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Effects of IFN- γ on the cell growth and the expression of ADAM33 gene in human embryonic lung Mrc-5 fibroblasts in vitro

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Abstract

Objective: To investigate the effects of interferon- γ (IFN- γ) on the proliferation and viability of human embryonic lung Mrc-5 fibroblasts *in vitro* and the expression of the A Disintegrin and Metalloprotease 33 (ADAM33) gene and to explore the mechanism of airway remodeling.

Methods: Mrc-5 fibroblasts were sensitized with *Dermatophagoides farinae* 1 (Derf1) *in vitro* to mimic *in vivo* conditions observed in bronchial asthma. An inverted fluorescence microscope was used to observe changes in cell morphology before and after treatment. The viability of Mrc-5 cells was tested using the Cell Counting kit-8 (CCK8). Expression of the ADAM33 gene and protein in Mrc-5 cells was assessed using qPCR and Western blotting, respectively.

Results: Different concentrations of Derf1 increased cell growth and the expression of the ADAM33 gene in Mrc-5 cells, and these changes were most obvious in the 10 $\mu\text{g/ml}$ group. In contrast, IFN- γ decreased cell growth and the expression of the ADAM33 gene in both Mrc-5 cells and Derf1-induced Mrc-5 cells, and these changes were most obvious in the 10 ng/ml group. The negative effects of 10 ng/ml IFN- γ were the most significant at 32 h.

Conclusions: Derf1-induced Mrc-5 cells successfully imitated the *in vivo* conditions observed in patients with asthma. IFN- γ inhibited the proliferation and viability of Mrc-5 cells, and Derf1-induced Mrc-5 cells were more sensitive to IFN- γ treatment. IFN- γ treatment significantly downregulated the expression of the ADAM33 gene in a concentration- and time-dependent manner. IFN- γ may participate in airway remodeling in asthma by regulating the expression of the ADAM33 gene.

Key words

Mrc-5 cells; Derf1; Cell proliferation; ADAM33 Gene; IFN- γ

Introduction

Bronchial asthma (asthma) is the most common chronic respiratory disease in childhood, and the morbidity of asthma has increased in recent years^[1]. In China, the prevalence of bronchial asthma has climbed to 1.24%. However, the disease control rate is far from the goal proposed by the Global Initiative for Asthma (GINA), due to the lack of proper attention to the guidelines^[2]. Asthma pathogenesis is mainly associated with complex immune and genetic factors. Among the numerous underlying factors, an imbalance in Th1/Th2 cells is one of the commonly accepted immune factors. An imbalance in Th1/Th2 cells presents as a decrease in the expression of Th1 cytokines (IFN- γ) and the excess secretion of Th2 cytokines (IL-4 and IL-13). IFN- γ reverses the imbalance and inhibits the type I allergy response mediated by Th2 cytokines. On the other hand, fibroblasts are closely related to the development of airway inflammation and airway remodeling in patients with asthma. Airway inflammation, tissue injury and subsequent abnormal repair result in structural changes in the airway wall in patients with asthma. The structural changes are known as airway remodeling. In recent years, the function of fibroblasts in asthma remodeling has attracted increasing attention. Fibroblasts may participate in asthma remodeling by producing collagen fibers, elastic fibers and reticular fibers. Moreover, as important immunological effector cells, fibroblasts may enhance and maintain inflammation by releasing cytokines and inflammatory factors. The number of fibroblasts is related to the thickness of the airway basement membrane^[3,4]. IFN- γ inhibits fibroblasts through multiple pathways, such as

proliferation, differentiation and collagen synthesis, which promote airway remodeling^[5].

A Disintegrin and Metalloprotease 33 (ADAM33) has been proposed as an asthma-related gene that is closely associated with airway remodeling. It is mainly expressed in lung smooth muscle cells and lung fibroblasts. Its protein product (a metalloprotease) is associated with the repair of injured airway epithelial cells. The function of smooth muscle cells and fibroblasts changes as ADAM33 activity changes. Therefore, we believe that ADAM33 is associated with airway remodeling^[6]. However, the upstream mechanism of ADAM33 has not yet been identified. As shown in our previous study, ADAM33 is up-regulated in the peripheral blood mononuclear cells (PBMCs) from children with asthma^[7]. According to the study by Ito, the ADAM33 mRNA and protein are expressed at higher levels in ASM cells obtained from biopsied specimens from patients with asthma than in cells from normal subjects. Cultured ASM cells expressed both the ADAM33 mRNA and protein. IFN- γ reduces the expression of ADAM33 mRNA in a dose- and time-dependent manner. Thus, the expression of the ADAM33 gene may be regulated by IFN- γ at the transcriptional level.

As an important allergen, *Dermatophagoides farinae* is very common in daily life. More than 16 kinds of *Dermatophagoides farinae* allergens have currently been reported. Among these allergens, *Dermatophagoides farinae* 1 (Derf1) is regarded as the most important allergen. Derf1 acts on several cells, such as tracheal epithelial cells or T lymphocytes, and induces type I allergic reactions. Subsequently, it may induce various types of allergic diseases, such as asthma,

rhinitis, and chronic urticaria. Derf1 has been widely used as an allergen in asthma models.

Currently, few *in vitro* and *in vivo* data are available that describe the relationship between ADAM33 expression in fibroblasts and allergic inflammation. In the present study, we aimed to investigate the effects of different concentrations of IFN- γ on ADAM33 expression and the proliferation and viability of human embryonic lung fibroblasts at different times to confirm that IFN- γ downregulates the inflammatory response. Through these investigations, we explored the therapeutic potential of using IFN- γ to treat bronchial asthma.

