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DNA methylation-mediated *FGFR1* silencing enhances *NF-κB* signaling: implications for asthma pathogenesis

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Background: Fibroblast growth factor receptor 1 (*FGFR1*) is known to play a crucial role in the pathogenesis of asthma, although the precise mechanism remains unclear. This study aims to investigate how DNA methylation-mediated silencing of *FGFR1* contributes to the enhancement of *NF-κB* signaling, thereby influencing the progression of asthma.

Methods: RT-qPCR was utilized to assess *FGFR1* mRNA levels in the serum of asthma patients and BEAS-2B, HBEpiC, and PCS-301-011 cells. CCK8 assays were conducted to evaluate the impact of *FGFR1* overexpression on the proliferation of BEAS-2B, PCS-301-011, and HBEpiC cells. Dual-luciferase and DNA methylation inhibition assays were performed to elucidate the underlying mechanism of *FGFR1* gene in asthma. The MassARRAY technique was employed to measure the methylation levels of the *FGFR1* DNA.

Results: Elevated *FGFR1* mRNA levels were observed in the serum of asthma patients compared to healthy controls. Overexpression of *FGFR1* in BEAS-2B cells significantly enhanced cell proliferation and stimulated *NF-κB* transcriptional activity in HERK-293T cells. Furthermore, treatment with 5-Aza-CdR, a DNA demethylating agent, markedly increased the expression of *FGFR1* mRNA in BEAS-2B, PCS-301-011, and HBEpiC cells. Luciferase activity analysis confirmed heightened *NF-κB* transcriptional activity in *FGFR1*-overexpressing BEAS-2B cells and BEAS-2B cells treated with 5-Aza-CdR. Additionally, a decrease in methylation levels in the *FGFR1* DNA promoter was detected in the serum of asthma patients using the MassARRAY technique.

Conclusion: Our findings reveal a potential mechanism involving *FGFR1* in the progression of asthma. DNA methylation of *FGFR1* inactivates the *NF-κB* signaling pathway, suggesting a promising avenue for developing effective therapeutic strategies for asthma.

KEYWORDS

FGFR1, *NF-κB*, DNA methylation, asthma, 5-aza-CdR

1 Introduction

Asthma is a heterogeneous and complex disease, characterized by diverse phenotypes and endotypes, each with distinct underlying mechanisms (Wenzel, 2012). These phenotypes and endotypes are influenced by a combination of genetic, environmental, and immunological factors (Lehto et al., 2019; BAJCOiP, 2019). Understanding the molecular basis of these variations is crucial for developing targeted therapeutic strategies.

Fibroblast growth factor receptor 1 (*FGFR1*) is a cytoplasmic protein-tyrosine kinase that belongs to the FGFR gene family (*FGFR1–4*). *FGFR1* is activated by binding with the essential fibroblast growth factor (bFGF) and is crucial for various cellular processes, including mitosis and differentiation. Recent research has highlighted the role of *FGFR1* signaling as a pivotal regulator of inflammation, implicating its involvement in numerous chronic inflammation-related disorders, such as cancer (Chen et al., 2020; Wang et al., 2020). Studies on lung cancer models have demonstrated that *FGFR1* promotes cancer progression and is linked to risk factors for respiratory conditions like bronchiolitis and chronic inflammation. Inhibiting *FGFR1* has been shown to downregulate the expression of genes associated with asthma pathogenesis, including SOX2, a marker of epithelial-mesenchymal transition (EMT), as well as the mesenchymal markers N-cadherin and Vimentin (Wang K. et al., 2018). Elevated levels of *FGFR1* expression in airway epithelial cells suggest its involvement in airway remodeling, a significant characteristic of asthma (Balasooriya et al., 2016; Tsai et al., 2018; Yuan et al., 2020). (Loffredo et al., 2017). Despite these findings, the precise role of *FGFR1* in bronchial epithelial cells and its mechanisms contributing to asthma pathogenesis remain inadequately elucidated.

The NF- κ B signaling pathway regulates various multicellular developmental processes, including cell cycle progression, cell adhesion, inflammation, and angiogenesis (TJERCIWoS, 2009; El-Nagar and Elsis, 2023; Chusongdam et al., 2024). Activation of the canonical pathway occurs through Toll-like receptors (TLRs) and proinflammatory cytokines, such as TNF- α and IL-1, leading to the activation of the IKK α -IKK β -NEMO kinase complex via TRAF complexes. This complex then phosphorylates and ubiquitinates I κ B, releasing NF- κ B dimers (such as p50-p65) to translocate into the nucleus and initiate the transcription of target genes. Negative feedback regulation is achieved through synthesizing I κ B proteins and binding TNF receptor-associated factors (TRAFs) to inhibit NF- κ B activity, thereby establishing a regulatory loop. In contrast, specific receptors and ligands, such as CD40L and BAFF, activate the non-canonical pathway, resulting in the phosphorylation of p100 by inhibitory IKK α , leading to its partial proteasomal degradation into p52. The RelB-bound p52 then translocates to the nucleus to modulate gene transcription (Pisani et al., 2022; Sun, 2017). Dysregulation of the NF- κ B system has been implicated in various human diseases, notably inflammatory and fibrotic disorders. Extensive research has underscored the pivotal role of the NF- κ B system in inflammation and immunity (Zhang et al., 2021). Lin et al. proposed a link between the NF- κ B system and mucus hypersecretion, a hallmark feature of asthma (Lin et al., 2015). Moreover, studies have shown that activation of the NF- κ B system contributes to airway remodeling and exacerbates neuroinflammation in a murine

model of asthma (Kim et al., 2018). Our findings contribute to the understanding of asthma's molecular complexity by suggesting that *FGFR1* hypomethylation and the subsequent activation of NF- κ B signaling may be associated with specific asthma endotypes, particularly those linked to airway remodeling and chronic inflammation.

