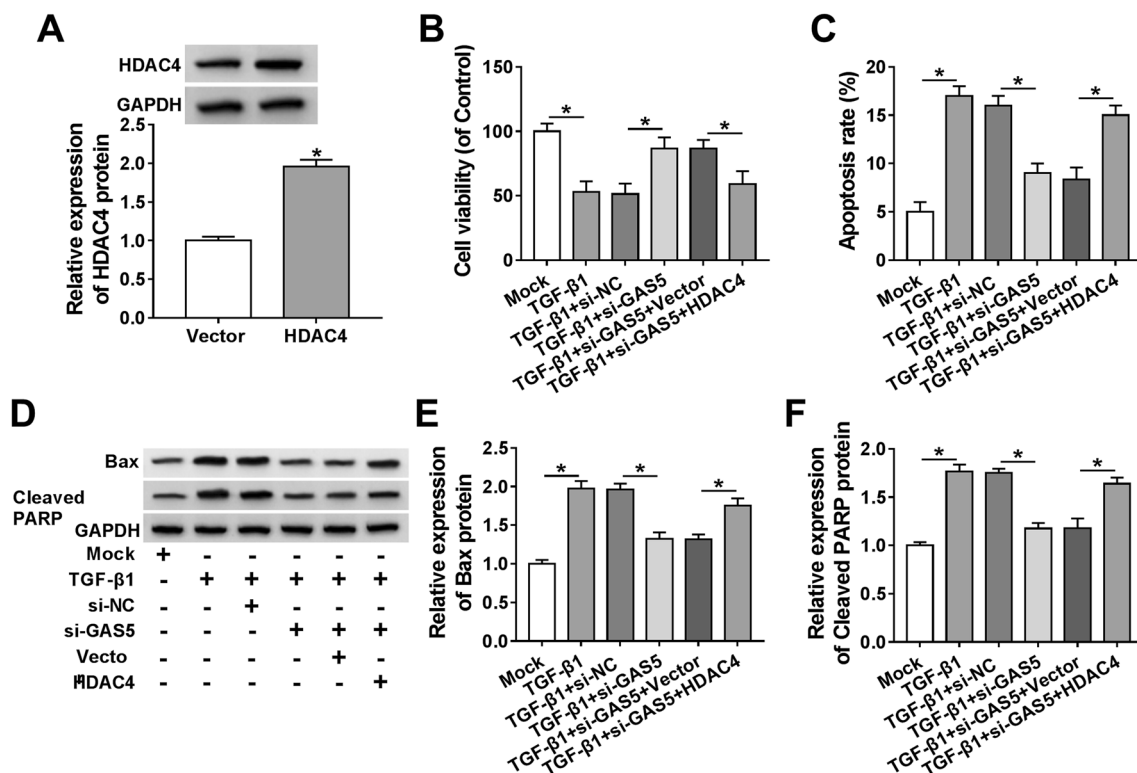
### HDAC4 overexpression partially reversed the effects of GAS5 knockdown on viability and apoptosis of TGF- $\beta$ 1-induced BEAS-2B cells

To investigate whether GAS5 regulated cell viability and apoptosis by regulating HDAC4 in TGF- $\beta$ 1-induced BEAS-2B cells. HDAC4 overexpression vector (HDAC4) and si-GAS5 were co-transfected into TGF- $\beta$ 1-treated BEAS-2B cells. Firstly, western blot showed that HDAC4 protein expression was remarkably elevated by transfection of HDAC4 in BEAS-2B cells (Fig. 6a). Next, we observed that interference with GAS5 increased cell activity (Fig. 6b) and inhibited apoptosis (Fig. 6c), which could be reversed by overexpression of HDAC4 in TGF- $\beta$ 1-treated BEAS-2B cells. In addition, the inhibition effect of si-GAS5 on expression of Bax and Cleaved PARP was neutralized by overexpressing HDAC4 (Fig. 6d–f). The results proved that GAS5 knockdown could induce cell viability and suppress apoptosis in TGF- $\beta$ 1-induced BEAS-2B cells by decreasing HDAC4. Besides, we also evaluated the expression of GAS5, miR-217, and HDAC4 in BEAS-2B cells with LY-215 (an inhibitor of TGF- $\beta$ 1) treatment. As shown in

Supplementary Fig. 3, the expression of GAS5 and HDAC4 were elevated while miR-217 was inhibited by TGF- $\beta$ 1, however, co-treatment of LY-215 partly reversed the effect of TGF- $\beta$ 1, indicating that TGF- $\beta$ 1 regulated cell viability and apoptosis by GAS5/miR-217/HDAC4 axis in BEAS-2B cells.