Methods

1. Materials and instruments

Primary embryonic lung fibroblasts were purchased from the Shanghai Cell Bank (Chinese Academy of Sciences, China). Dulbecco's Modified Eagle's Medium (DMEM), penicillin and streptomycin were purchased from Gibco Co., Ltd. (Grand Island, NY, USA). Fetal bovine serum (FBS) was obtained from BioInd (Israel). Glutamax and sodium pyruvate were purchased from Gibco (Waltham, MA, USA). Derf1 was purchased from Prospec Company (Israel). IFN- γ was purchased from PeproTech Company (Rocky Hill, USA), and the Cell Counting kit-8 (CCK-8) was obtained from the Japan Research Institute of Chemistry (Japan). Trizol reagent, SYBR Green PCR Master mix and PrimeScript RT Master Mix were obtained from TaKaRa (Dalian, China). The RIPA kit was purchased from Thermo Fisher Scientific (Waltham, MA, USA). ADAM33 and beta-actin primers were synthesized by Shanghai Sangon Biotech Co., Ltd.

(Shanghai, China). The ADAM33 polyclonal antibody was purchased from Abcam (Cambridge, USA). The beta-actin antibody was purchased from Beijing Bioss Antibody Co., Ltd. (Beijing, China). The goat anti-rabbit antibody was obtained from Cell Signaling Technology Company (Danvers, USA). The goat anti-rat antibody was obtained from Beijing Bioss Antibody Co., Ltd. (Beijing, China). The ECL Plus Western Blotting Detection Reagent was obtained from Millipore (Mabumveryussetts, USA).

An inverted fluorescence microscope was obtained from N-STORM (Nikon, Japan). A SpectraMax M5 Microplate Reader was obtained from Molecular Devices (Silicon Valley, USA). The LC480 instrument was purchased from Roche (Basel, Switzerland), and a Hyperfilm G:BOX XT4 was purchased from Syngene (Cambridge, UK).

2. Methods

2.1 Cell culture and passage:

Mrc-5 cells were cultured in optimal medium supplemented with 10% FBS, 100 U/ml penicillin, 100 mg/ml streptomycin, 1% Glutamax and 1% sodium pyruvate in a humidified 37°C incubator with 5% CO₂. The dilution for cell passaging was 1:2 or 1:3.

2.2 Establishment of asthmatic changes *in vitro*:

The induction of asthmatic changes *in vitro* was performed using a modified version of a previously reported protocol^[8,9]. Mrc-5 cells were seeded in 6-well plates at a density of 1.5×10^5 cells per well. Different concentrations (0 µg/ml, 10 µg/ml, or 100 µg/ml) of Derf1 were added

to each well on the next day. After 24 h of induction, cells were harvested to measure cell proliferation and viability, as well as ADAM33 expression.

2.3 IFN- γ intervention

Mrc-5 cells and Derf1-induced Mrc-5 cells were grown in optimal medium and treated with various concentrations of IFN- γ (0.1 ng/ml, 1 ng/ml, 10 ng/ml, or 100 ng/ml) after the cells had adhered to the plate.

2.4 Observation of cell morphology

Cell morphology was observed under an inverted fluorescence microscope before and after treatment with IFN- γ or Derf1.

2.5 Measurement of Mrc-5 cell viability using the CCK-8 assay

Cells were resuspended and then seeded into 96-well plates (1×10^5 cells per well). The plates were incubated in a humidified incubator (37°C, 5% CO₂) overnight to allow the cells to adhere. Following treatments of varying durations, CCK-8 was added to each well at 10% of the total volume. The blank control group was established simultaneously. Cells were incubated for 2.5 h, and the absorbance was measured at 450 nm using a microplate reader. The following formula describes the relationship between absorbance and cell viability:

$$\text{cell viability} * (100\%) = [\text{OD (treated cells)} - \text{OD (blank)}] / [\text{OD (untreated cells)} - \text{OD (blank)}] * 100.$$

2.6 Measurement of the expression of ADAM33 mRNA using qPCR

Total RNA was extracted with Trizol reagent according to the manufacturer's protocol. The PrimeScript RT Master Mix was used to synthesize cDNAs. The SYBR Green PCR Master mix and the LC480 instrument were used to perform qPCR to detect the expression of ADAM33 mRNA. Beta-actin was chosen as an internal reference to normalize the mRNA levels in different cell samples. Primer sequences for the ADAM33 gene were as follows: sense: 5'-GGA ACC CCG AGC CAC ATT AG-3' and antisense: 5'-AGC ACT ACC CAG CCT CCA CT-3'. The reaction volume was 10 μ l, and the following reaction conditions were used: 95°C for 3 min and 40 cycles of 95°C for 10 s and 55°C for 30 s. Based on the CT (cycle threshold) value, the $\Delta\Delta$ Ct method was used to calculate gene expression.

2.7 Measurement of the expression of the ADAM33 protein using Western blotting

A mixture of the cell lysis buffer (RIPA) and phenylmethylsulfonyl fluoride (PMSF) (ratio of 100:1) was added to the culture dishes, and the proteins were collected after a 30 min incubation on ice. Proteins (40 μ g per well) were electrophoresed on 10% polyacrylamide gel and transferred to a PVDF membrane. Membranes were blocked with 5% dry non-fat milk for 1 h at room temperature and incubated with the primary antibody (1:2,000 dilution) overnight at 4°C. After 3 10-min washes with TBST, membranes were incubated with a secondary antibody for 1 h at room temperature. ECL Plus Western Blotting Detection Reagent was used for detection, and the blots were digitally scanned using Hyperfilm. Image-Pro Plus 6.0 was used to analyze the images.

3. Statistical analysis

All experiments were performed with at least 4 replicates. Data were expressed as the means and standard deviations. All data were analyzed using SPSS 13.0 and GraphPad Prism 6 statistical software. Statistical analyses were performed using one-way ANOVA, and comparisons between groups were performed using the LSD method. $P < 0.05$ was considered statistically significant.