Gene-environment interactions are pivotal in the development and progression of complex diseases, including asthma. Epigenetic mechanisms, such as DNA methylation, serve as critical mediators in these interactions by regulating gene expression in response to environmental stimuli. This dynamic interplay between genetic predisposition and environmental factors underscores the importance of epigenetics in understanding asthma pathogenesis (Potaczek et al., 2017). Given that epigenetic regulation, particularly DNA methylation, plays a significant role in asthma by modulating gene expression (Yang, 2019), we aimed to investigate the interaction between *FGFR1* and NF- κ B signaling in bronchial epithelial cells. Specifically, we examined the effect of 5-Aza-CdR, a DNA methylation inhibitor, on this regulatory axis in BEAS-2B cells, following evidence of elevated *FGFR1* expression in asthma patients. Additionally, we assessed the clinical relevance of DNA methylation in regulating *FGFR1* expression in the serum of asthma patients.

2 Methods

2.1 Subjects and blood samples

Between January and April 2020, 43 asthma patients from the Affiliated Hospital of Youjiang Medical University for Nationalities were included in our study. A physician diagnosed these patients according to the criteria outlined in the Chinese guidelines for the prevention and treatment of bronchial asthma (2016 Edition). Serum samples (500 μ L) were obtained from these patients prior to treatment. Additionally, 47 healthy donors matched in age, sex, and BMI were included as control subjects.

2.2 Cells and plasmids

Human normal lung epithelial cells (BEAS-2B cells), normal human bronchial epithelial cells (HBEPiC), and Primary Small Airway Epithelial Cells (PCS-301-011) were procured from the China Center for Type Culture Collection (Wuhan, China). The cells were cultured in Dulbecco's modified Eagle's medium/F12 (DMEM/F12) at 37°C in a 5% CO₂ atmosphere. PCS-301-011 cells were maintained following the instructions in the Bronchial Epithelial Growth Kit (ATCC, USA). NF- κ B luciferase reporter plasmids (NF- κ B Luc) and pCMV-N-Flag plasmids were purchased from Yeasen Co., Ltd., Shanghai, China.

2.3 Cell transfection and overexpression of *FGFR1*

To upregulate *FGFR1* expression, reverse transcription-PCR (RT-PCR) was performed to amplify its cDNA sequences in BEAS-2B cells using specific primers: forward primer

5'TGGCACCCGAGGCATTATTT3' and reverse primer 5'GTACAAGAAAGTTGGGCAGCG3'. The PCR-amplified cDNA was gel-purified and extracted using a QIA quick gel extraction kit. The *FGFR1* gene was cloned into pCMV-N-Flag vectors (Biovector Co., LTD, China). The resulting pCMV-N-Flag-*FGFR1* plasmid and empty vectors were used to transfect 5×10^5 sub-confluent HEK293T cells and virus skeleton plasmids. The HEK293T medium was centrifuged and sterile-filtered before using the viral supernatant to transduce BEAS-2B, HBEpiC, and PCS-301-011 cells for 48 h. Transformed colonies were selected based on ampicillin resistance on plates containing 100 $\mu\text{g}/\text{mL}$ of ampicillin for 8 days. Surviving clones were further validated through Western blotting and Sanger sequencing.

2.4 RT-qPCR

Total RNA (100 ng) was extracted from serum samples, BEAS-2B cells, HBEpiC, and PCS-301-011 using the Plasma/Serum Circulating and Exosomal RNA Purification Kit (Amyjet Scientific Inc., China). Subsequently, 1 μg of total RNA was reverse-transcribed into cDNA with the ReverTra Ace qPCR RT kit (Toyobo, Japan) to detect *FGFR1* mRNA expression via a quantitative polymerase chain reaction. The relative expression of *FGFR1* mRNA compared to GAPDH was quantified using the comparative ($2^{-\Delta\Delta}$) Ct method.

2.5 Western blot analysis

Total protein extracts from BEAS-2B, HBEpiC, and PCS-301-011 cells were obtained using RIPA lysis buffer and quantified with a BCA kit (Beyotime, China). Subsequently, 10 μg of protein samples were separated on a 10% SDS-PAGE gel and transferred onto a nitrocellulose membrane. Following blocking with 5% non-fat skim milk, the membranes were probed with primary antibodies: anti-flag (Cat#FNab03154, Fine Test, Wuhan, China, 1:1,000), anti-Ikba (Cat#FNab04198, Fine Test, Wuhan, China, 1:1,000), and anti-GAPDH (Cat#FNab03343, Fine Test, Wuhan, China, 1:1,000). Detection of signals was achieved using a western chemiluminescent HRP substrate (Cat#FNSA-0162/FNSA-0160, Fine Test, Wuhan, China, 1:1,000) and an enhanced chemiluminescence system.

2.6 Luciferase reporter assays

The NF- κB luciferase reporter plasmid was procured from QCBio Science and Technologies Co., Ltd., China and subsequently transfected into BEAS-2B, HBEpiC, and PCS-301-011 cells along with Pcmv-n-Flag-*FGFR1* plasmid or control vectors using Lipofectamine 3,000 Transfection Reagent (Invitrogen, L3000). Luciferase activity was measured 48 h post-transfection using the Promega Luciferase Assay System (Promega, United States).

2.7 DNA extraction and methylation assay

DNA was extracted from extracellular DNA in peripheral blood samples using a cfDNA Library Kit from Beijing Baiaolaibo

Technology Co., Ltd., China. Quantification of DNA was performed using a NANODROP 1000. Methylation analysis of *FGFR1* at a concentration of 300 ng/ μL was carried out by BioMiao Biological Technology (Beijing) Co., Ltd. The isolated DNA underwent sodium bisulfite treatment and PCR amplification using the PyroMark PCR Kit to enrich *FGFR1* sequences. The extracted extracellular DNA was then subjected to methylation. After SAP treatment (2 μg DNA), the samples (500 ng) were analyzed for targeted CpG island methylation using the MALDI-TOF MassARRAY system on a SpectroCHIP. The specific primers used were: 5'AGGAAGAGAGTTATTGTAGGTTGGAGATTTTTTGA3'; 3'CAGTAATACGACTCACTATAGGGAGAAGGCTACCCCTCTCTTCTTCTACAACCTAATC5'.