## Discussion

In this work, we found that TGF- $\beta$ 1 might promoted apoptosis and inhibited cell viability through GAS5/miR-217/HDAC4 axis in bronchial epithelial cells. It has shown that epithelial cells injury was more severe in severe asthma patients than in mild asthma patients, suggesting the importance of epithelial function in asthma patients (Semlali et al. 2010), and reduced antiviral signaling was observed in bronchial epithelial cells in asthma patients (Menzel et al. 2019). Therefore, exploring the mechanism of airway epithelial cells injury might have important significance in improving childhood asthma. Earlier studies have suggested that TGF- $\beta$ 1 could regulate both of airway epithelial apoptosis and EMT, thus affect the development of asthma (Pu et al. 2020). In our research, TGF- $\beta$ 1 treated BEAS-2B cells were used as a research object to



**Fig. 6** HDAC4 overexpression partially reversed the effects of GAS5 knockdown on viability and apoptosis of TGF-β1-induced BEAS-2B cells. **a** HDAC4 protein expression in BEAS-2B cells transfected with Vector or HDAC4 was measured using western blot assay. **b–f** BEAS-2B cells were treated with Mock, TGF-β1, TGF-β1+si-NC

and TGF-β1+si-GAS5, TGF-β1+si-GAS5+Vector and TGF-β1+si-GAS5+HDAC4. **b, c** Cell viability and apoptosis were assessed through MTT assay and Flow cytometry, respectively. **d–f** The levels of Bax and Cleaved PARP were analyzed by western blot. \* $P < 0.05$

investigate the mechanism underlying TGF-β1 in asthma. In accord with previous research, TGF-β1 was upregulated in bronchial mucosal tissues of asthmatic children. And TGF-β1 treatment could inhibit cell viability and induced apoptosis in BEAS-2B cells, which indicating that TGF-β1 induced airway epithelial injury in asthma.

Previous studies have identified that GAS5 is an important biomarker in many types of diseases, such as cerebral infarction (Zhao et al. 2019), diabetic nephropathy (Ge et al. 2019) and B lymphocytic leukaemia (Jing et al. 2019), while the role and potential mechanism of GAS5 in childhood asthma were not fully validated. In our study, GAS5 was up-regulated in bronchial mucosal tissues of asthmatic children and TGF-β1-treated bronchial epithelial cells, and GAS5 knockdown reversed the inhibitory effect of TGF-β1 on cell viability and the promotion on cell apoptosis, which was in accordance with previous reports. Keenan et al. (2015) indicated that pro-inflammatory mediators could raise GAS5 level in airway epithelial and smooth muscle cells, and GAS5 was found to be significantly augmented in T cells in patients with rheumatoid arthritis (Moharamoghi et al. 2019). These findings suggested that GAS5 might play a pivotal regulatory role in childhood asthma.

miR-217, which has the opposite expression pattern to GAS5 in bronchial mucosal tissues of asthmatic children and TGF-β1-treated BEAS-2B cells, was the target gene for GAS5. Importantly, miR-217 expression was enormously reduced in TGF-β1-stimulated ASMCs, and miR-217 overexpression could hinder TGF-β1-induced proliferation and promote apoptosis in ASMCs (Gao et al. 2018). In our research, miR-217 was downregulated in airway epithelial in bronchial mucosal tissues of asthmatic children.

It has been revealed that HDAC4 expression was increased in TGF-β1-stimulated ASMCs (Pan et al. 2018), and its activity is required for TGF-β1-induced lung fibroblast-to-myofibroblast differentiation (Guo et al. 2009). In this research, HDAC4 was enhanced in bronchial mucosal tissues of asthmatic children and TGF-β1-treated BEAS-2B cells, and HDAC4 was directly targeted by miR-217. We also found that GAS5 served as a molecular sponge for miR-217 to regulate HDAC4 expression. HDAC4 elevation partly restored the effects of GAS5 knockdown on cell viability and apoptosis in TGF-β1-induced BEAS-2B cells. Besides, the elevated expression of GAS5 and HDAC4, and decreased expression of miR-217 that mediated by TGF-β1 was partly reversed by TGF-β1 inhibitor LY-215.



## Conclusions

In summary, we uncovered that TGF- $\beta$ 1 treatment could increase GAS5 and HDAC4 expression, but inhibit miR-217 expression in airway epithelial cells. GAS5 knockdown might alleviate TGF- $\beta$ 1-induced bronchial epithelial cells injury by regulating miR-217/HDAC4 axis. Our results may provide a new insight into understanding the regulation mechanism of TGF- $\beta$ 1 in asthma pathogenesis.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s13258-021-01092-1>.

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## Declarations

**Conflict of interest** Sihui Zhao, Yunfang Ning, Na Qin, Nan Ping, Yong Yu and Guoyan Yin declare that they have no conflict of interest.

**Ethical approval** The research has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the Ethics Committee of Heping Hospital Affiliated to Changzhi Medical College.

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