Results

1. Effects of Derf1 on the proliferation and viability of Mrc-5 cells

Mrc-5 cells were treated with different concentrations (0 $\mu\text{g/ml}$, 10 $\mu\text{g/ml}$ or 100 $\mu\text{g/ml}$) of Derf1 for 24 h. Both the 10 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$ Derf1 groups grew faster, as a smaller intercellular space was observed in these groups compared with the blank group (Fig 1A).

Various concentrations of Derf1 were added to the cells for 24 h. According to cell viability measured using the CCK-8 assay, 10 $\mu\text{g/ml}$ Derf1 increased cell viability compared with the blank group ($P < 0.05$), whereas 100 $\mu\text{g/ml}$ had little effect on cell viability (Fig 1B and Table 1, $P > 0.05$). Thus, certain concentrations of Derf1 increased cell viability.

2. Effects of IFN- γ on the proliferation and viability of Mrc-5 cells

Mrc-5 cells were treated with different concentrations (0 ng/ml, 0.1 ng/ml, 1 ng/ml, 10 ng/ml and 100 ng/ml) of IFN- γ for 24 h, and cell morphology was observed under a microscope. All treated groups grew more slowly than the blank group. The 10 ng/ml and 100 ng/ml groups showed the most obvious changes (Fig 2A).

Regarding the viability of the Mrc-5 cells, 100 ng/ml IFN- γ exerted an obvious negative effect on cell viability at 24 and 48 h ($P<0.05$). Additionally, a negative effect of 10 ng/ml IFN- γ on cell viability was observed at 72 h ($P<0.05$). However, no significant differences were observed among the four treated groups and the blank group at 96 h (Table 2 and Fig 2B, $P>0.05$).

3. Effects of IFN- γ on the proliferation and viability of Derf1-induced Mrc-5 cells

Mrc-5 cells were pretreated with 10 μ g/ml Derf1 for 24 h. Subsequently, various concentrations (0 ng/ml, 0.1 ng/ml, 1 ng/ml, 10 ng/ml and 100 ng/ml) of IFN- γ were added to the cells. Based on the cell morphology, the 10 ng/ml and 100 ng/ml IFN- γ treatments significantly suppressed cell proliferation and increased the intercellular space (Fig 3A).

Regarding cell viability, the 10 ng/ml and 100 ng/ml IFN- γ treatments remarkably inhibited cell viability after 24 h, and the negative effect of 10 ng/ml IFN- γ was significant after 48 h ($P<0.05$). Furthermore, the effect of 10 ng/ml IFN- γ was still obvious after 72 h ($P<0.05$). No significant differences were observed among cells treated with the four concentrations of IFN- γ at 96 h (Table 3 and Fig 3B, $P>0.05$).

4. Effects of Derf1 on ADAM33 expression in Mrc-5 cells

Mrc-5 cells were treated with different concentrations of Derf1 for 24 h, and then the expression of ADAM33 mRNA was determined using qPCR. The expression of ADAM33 mRNA was significantly increased in Mrc-5 cells treated with 10 μ g/ml Derf1 compared the blank control group (Fig 4A, $P<0.05$). Consistent with the mRNA expression levels, Mrc-5 cells treated with

10 µg/ml Derf1 expressed significantly higher levels of the ADAM33 protein than the control group (Fig 4B-4C, $P<0.05$).

5. Effects of IFN- γ on ADAM33 expression in Mrc-5 cells

Mrc-5 cells were treated with four different concentrations of IFN- γ for 24 h. The expression of ADAM33 mRNA was decreased in Mrc-5 cells in the 10 ng/ml IFN- γ group compared with the blank control group (Fig 5A, $P<0.05$). Consistent with the mRNA abundance, the levels of the ADAM33 protein were significantly decreased in the 10 ng/ml IFN- γ group (Fig 5B-5C, $P<0.05$). On the other hand, the other treated groups showed little change in ADAM33 expression compared with the blank control group ($P>0.05$).

6. Effects of IFN- γ on ADAM33 expression in Derf1-induced Mrc-5 cells

Derf1-induced Mrc-5 cells were treated with various concentrations of IFN- γ for 24 h. The expression of ADAM33 mRNA was decreased in the 10 ng/ml IFN- γ group compared with the blank control group (Fig 6A). Meanwhile, the expression of ADAM33 protein was significantly decreased in each group compared to the blank control group (Fig 6B-6C, $P<0.05$). Sensitized Mrc-5 cells were treated with 10 ng/ml IFN- γ , and the expression of ADAM33 mRNA was measured every 8 h. The expression of ADAM33 mRNA was strikingly decreased at 32 h compared with the blank control group (Fig 7A). Correspondingly, the level of ADAM33 protein decreased at 32 h compared with the blank control group (Fig 7B-7C, $P<0.05$).

Discussion

Airway fibrosis is an important pathological change observed during the progression of asthma. Fibroblasts participate in the progression of airway inflammation and are closely related to cell proliferation and collagen deposition, which thicken the basement membrane and worsen epithelial fibrosis. Fibroblasts have been shown to play a crucial role during *in situ* inflammation by releasing various inflammatory mediators, such as IL-6 and IL-8. On the other hand, fibroblasts also play an important role in tissue repair and airway remodeling by producing a variety of regulatory factors, such as TGF- β , fibronectin, and vascular endothelial growth factor^[10]. Cytokines from patients' PBMCs may mediate the function of fibroblasts. Even small amounts of cytokines in the supernatant of PBMCs induce fibroblasts to produce chemokines^[11]. Thus, fibroblasts play a crucial role in the development of asthma by participating in a series of inflammatory processes.