2.8 Statistical analysis

Prism 9.0 software facilitated data analysis. For data with normal distributions, parametric tests were conducted. Specifically, an unpaired *t*-test was used for two-group comparisons, while one-way ANOVA followed by Dunnett's *post hoc* test was applied for multiple group comparisons. For non-parametric distributions, the Mann-Whitney U test was employed. For categorical or qualitative parameters, such as sex, comparisons were performed using the chi-square test. Statistical significance was designated at $p < 0.05$, with each experiment conducted at least three times in triplicate.

2.9 Ethical clearance

The Medical Ethics Committee of Youjiang Medical University for Nationalities obtained the ethical approval. (No.2017030501).

3 Results

3.1 Elevated expression of serum *FGFR1* in asthma patients

Our study observed no significant variances in sex, age, and BMI between the case and control groups. Nevertheless, upon adjusting for these factors, individuals with asthma exhibited 2.52 times higher levels of *FGFR1* mRNA compared to the control group, as indicated in [Table 1](#).

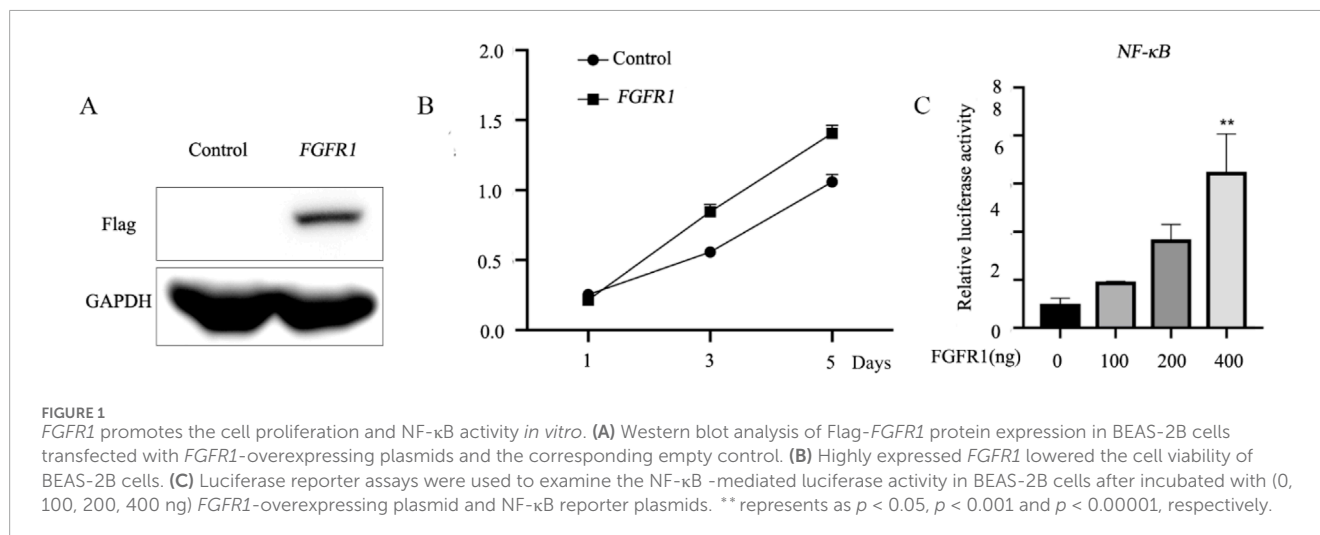
3.2 *FGFR1* promotes cell proliferation and NF- κB activity *in vitro*

The recent study indicates that upregulated *FGFR1* expression in H1581 and DMS114 cells induces epithelial-mesenchymal transition (EMT), potentially contributing to asthma pathology. Our data showed that *FGFR1*-overexpression vectors were constructed and transfected into BEAS-2B cells, with overexpression confirmed via Western blotting ([Figure 1A](#)). Cell Counting Kit-8 (CCK8) assays were conducted to assess BEAS-2B cell proliferation, revealing that increased *FGFR1* expression enhanced cell proliferative capacity *in vitro* ([Figure 1B](#)). Furthermore, demethylation of *FGFR1* was

TABLE 1 Baseline characteristics of the patient: Cases (n = 47) and control (n = 43).

Characteristics	Control n (%)	Case (%)	Crude OR	Adjusted OR	95% CI	p-value
Sex						0.413
Female	21(53.85)	18(46.15)	1	N/A	N/A	
Male	22(43.14)	29(56.86)	1.54	1.43	0.61 to 3.35	
Age						
Mean ± SD	49.79 ± 2.05	47.62 ± 2.54	-2.17	0.99	0.97 to 1.02	0.544
MBI						
Mean ± SD	22.49 ± 0.52	21.77 ± 0.39	-0.72	0.93	0.81 to 1.07	0.335
<i>FGFR1</i> mRNA levels						
Mean ± SD	0.98 ± 0.03	3.50 ± 0.09	2.52	2.52	2.32 to 2.73	<0.001

BMI: body mass index.



linked to heightened NF- κ B activity, as demonstrated by dual-luciferase assays (Figure 1C).

3.3 5-Aza-CdR increases *FGFR1* mRNA expression and activates the NF- κ B signaling pathway

Numerous studies have confirmed the involvement of DNA methylation in the pathogenesis of asthma, specifically through gene silencing via CpG island methylation in the gene promoter (Hudon Thibeault and Laprise, 2019; Reese et al., 2019; Xu et al., 2018). The methyltransferase inhibitor 5-Aza-CdR has been shown to induce DNA hypomethylation, leading to increased gene transcription. This study, BEAS-2B cells were treated with 1 μ m 5-Aza-CdR for 24 h, followed by RT-qPCR analysis of *FGFR1* mRNA levels. The results, depicted in Figure 2A, revealed an

approximately five-fold elevation in *FGFR1* expression in BEAS-2B cells post 5-Aza-CdR treatment. Subsequently, the relative luciferase activity of NF- κ B in BEAS-2B cells was assessed after treatment with 5-Aza-CdR. Overexpression of *FGFR1* in BEAS-2B cells resulted in a notable increase in NF- κ B transcriptional activity, with a more pronounced effect observed in cells treated with 5-Aza-CdR (Figure 2B). Western blot analysis was then conducted to assess I κ B α protein expression, showing that both elevated *FGFR1* expression and 5-Aza-CdR treatment led to increased I κ B α expression levels (Figure 2C).