Currently, the use of *in vivo* and *ex vivo* asthma models is very common. The allergens used in these models include ovalbumin, house dust mites, *Dermatophagoides farinae*, and viruses. Since environmental allergens such as house dusts and grass pollen are closely related to clinical features, these allergens have been widely used^[12]. Among these allergens, Derf1 is the most important allergenic component and possesses strong allergenicity. Among patients who are allergic to dust mites, 70% - 80% produce serum IgE, which binds to this type of antigen^[13]. As the main allergenic component of *Dermatophagoides farinae*, Derf1 induces a type I allergic reaction. As shown in the study by Sugiura H et al, dust mites increase epithelial cell

permeability and destroy the barrier of the bronchial epithelium^[14]. In their study, airway epithelial cells were stimulated with house dust mites. The supernatant of stimulated cells was collected and used to treat human embryonic lung fibroblasts, leading to excess fibronectin secretion, which is related to multiple essential factors involved in airway remodeling, such as TGF- β 1 and Smad2^[10]. Chun K. Wong et al^[8] used the house dust mite allergen to mimic the *in vivo* conditions observed in patients with bronchial allergic asthma. The house dust mite allergen increases the release of inflammatory cytokines and the expression of adhesion molecules, consistent with the study by Byoung Chul Kwon^[9]. Based on these results, we attempted to imitate asthmatic changes in a cell line. The main focus of our study was the airway remodeling mechanism of asthma and the important role of fibroblasts in airway remodeling. Therefore, we used fibroblasts for sensitization. Derf1 was used to stimulate human embryonic lung fibroblasts (Mrc-5 cells), imitating the allergic reaction observed in patients with bronchial asthma.

Based on the results of our initial study, Mrc-5 human embryonic lung fibroblasts showed increased proliferation and viability after treatment with different concentrations of Derf1. Moreover, the expression of ADAM33, a gene identified as a positive factor related to asthma, was significantly up-regulated following the Derf1 treatment. Thus, asthmatic changes were successfully imitated. However, the allergen had a certain concentration limit that would stimulate fibroblasts. Excessive stimulation (100 μ g/ml) did not sensitize the cells and failed to trigger a corresponding significant change in cell proliferation or gene expression. Thus, we

speculated that airway fibroblasts in asthmatic children have the potential to proliferate in response to chronic allergen stimulation and participate in the process of airway remodeling by secreting associated cytokines. To some extent, our cell-based experiment in which Mrc-5 cells were treated with Derf1 cannot completely mimic asthma inflammation. We will further examine our conclusions in animal models in a future study.

An imbalance in the ratio of Th1/Th2 cells is generally considered a critical factor in patients with asthma. Among the numerous inflammatory mediators involved in this imbalance, IFN- γ suppresses the type I allergic reaction mediated by Th2 cytokines. IFN- γ improves the lung function of patients with idiopathic pulmonary fibrosis. This finding may be associated with a decrease in the expression of TGF- β 1 and connective tissue growth factor^[15]. Moreover, IFN- γ dramatically inhibits fibroblast proliferation, differentiation and collagen synthesis. Multiple pathways that are inhibited by IFN- γ lead to airway remodeling. IFN- γ has been shown to act as a positive regulator of asthma. We directly treated fibroblasts with IFN- γ to investigate the effects of IFN- γ on fibroblasts. IFN- γ regulated the proliferation and differentiation of normal fibroblasts *in vitro*. Based on these data, we hypothesized that IFN- γ potentially regulated the abnormal proliferation, differentiation and inflammation of allergen-induced fibroblasts, which might effectively hinder the development of asthma to some extent.

ADAM33, an asthma susceptibility gene, is mainly expressed in airway fibroblasts and airway smooth muscle cells. Polymorphisms in the ADAM33 gene are associated with asthma

prevalence in the Han population^[16,17]. Therefore, polymorphisms in the ADAM33 gene may participate in proliferation, differentiation and migration, functions that are closely related to airway remodeling. In asthmatic patients, ADAM33 is associated with airway hyperresponsiveness, airway remodeling, the deterioration of lung function, the progression of wheeze, and a poor prognosis. In this study, the ADAM33 gene was expressed in normal human embryonic lung fibroblasts (Mrc-5 cells) at a relatively low level. The onset of asthma is closely related to environmental factors (allergens, airway infection, smoking, environmental pollution, etc.), which interact with genetic factors (genetic susceptibility, etc.). In the early stage, asthma manifests as intermittent attacks. Subsequently, consistent onset leads to a chronic, persistent disease^[18]. Based on the results of our study, Derf1 has significant effects on ADAM33; Derf1 up-regulated ADAM33 expression, indicating that sensitization increased ADAM33 expression and contributed to the airway remodeling that is typically observed in patients with asthma.

Cytokines, such as TGF- β 2, inhibit the expression of the ADAM33 gene by changing the chromatin structure rather than methylating the DNA^[19]. Th2 type cytokines, such as IL-4 and IL-13, stimulate the expression of the ADAM33 mRNA *in vitro*, implying that they are involved in asthma onset^[20]. Further studies are needed to understand the mechanisms by which ADAM33 induces asthma progression and its upstream regulation of cytokines. The IFN- γ levels in patients with asthma are relatively low. According to our previous study, the IFN- γ levels in peripheral blood from patients in the remission stage were lower than in patients in the acute attack stage,

and polymorphisms in the IFN- γ gene at the +874 A/T locus are associated with its expression levels. However, polymorphisms in the IFN- γ gene may not be associated with the susceptibility to asthma^[21,22]. The mechanism downstream of IFN- γ that regulates asthma has not been completely elucidated. Vishweswaraiah et al studied the interaction between ADAM33 and a variety of molecules using a network information analysis, GeneMANIA tools and literature searches. The ADAM33 protein was located on the cell membrane area and interacted with 17 proteins, including 8 extracellular matrix proteins (6 located in the cell membrane, 2 located in the cytoplasm, and 1 located in the nucleus). IFN- γ was one of the candidates^[23]. IFN- γ participates in biological processes and molecular functions related to ADAM33, such as cell adhesion, cell differentiation and receptor binding.