3.4 *FGFR1* promotes the growth of HBEpiC and PCS-301-011 cells

The expression of *FGFR1* was assessed in HBEpiC and PCS-301-011 cells following treatment with 5-Aza-CdR. RT-qPCR analysis revealed increased *FGFR1* levels in HBEpiC cells post 5-Aza-CdR

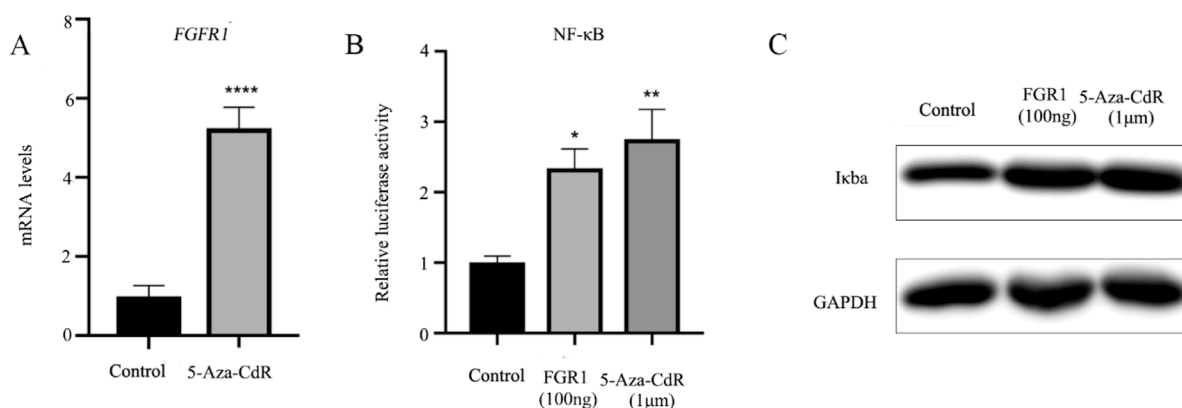


FIGURE 2

5-Aza-CdR increases the *FGFR1* mRNA expression and activates the NF- κ B signaling pathway. (A) RT-qPCR examined *FGFR1* mRNA levels in BEAS-2B cells incubated with 1 μ M 5-Aza-CdR for 48 h or not. (B) Luciferase-based NF- κ B was determined in BEAS-2B cells incubated with 1 μ M 5-Aza-CdR for 48 h or transfected with *FGFR1*-overexpression plasmids. (C) Western blot detection of *Ikba* protein expression in BEAS-2B cells incubated with 1 μ M 5-Aza-CdR for 48 h or transfected with *FGFR1*-overexpressing plasmids. *, **, **** represents as $p < 0.05$, $p < 0.001$ and $p < 0.00001$, respectively.

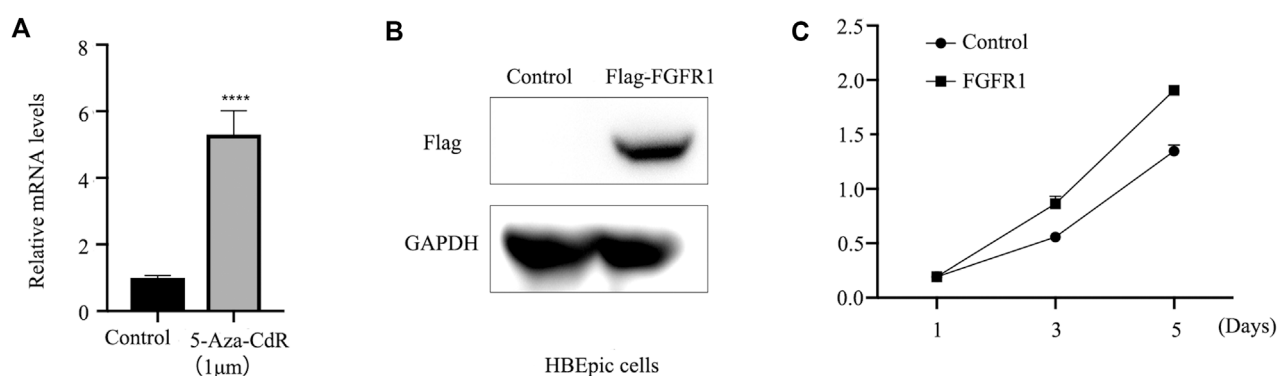


FIGURE 3

FGFR1 promotes the growth of HBEpiC cells. (A) RT-qPCR examined *FGFR1* mRNA levels in HBEpiC cells incubated with 1 μ M 5-Aza-CdR for 48 h or not. (B) western blots examined *FGFR1* protein levels in HBEpiC cells transfected with *FGFR1*-overexpressing plasmids. (C) CCK8 assays demonstrating the cell proliferation, **** represents $p < 0.001$.

treatment (Figure 3A). Overexpression of *FGFR1* (Figure 3B) was associated with enhanced cell viability (Figure 3C).

To further elucidate the role of *FGFR1* in asthma, the impact of 5-Aza-CdR on *FGFR1* expression in PCS-301-011 cells was investigated. As depicted in Figure 4A, treatment with 5-Aza-CdR led to upregulation of *FGFR1* expression. Subsequently, *FGFR1* was overexpressed in PCS-301-011 cells (Figure 4B). CCK8 assays demonstrated that *FGFR1* overexpression promoted the proliferation of PCS-301-011 cells (Figure 4C).

3.5 *FGFR1* DNA methylation in the serum of asthma patients

The impact of 5-Aza-CdR on *FGFR1* expression was assessed by investigating *FGFR1* DNA methylation in five asthma cases and five healthy controls. Only one CpG island was initially anticipated in the *FGFR1* DNA promoter using the CpG Island Searcher

tool (accessible at <http://www.cpgislands.com>) (Figures 5A,B). Subsequently, genomic DNA from *FGFR1* was isolated from serum samples and analyzed using MassARRAY spectrometry to quantify *FGFR1* DNA methylation, revealing twelve CpG sites (Figure 5C). Upon examination, a reduction in methylation levels was observed in asthma patients compared to healthy controls (Figure 5D).

4 Discussion

Higher levels of *FGFR1* mRNA were observed in the serum of asthma patients compared to healthy controls in our study. Overexpression of *FGFR1* notably enhanced the proliferative capacity of BEAS-2B cells and triggered NF- κ B transcriptional activity. Our investigation into the DNA methylation mechanism of *FGFR1* expression revealed that treating BEAS-2B cells with 5-Aza-CdR led to increased *FGFR1* mRNA levels. This demethylation treatment also significantly activated NF- κ B

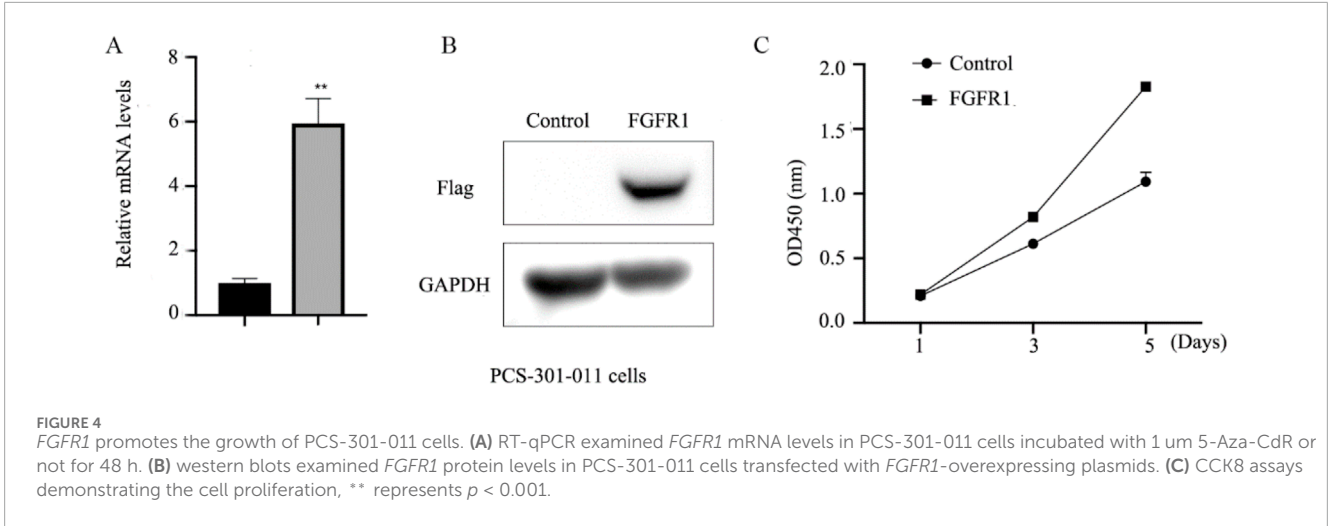


FIGURE 4
FGFR1 promotes the growth of PCS-301-011 cells. **(A)** RT-qPCR examined *FGFR1* mRNA levels in PCS-301-011 cells incubated with 1 μm 5-Aza-CdR or not for 48 h. **(B)** western blots examined *FGFR1* protein levels in PCS-301-011 cells transfected with *FGFR1*-overexpressing plasmids. **(C)** CCK8 assays demonstrating the cell proliferation, ** represents $p < 0.001$.