As shown in the present study, IFN- γ plays a role in the proliferation and activity of normal and sensitized MRC-5 cells. It downregulated the expression of ADAM33 at the mRNA and protein levels in a concentration- and time-dependent manner, consistent with the results of the study by Ito^[24]. Consistent with our result, the results from the study by Han identified an antagonistic effect of IFN- γ on type 2 innate lymphoid cell (ILC2) expansion and gene expression, the absence of which may contribute to the development of an asthma-like phenotype after an early-life RV infection^[25]. However, the role of IFN- γ in asthma still remains controversial. According to some studies, IFN- γ is not always protective but may exert pathogenic effects on the development of asthma in some cases. In a study by Deng, IFN- γ

mediated airway inflammation and AHR in OVA-induced asthmatic mice by inhibiting IL-17. Thus, IFN- γ plays a pathogenic role in the development of asthma inflammation and AHR^[26]. Furthermore, as shown in the study by Haapakoski, treatment with a soluble IFN- γ neutralizing mAb decreased IFN- γ -mediated AHR in a murine model^[27].

Thus, IFN- γ may exert both pro-asthma and anti-asthma effects in different situations that may depend on the following: (1) the upstream regulatory mechanism; (2) the differences in the experiment model, such as the BALB/c mouse model of asthma and asthmatic changes in Mrc-5 cells, which may affect the results of experiment; (3) the type of IFN- γ used in the experiment; and (4) the concentration of IFN- γ , as our study revealed a concentration limit. Overall, the specific mechanism of IFN- γ is still not clear and should be assessed using animal experiments and even *in situ* experiments to verify our findings.

The mechanism by which the ADAM33 gene affects asthma has not yet been clarified, but epigenetic modification has been regarded as the underlying mechanism. Although a difference in the DNA methylation status was not observed between the asthma group and control group in a previous study, evidence for DNA methylation in patients with asthma has gradually accumulated^[28]. According to a novel study, epigenetic regulation might be involved in the development of asthma. Differences in the methylation status of the ADAM33 gene might be associated with environmental exposure and the asthma phenotype^[29]. As shown in the study by Yang et al, the ADAM33 promoter resides in a methylated CpG island, which might silence

ADAM33 expression^[19]. The JAK/STAT pathway is an intracellular signaling pathway induced by IFN- γ *in vivo*. Moreover, the MAPK and ERK pathways play important roles in the inflammation process and are induced by IFN- γ ^[24]. These pathways may have potential roles in the methylation of the ADAM33 gene. IFN- γ might affect the expression of ADAM33 mRNA by changing the epigenetic status of the ADAM33 promoter. The complete mechanism by which IFN- γ induces asthma via ADAM33 has not been completely characterized.

In conclusion, after an optimal treatment duration, induction with specific concentrations of IFN- γ effectively decreased the proliferation and viability of Derf1-induced Mrc-5 cells, as well as the expression of the ADAM33 gene. Therefore, IFN- γ may represent a potential treatment for asthma in the future.

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Table.1. Effects of Derf1 on cell viability of Mrc-5 cells(Means±SEMs, n=12)

Derf1(μg/ml)	Cell Viability(%)
0	100.00±8.34
10	117.48±8.27*
100	104.70±5.40

* $P < 0.05$, compared with the blank control

Table.2. Effects of IFN- γ on cell viability of Mrc-5(Means $\pm$ SEMs, n=12)

IFN- γ (ng/ml)	Cell Viability(%)			
	24h	48h	72h	96h
0	100.00 $\pm$ 11.66	100.00 $\pm$ 3.87	100.00 $\pm$ 6.27	100.00 $\pm$ 7.16
0.1	115.99 $\pm$ 8.08	98.42.0 $\pm$ 1.13	86.37 $\pm$ 12.86	89.97 $\pm$ 1.91
1	119.41 $\pm$ 7.71	101.89 $\pm$ 10.55	94.01 $\pm$ 5.36	91.47 $\pm$ 3.27
10	117.56 $\pm$ 3.48	89.97 $\pm$ 12.60	79.77 $\pm$ 4.62 [#]	91.22 $\pm$ 3.25
100	58.65 $\pm$ 13.42 [#]	72.02 $\pm$ 11.69 [#]	94.06 $\pm$ 5.78	98.19 $\pm$ 2.02

[#] $P < 0.05$, compared with the blank control

Table.3.Effects of IFN- γ on cell viability of sensitized Mrc-5(Means $\pm$ SEMs,n=12)

Derf1+IFN- γ (ng/ml)	Cell Viability(%)			
	24h	48h	72h	96h
0	100.00 $\pm$ 8.70	100.00 $\pm$ 8.46	100.00 $\pm$ 5.43	100.00 $\pm$ 10.37
0.1	99.68 $\pm$ 7.80	104.28 $\pm$ 4.32	98.41 $\pm$ 4.77	103.11 $\pm$ 8.96
1	102.23 $\pm$ 10.31	101.07 $\pm$ 8.47	97.03 $\pm$ 5.58	105.83 $\pm$ 14.22
10	65.39 $\pm$ 9.00 [#]	78.29 $\pm$ 2.51 [#]	83.09 $\pm$ 12.32 [#]	100.12 $\pm$ 11.36
100	57.79 $\pm$ 9.05 [#]	91.44 $\pm$ 4.03	98.19 $\pm$ 6.20	79.41 $\pm$ 17.12