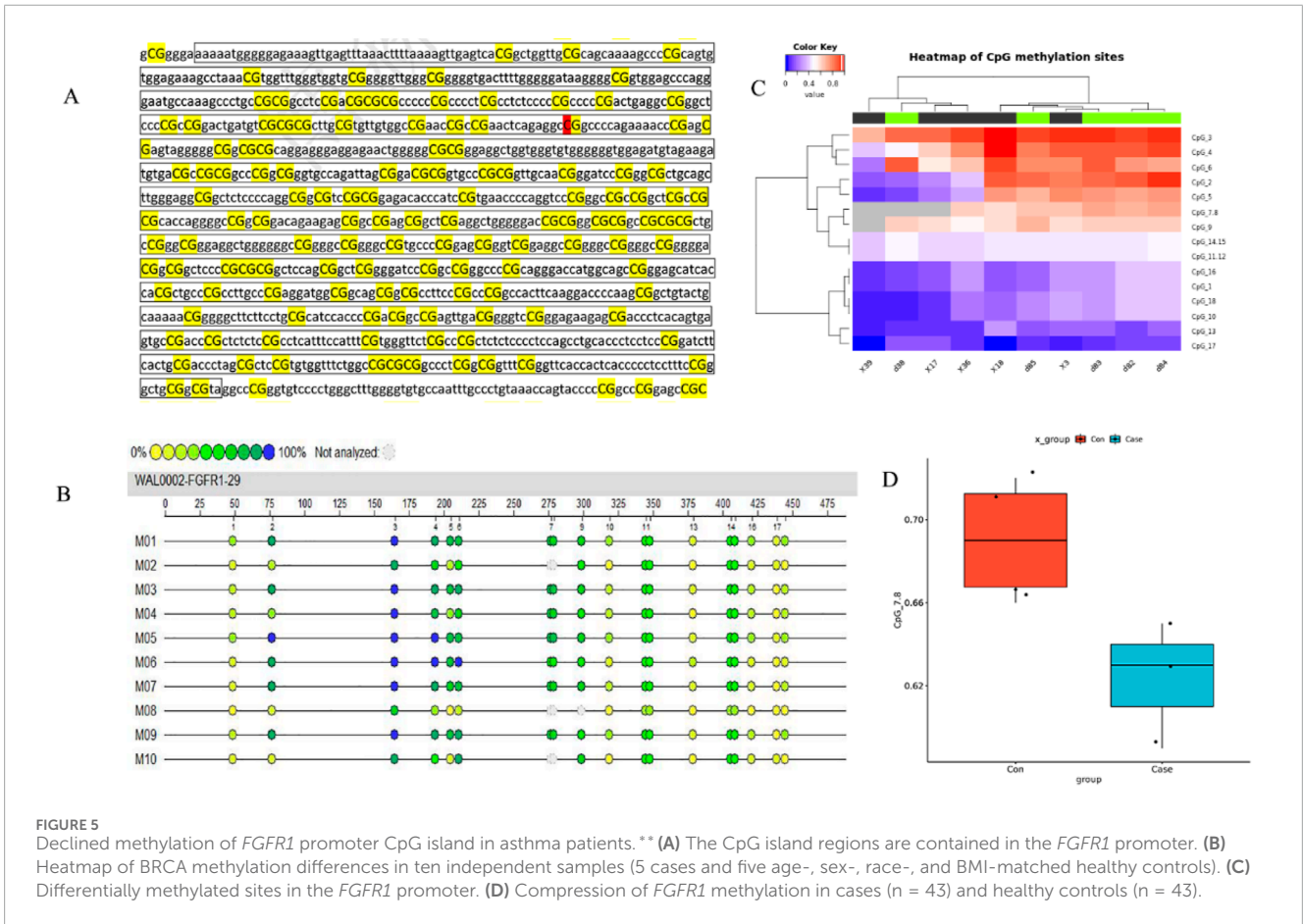


FIGURE 5
 Declined methylation of *FGFR1* promoter CpG island in asthma patients. **(A)** The CpG island regions are contained in the *FGFR1* promoter. **(B)** Heatmap of BRCA methylation differences in ten independent samples (5 cases and five age-, sex-, race-, and BMI-matched healthy controls). **(C)** Differentially methylated sites in the *FGFR1* promoter. **(D)** Compression of *FGFR1* methylation in cases ($n = 43$) and healthy controls ($n = 43$).

cascades. Methylation analysis showed hypomethylation of the *FGFR1* DNA promoter in asthma samples compared to healthy controls. This study highlights the importance of investigating extracellular RNA and DNA, particularly in understanding the molecular mechanisms underlying asthma. The hypomethylation observed in the extracellular DNA of *FGFR1* suggests that DNA methylation may serve as a crucial regulatory mechanism,

influencing gene expression and the progression of inflammatory processes in asthma.

A recent bioinformatics analysis identified *FGFR1* as a potential dysregulated gene in asthma based on consensus gene expression patterns (Loffredo et al., 2017). *FGFR1* plays a central role in modulating epithelial-mesenchymal signaling dysregulation, which may be a shared pathophysiological mechanism among

individuals with different asthma severities (Kitai et al., 2016). Wang et al.'s study on lung cancer cell lines revealed elevated *FGFR1* expression, which was associated with the upregulation of genes linked to asthma development, such as ETM-related genes and SOX2 (Wang K. et al., 2018). Notably, our comparison of asthma and healthy samples showed a significant increase in *FGFR1* mRNA levels in asthma samples, supporting the notion of *FGFR1*'s involvement in asthma progression.

NF- κ B signaling is pivotal in driving various biological processes implicated in developing inflammatory and immune disorders, notably asthma. For instance, its activation in BEAS-2B cells is essential for eliciting adaptive inflammatory responses against intracellular pathogens like PM2.5 and PM1 (Dang et al., 2020). In asthma animal models, inhibition of the NF- κ B signaling pathway has markedly reduced airway inflammation and remodeling (Chauhan et al., 2018). Conversely, prior research has highlighted *FGFR1* as a proinflammatory factor, activating the NF- κ B signaling cascade (Wang C. et al., 2018). To delve deeper into the contribution of *FGFR1* in asthma progression, we conducted experiments involving the overexpression of *FGFR1* in BEAS-2B cells to investigate its interplay with NF- κ B. Consistent with findings by Wang et al. (Reese et al., 2019), our results demonstrated that heightened expression of *FGFR1* significantly enhanced cell proliferation and upregulated NF- κ B transcription, providing novel insights into the association between *FGFR1* expression and asthma (Bae et al., 2020). Furthermore, epigenetic studies in asthma have underscored the significant role of epigenetic regulation as a primary driver of asthma pathogenesis.

Our findings indicate that *FGFR1* hypomethylation amplifies NF- κ B signaling, contributing to the inflammatory processes linked to asthma. This observation is consistent with prior research emphasizing the pivotal role of NF- κ B in asthma pathogenesis. By establishing a connection between *FGFR1* methylation and NF- κ B activity, our study offers fresh insights into the molecular mechanisms of asthma and proposes potential targets for therapeutic strategies. Subsequent investigations should prioritize *in vivo* models to validate and expand upon these findings. It is important to acknowledge certain limitations of our study. The modest sample size, particularly in the methylation analyses, may compromise the strength of our conclusions, necessitating more extensive studies for validation.

Our findings suggest that DNA methylation of *FGFR1* may be a key epigenetic mechanism through which environmental factors influence asthma development (Potaczek et al., 2022). Given that epigenetic modifications are responsive to environmental changes, the observed hypomethylation of *FGFR1* in asthma patients could reflect an interaction between genetic susceptibility and environmental exposures. This highlights the potential for targeting epigenetic modifications in therapeutic strategies aimed at mitigating the impact of environmental factors on asthma. Our results showed that using 5-aza-CdR, a broad-spectrum demethylation agent, may lead to the demethylation of multiple loci, potentially impacting the expression of genes beyond *FGFR1*. For instance, 5-aza-CdR has been reported to decrease methylation of the *ORMDL3* promoter, thereby promoting inflammation in asthma (Schedel et al., 2015; Toncheva et al., 2015). Polymorphisms related to *ORMDL3* are associated with asthma susceptibility. Despite these constraints, our study underscores the potential importance

of *FGFR1* demethylation in activating NF- κ B signaling, which could exacerbate the pathological inflammation associated with asthma. Future investigations, incorporating *in vivo* studies and more targeted epigenetic strategies, are imperative to validate and further elucidate these mechanisms.

5 Conclusion

Our findings suggest that extracellular RNA and DNA, particularly through the mechanism of DNA methylation, play a significant role in the regulation of *FGFR1* expression and the activation of NF- κ B signaling in asthma. These results open new avenues for the development of targeted therapies that focus on epigenetic modifications in extracellular DNA as a means to manage asthma more effectively. Therefore, our study not only underscores the importance of *FGFR1* methylation in asthma but also emphasizes the broader role of epigenetic mechanisms in mediating gene-environment interactions, offering new insights into potential intervention strategies. However, we acknowledge that our study lacks detailed clinical and laboratory data, such as asthma phenotypes, allergies, accompanying disorders, asthma severity, treatment, and relevant laboratory tests (e.g., IgE levels, specific IgE/skin prick testing, eosinophilia, spirometry). While these data would have provided additional context and depth to our findings, the focus of our research was on exploring the molecular mechanisms underlying *FGFR1* methylation in asthma. Future studies with a more comprehensive clinical dataset are needed to validate and extend our findings. Additionally, in addition to a small number of total plasma samples, the statistical power was insufficient because the number of normal plasma samples was small compared to that for lung cancer patients.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here https://figshare.com/articles/dataset/Data_Set_Article_ID_1433557/26894038?file=48934777.

Ethics statement

The studies involving humans were approved by Medical Ethics Committee of Youjiang Medical University for Nationalities (No.2017030501). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

RM: Conceptualization, Data curation, Formal Analysis, Methodology, Software, Supervision, Validation, Visualization, Writing—original draft, Writing—review and editing. MM: Conceptualization, Data curation, Formal Analysis, Investigation,

Methodology, Software, Supervision, Validation, Visualization, Writing—original draft, Writing—review and editing. YM: Conceptualization, Data curation, Methodology, Software, Supervision, Validation, Visualization, Writing—original draft, Writing—review and editing. JX: Conceptualization, Data curation, Formal Analysis, Methodology, Supervision, Validation, Visualization, Writing—original draft, Writing—review and editing. GC: Conceptualization, Data curation, Formal Analysis, Methodology, Software, Supervision, Validation, Visualization, Writing—original draft, Writing—review and editing.