[#] $P < 0.05$, compared with the blank control

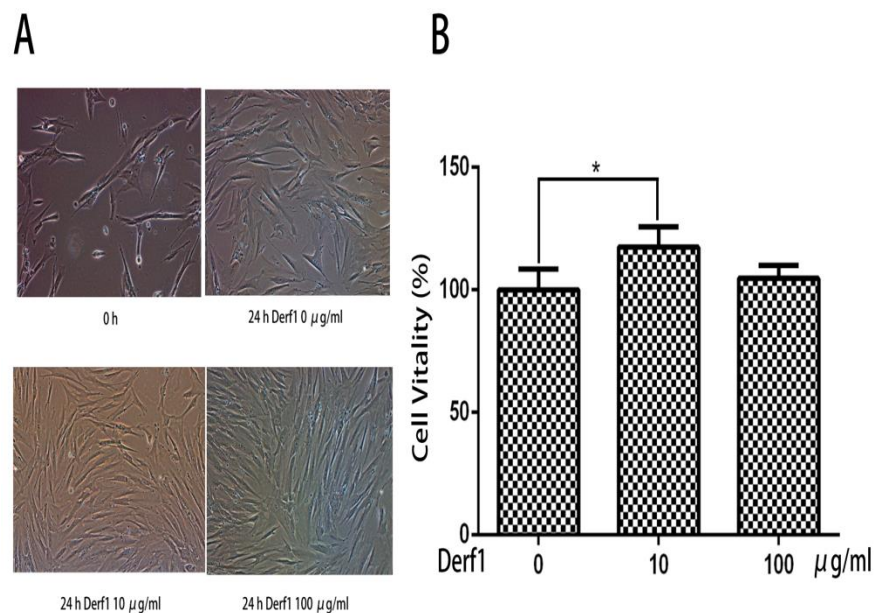


Figure1. Effects of Derf1 on the proliferation and vitality of Mrc-5 cells, A. Morphological changes of Mrc-5 cells under phase contrast microscope treated with different concentrations of Derf1 for 24 h ($\times 100$), B. Effects of Derf1 on cell viability of Mrc-5 cells $*P < 0.05$

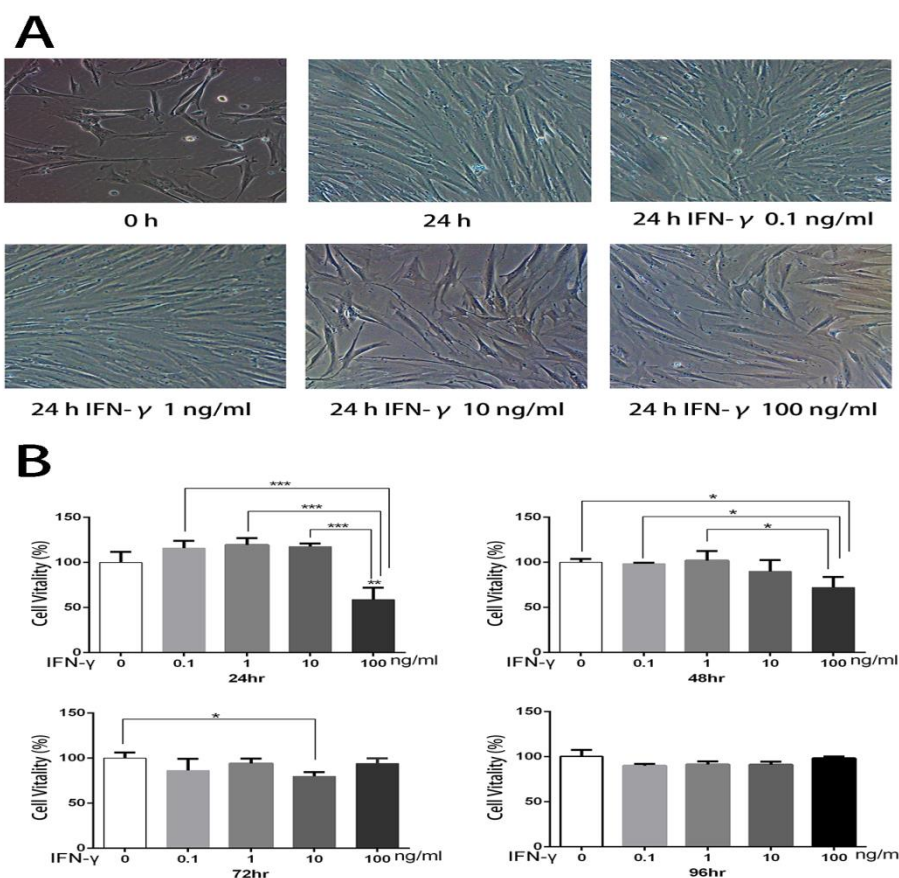


Figure2. Effects of IFN- γ on the cell proliferation and vitality of Mrc-5 cells, A. Morphological changes of Mrc-5 cells under phase contrast microscope treated with different concentrations of IFN- γ for 24 h ($\times 100$), B. Effects of IFN- γ on cell viability of Mrc-5 cells at different time points $*P < 0.05$, $***P < 0.001$