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References

- Bae, D.-J., Jun, J. A., Chang, H. S., Park, J. S., Park, C.-S. J. T., and Diseases, R. (2020). Epigenetic changes in asthma: role of DNA CpG methylation. *Tuberc. Respir. Dis.* 83, 1–13. doi:10.4046/trd.2018.0088
- BAJCOiP, R. (2019). Asthma severity, nature or nurture: genetic determinants. *Curr. Opin. Pediatr.* 31, 340–348. doi:10.1097/MOP.0000000000000758
- Balasoorya, G. I., Johnson, J.-A., Basson, M. A., and Eljdc, R. (2016). An FGFR1-SPRY2 signaling axis limits basal cell proliferation in the steady-state airway epithelium. *Dev. Cell.* 37, 85–97. doi:10.1016/j.devcel.2016.03.001
- Chauhan, P. S., Singh, D., Dash, D., and Singh, R. J. P. (2018). Intranasal curcumin regulates chronic asthma in mice by modulating NF- κ B activation and MAPK signaling. *Phytomedicine* 51, 29–38. doi:10.1016/j.phymed.2018.06.022
- Chen, X., Zhang, X., Xu, J., Zhao, Y., Bao, J., Zheng, Z., et al. (2020). AZD4547 attenuates lipopolysaccharide-induced acute kidney injury by inhibiting inflammation: the role of FGFR1 in renal tubular epithelial cells. *Drug Des. devel. Ther.* 14, 833–844. doi:10.2147/DDDT.S224343
- Chusongdam, S., Woonnoi, W., Moolsup, F., Aenglong, C., Chonpathompikunlert, P., Tanasawet, S., et al. Suppression of inflammation in adipocyte-macrophage coculture by passion fruit seed extract: insights into the p38 and NF- κ B pathway. (2024) 2024.
- Dang, X., He, B., Ning, Q., Liu, Y., Guo, J., Niu, G., et al. (2020). Alantolactone suppresses inflammation, apoptosis and oxidative stress in cigarette smoke-induced human bronchial epithelial cells through activation of Nrf2/HO-1 and inhibition of the NF- κ B pathways. *Respir. Res.* 21, 95–111. doi:10.1186/s12931-020-01358-4
- El-Nagar, M. M. F., and Elsis, A. E. (2023). Exposure to bromoxynil octanoate herbicide induces oxidative stress, inflammation, and apoptosis in testicular tissue via modulating NF- κ B pathway. *Food Chem. Toxicol.* 180, 114008. doi:10.1016/j.fct.2023.114008
- Hudon Thibeault, A.-A., and Laprise, C. J. G. (2019). Cell-specific DNA methylation signatures in asthma. *Genes (Basel)*. 10, 932. doi:10.3390/genes10110932
- Kim, J.-M., Kim, H.-K., Im, Y.-N., Bae, Y.-S., Im, S.-Y., Lee, H.-KJIA, A., et al. (2018). Fc γ R/ROS/CK2 α is the key inducer of NF- κ B activation in a murine model of asthma. *Int. Arch. Allergy Immunol.* 175, 16–25. doi:10.1159/000485621
- Kitai, H., Ebi, H., Tomida, S., Floros, K. V., Kotani, H., Adachi, Y., et al. (2016). Epithelial-to-mesenchymal transition defines feedback activation of receptor tyrosine kinase signaling induced by MEK inhibition in KRAS-mutant lung cancer. *Cancer Discov.* 6, 754–769. doi:10.1158/2159-8290.CD-15-1377
- Lehto, K., Pedersen, N. L., Almqvist, C., Lu, Y., and Brew, BKJERJ (2019). *Asthma and affective traits in adults: a genetically informative study*, 53.
- Lin, J., Jiang, Y., Li, L., Liu, Y., Tang, H., and Jiang, D. J. E. (2015). TMEM16A mediates the hypersecretion of mucus induced by Interleukin-13. *Exp. Cell. Res.* 334, 260–269. doi:10.1016/j.yexcr.2015.02.026
- Loffredo, L. F., Abdala-Valencia, H., Anekalla, K. R., Cuervo Pardo, L., Gottardi, C. J., and Berdnikovs, S. J. A. Beyond epithelial-to-mesenchymal transition: common suppression of differentiation programs underlies epithelial barrier dysfunction in mild, moderate, and severe asthma. (2017) 72, 1988–2004. doi:10.1111/all.13222
- Pisani, L. F., Tontini, G., Vecchi, M., Croci, G. A., and LjoiMR, P. (2022). NF- κ B pathway is involved in microscopic colitis pathogenesis. *J. Int. Med. Res.* 50, 03000605221080104. doi:10.1177/03000605221080104
- Potaczek, D. P., Alashkar Alhamwe, B., Mieth, S., and Garn, H. (2022). Epigenetic mechanisms in allergy development and prevention. *Handb. Exp. Pharmacol.* 268, 331–357. doi:10.1007/164_2021_475
- Potaczek, D. P., Harb, H., Michel, S., Alhamwe, B. A., Renz, H., and Tost, J. (2017). Epigenetics and allergy: from basic mechanisms to clinical applications. *Epigenomics* 9, 539–571. doi:10.2217/epi-2016-0162
- Reese, S. E., Xu, C.-J., Den Dekker, H. T., Lee, M. K., Sikdar, S., Ruiz-Arenas, C., et al. (2019). Epigenome-wide meta-analysis of DNA methylation and childhood asthma. *J. Allergy Clin. Immunol.* 143, 2062–2074. doi:10.1016/j.jaci.2018.11.043
- Schedel, M., Michel, S., Gaertner, V. D., Toncheva, A. A., Depner, M., Binia, A., et al. (2015). Polymorphisms related to ORMDL3 are associated with asthma susceptibility, alterations in transcriptional regulation of ORMDL3, and changes in TH2 cytokine levels. *J. Allergy Clin. Immunol.* 136, 893–903. doi:10.1016/j.jaci.2015.03.014
- Sun, S.-C. J. N. (2017). The non-canonical NF- κ B pathway in immunity and inflammation. *Nat. Rev. Immunol.* 17, 545–558. doi:10.1038/nri.2017.52
- TJERCiWoS, L. (2009). The nuclear factor NF- κ B pathway in inflammation. *Cold Spring Harb. Perspect. Biol.* 1, a001651. doi:10.1101/cshperspect.a001651
- Toncheva, A. A., Potaczek, D. P., Schedel, M., Gersting, S. W., Michel, S., Krajnov, N., et al. (2015). Childhood asthma is associated with mutations and gene expression differences of ORMDL genes that can interact. *Allergy* 70, 1288–1299. doi:10.1111/all.12652
- Tsai, M.-J., Chang, W.-A., Jian, S.-F., Chang, K.-F., Sheu, C.-C., Kuo, P.-L. J. P.-R., et al. (2018). Possible mechanisms mediating apoptosis of bronchial epithelial cells in chronic obstructive pulmonary disease—a next-generation sequencing approach. *Pathol. Res. Pract.* 214, 1489–1496. doi:10.1016/j.prp.2018.08.002
- Wang, C., Ke, Y., Liu, S., Pan, S., Liu, Z., Zhang, H., et al. (2018b). Ectopic fibroblast growth factor receptor 1 promotes inflammation by promoting nuclear factor- κ B signaling in prostate cancer cells. *prostate cancer cells* 293, 14839–14849. doi:10.1074/jbc.RA118.002907

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmolb.2024.1433557/full#supplementary-material>

- Wang, K., Ji, W., Yu, Y., Li, Z., Niu, X., Xia, W., et al. (2018a). FGFR1-ERK1/2-SOX2 axis promotes cell proliferation, epithelial–mesenchymal transition, and metastasis in FGFR1-amplified lung cancer. *Oncogene* 37, 5340–5354. doi:10.1038/s41388-018-0311-3
- Wang, S., Cao, S., Arhatte, M., Li, D., Shi, Y., Kurz, S., et al. (2020). Adipocyte Piezo1 mediates obesogenic adipogenesis through the FGF1/FGFR1 signaling pathway in mice. *Nat. Commun.* 11, 2303. doi:10.1038/s41467-020-16026-w
- Wenzel, S. E. (2012). Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat. Med.* 18, 716–725. doi:10.1038/nm.2678
- Xu, C.-J., Söderhäll, C., Bustamante, M., Baiz, N., Gruziova, O., Gehring, U., et al. (2018). DNA methylation in childhood asthma: an epigenome-wide meta-analysis. *Lancet. Respir. Med.* 6, 379–388. doi:10.1016/S2213-2600(18)30052-3
- Yang, IVJTLRM (2019). DNA methylation signatures of atopy and asthma. *Lancet. Respir. Med.* 7, 289–290. doi:10.1016/S2213-2600(18)30504-6
- Yuan, T., Klinkhammer, K., Lyu, H., Gao, S., Yuan, J., Zhang, J.-S., et al. (2020). Temporospacial expression of Fgfr1 and 2 during lung development, homeostasis, and regeneration. *Homeost. Regen.* 11, 512004. doi:10.3389/fphar.2020.00120
- Zhang, T., Ma, C., Zhang, Z., Zhang, H., and Hu, H. J. M. (2021). NF- κ B signaling in inflammation and cancer. *MedComm* 2, 618–653. doi:10.1002/mco2.104