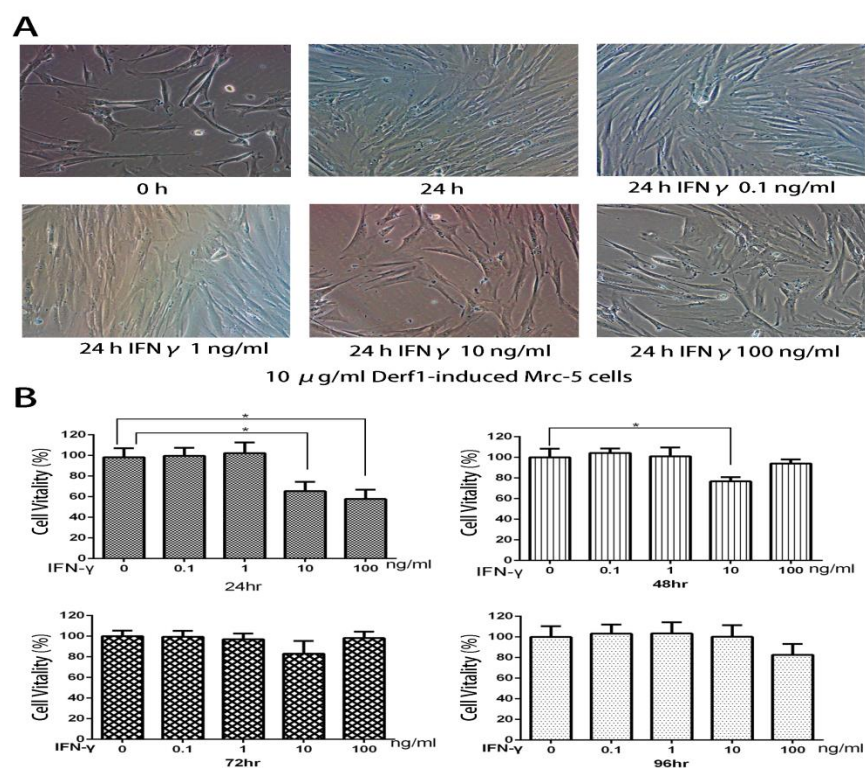


Figure3. Effects of IFN- γ on the cell proliferation and vitality of Derf1-induced Mrc-5 cells,

A. Morphological changes of Derf1-induced Mrc-5 cells under phase contrast microscope treated with different concentrations of IFN- γ for 24 h ($\times 100$), B. Effects of IFN- γ on cell viability of Derf1-induced Mrc-5 cells at different time points * $P < 0.05$

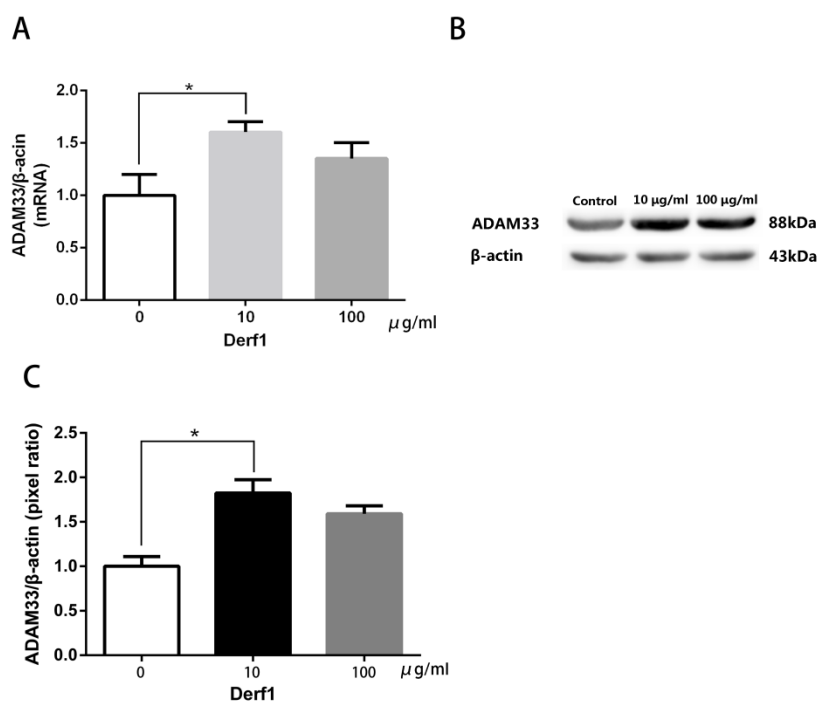


Figure4. Effects of Derf1 on the expression of the ADAM33 gene in Mrc-5 cells, A. Effects of Derf1 on the expression of ADAM33 mRNA in Mrc-5 cells * $P < 0.05$, B-C. Effects of Derf1 on the expression of ADAM33 protein in Mrc-5 cells * $P < 0.05$

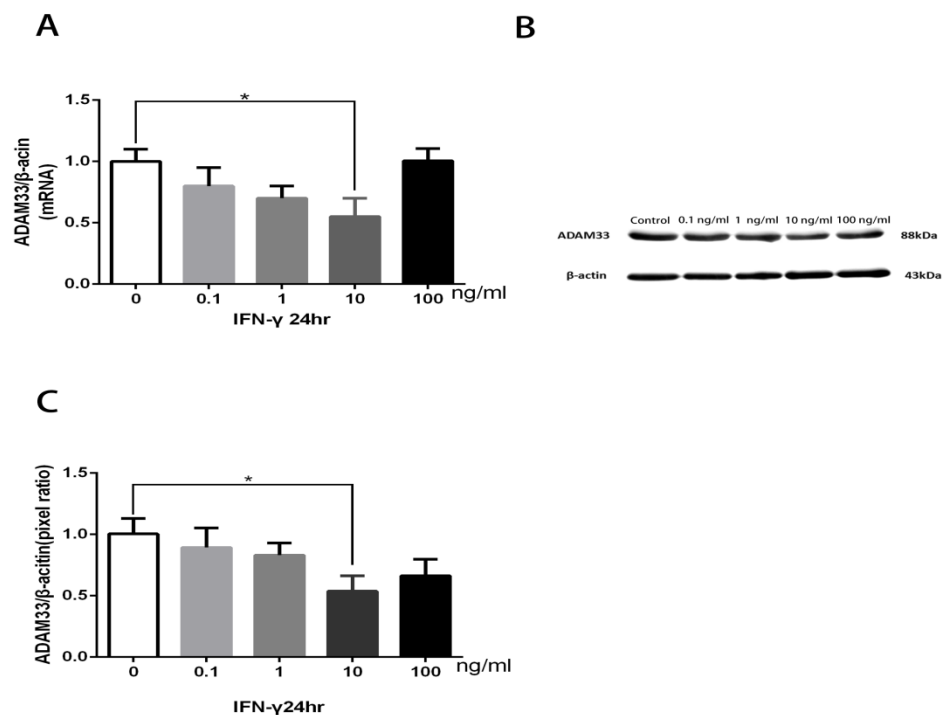


Figure5. Effects of IFN-γ on the expression of the ADAM33 gene in Mrc-5 cells, A. Effects of IFN-γ on the expression of ADAM33 mRNA in Mrc-5 cells * $P < 0.05$, B-C. Effects of IFN-γ on the expression of ADAM33 protein in Mrc-5 cells * $P < 0.05$

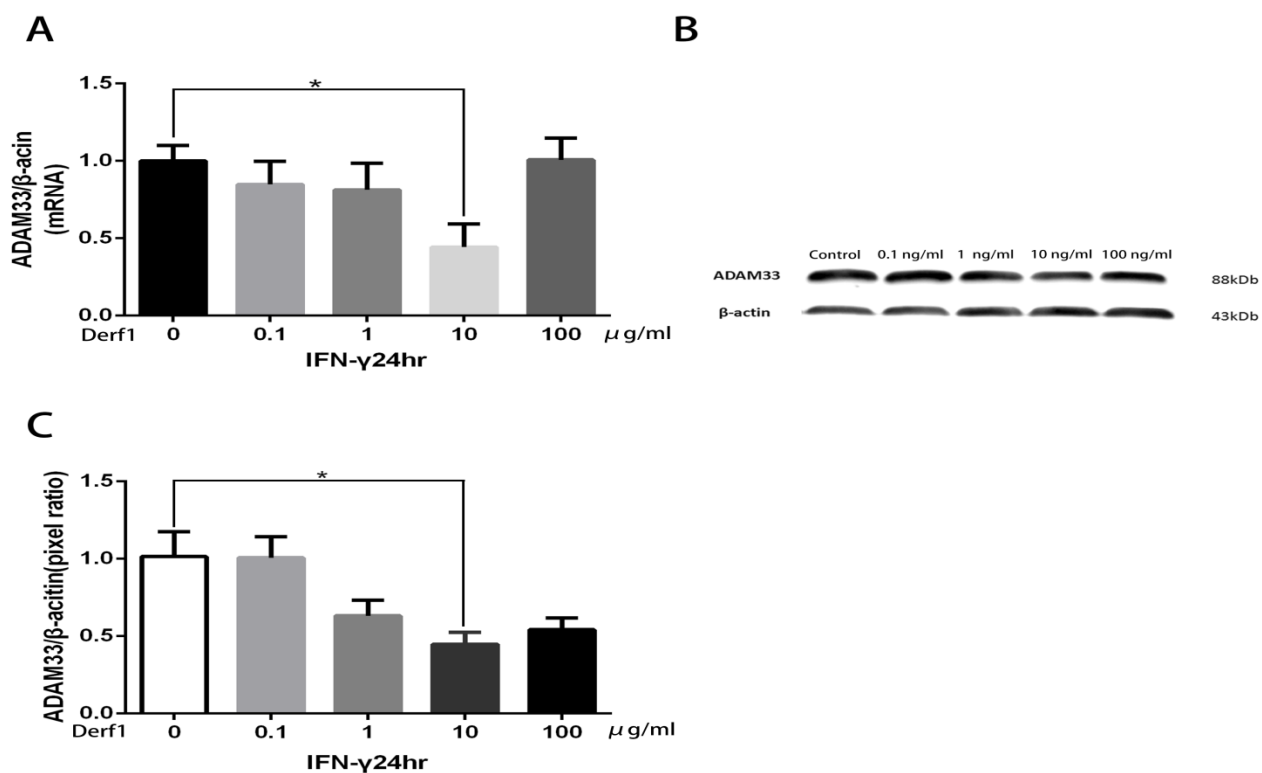


Figure6. Effects of IFN-γ on the expression of the ADAM33 gene in Derf1-induced Mrc-5 cells, A. Effects of IFN-γ on the expression of ADAM33 mRNA in Derf1-induced Mrc-5 cells
 $*P < 0.05$, B-C. Effects of IFN-γ on the expression of ADAM33 protein in Derf1-induced Mrc-5 cells $*P < 0.05$

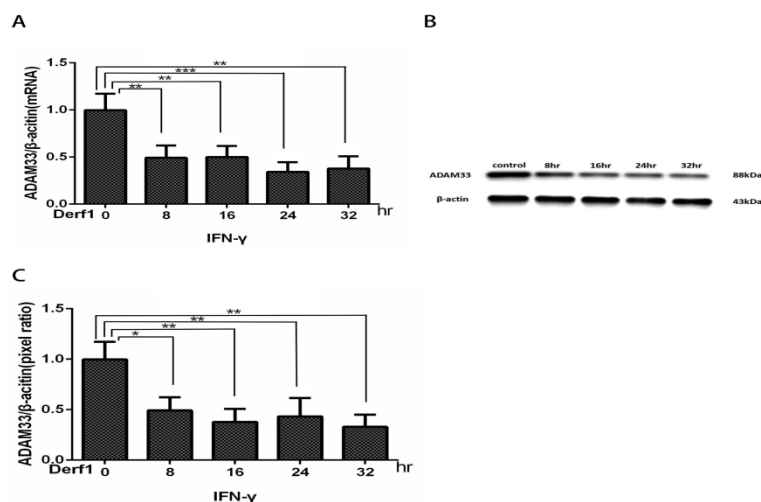


Figure7. Effects of IFN-γ on the expression of the ADAM33 gene in Derf1-induced Mrc-5 cells at different time points, A. Effects of IFN-γ on the expression of ADAM33 mRNA in Derf1-induced Mrc-5 cells at different time points * $P < 0.05$, ** $P < 0.01$, * $P < 0.001$, B-C. Effects of IFN-γ on the expression of ADAM33 protein in Derf1-induced Mrc-5 cells at different time points * $P < 0.05$**