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REDD1 mediates HDM-induced nuclear-cytoplasmic translocation and release of IL-33 in airway epithelial cells by downregulating Nrf2

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Abstract

Objective This study aims to investigate whether REDD1 (Regulated in Development and DNA Damage Responses 1) mediates the nuclear-to-cytoplasmic translocation and release of IL-33 in airway epithelial cells induced by house dust mites (HDM).

Methods REDD1 expression levels in bronchial asthma patients were validated using public databases, followed by immunohistochemical analysis of REDD1 protein in airway epithelial cells from these patients. An asthma model was then established using HDM-induced 16HBE cell lines, with REDD1 gene knockout performed. The relationship between varying levels of REDD1 expression, Nrf2, and related inflammatory factors was assessed using Western blot and qPCR. To further investigate the role of the REDD1-Nrf2-IL-33 axis in the development of asthma, we employed Nrf2 activators and inhibitors to reassess the impact of REDD1 on IL-33.

Results At both mRNA and protein levels, we found that REDD1 was significantly overexpressed in samples from asthma patients ($P < 0.05$). In vitro, 24-hour exposure to HDM induced a notable nuclear-to-cytoplasmic translocation of IL-33 and increased its levels in the culture medium of 16HBE cells. In addition, HDM treatment significantly upregulated the expression of both REDD1 and Nrf2. Knockdown of REDD1 markedly suppressed HDM-induced IL-33 release and the expression of TNF-α, IL-6, and IL-1β, while enhancing Nrf2 expression. Moreover, treatment with the Nrf2 agonist curcumin inhibited HDM-induced nuclear-to-cytoplasmic translocation and extracellular secretion of IL-33, whereas the opposite effect was observed when using the Nrf2 antagonist ML385.

Conclusion This study reveals the crucial regulatory role of the REDD1-Nrf2-IL-33 axis in the pathological process of bronchial asthma. REDD1 modulates the expression of IL-33 and other inflammatory factors through the Nrf2 signaling pathway, thereby influencing the onset and progression of asthma.

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Keywords REDD1, Nrf2, IL-33, Airway epithelium cells, Asthma

Introduction

Bronchial asthma is a widespread chronic inflammatory airway disease [1], characterized by hyperreactivity and inflammation of the airways, and manifested by recurrent symptoms such as wheezing, chest tightness, and difficulty breathing. Allergic asthma is the most common type, closely associated with airborne allergens such as house dust mites (HDM) [2]. In particular, moderate to severe persistent asthma significantly affects patients' quality of life and imposes a substantial economic burden on the public healthcare system [3, 4]. Furthermore, if asthma is not effectively controlled over the long term, it can lead to airway remodeling, further deteriorating the respiratory function of patients [5]. Therefore, early diagnosis and personalized treatment is of great importance for effective management of asthma [6, 7].

With the development of molecular biological techniques, multiple molecular markers associated with the progression and severity of asthma have been discovered [8]. In recent years, the airway epithelia-derived molecules has attracted increasing attention because of the fundamental role of airway epithelial cells in the pathogenesis of asthma as well as other airway diseases. When exposed to harmful stimuli including allergens, pathogens or pollutants the airway epithelial cells release a series of alarmins [9], such as IL-33 and TSLP, which could trigger downstream inflammatory cascades and facilitate the pathophysiological process of asthma [4]. Among these epithelial-derived alarmins, IL-33 has attracted special attention due to its dual functions in inflammatory responses. On one hand, nuclear IL-33 has been shown to inhibit inflammation [10]; on the other hand, extracellular IL-33 is a potent pro-inflammatory mediator [11]. Phase I and II clinical trials showed that monoclonal antibody itepekimab targeting extracellular IL-33 resulted in reduced blood eosinophil levels and improved lung function in patients with moderate to severe asthma, indicating that IL-33 is a promising therapeutic target for asthma [12, 13]. However, its upstream mechanisms are largely unknown.

REDD1 (Regulated in Development and DNA Damage Responses 1), also known as DDIT4 or rtp801, is an early response gene that regulates cellular responses to various stresses, including energy/nutrient deficiency, hypoxia, DNA damage, endoplasmic reticulum stress, and viral infection. It plays an important role in controlling cell growth, apoptosis, autophagy, and cancer development [14]. Recent data indicate that REDD1 is also involved in the regulation of immune and inflammatory responses. In a quiescent state, it is expressed at low levels in human tissues such as the lungs, bronchi, and kidneys [15], but is overexpressed in immune cells of patients with ulcerative colitis and systemic lupus erythematosus, as well as in the lungs of patients with emphysema [16–18]. Lack of REDD1 was shown to protect mice or cells against inflammatory responses in models of ulcerative colitis, allergic contact dermatitis, and emphysema [18, 19]. However, the role of REDD1 in asthma, especially whether it is involved in IL-33 release, remains unclear.

This study was aimed to evaluate the effects of REDD1 on IL-33 in airway epithelial cells exposed to HDM and to explore the underlying molecular mechanisms.

Materials and methods

Analysis of REDD1 expression in asthmatic tissues

Based on the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/gds), we retrieved gene expression profile data related to bronchial asthma and compared REDD1 expression levels among normal samples, asthma, and chronic obstructive pulmonary disease (COPD) patients, and explored the correlation between REDD1 and Nrf2, between REDD1 and inflammatory factors in asthma and COPD samples. Additionally, we also compared the expressed difference of REDD1 between mild asthma and severe asthma samples.

To validate our findings, we conducted immunohistochemical (IHC) analysis on airway epithelial tissue obtained via bronchoscopic biopsy from six asthma patients and three controls. Asthma samples were selected from subjects sensitive to house dust mites confirmed by either skin prick test or serum specific IgE measurement, and overall good disease control at the time of sample collection based on total asthma control test (ACT)score (≥ 20 points). Subjects were excluded if they had smoking history more than 10 packyears. Control samples were selected from early stage of peripheral lung cancer subjects who was required bronchoscopy to confirm staging. Airway epithelial tissue biopsy specimens are taken from lower segmental bronchus in asthma patients and from the healthy side segmental bronchus in control subjects. All patients provided informed consent prior to undergoing biopsy, and the study was approved by the Ethics Committee of Zhongshan People's Hospital (approval number: K2020-14) in accordance with the Declaration of Helsinki.

Immunohistochemistry

Transbronchial lung biopsy specimens were fixed in formalin.Samples were dehydrated in 50%, 70% and 90% absolute alcohol, cleared in xylene. The sections were

dewaxed and rehydrated, and antigen retrieval was performed with 10-mM sodium citrate (pH 6.1). Serial 4-μm sections were immunostained using a rabbit polyclonal antibody against REDD1 (1:200) (Proteintech, China) overnight at 4 ℃ and then processed with the corresponding second antibodies/horseradish peroxidase (1:100) for 60 min at room temperature.The nuclei were counterstained with hematoxylin. The intensity of labeling was evaluated in a blind manner by 2 independent investigators and graded by using a 5-scale system [20] (0, no signal; 1, weak; 2, moderate; 3, strong; 4, very strong).

Cell culture and treatments

Human bronchial epithelial cell line 16HBE (BioRad Laboratories (Shanghai) Co, Ltd, ATCC, Portland, Oregon) were cultured in RPMI-1640 medium (Gibco) containing 10% fetal calf serum (Gibco) at 37 ℃ and 5% CO_2. The culture medium was replaced every 48 h, and cells were passaged at a ratio of 1:3 when they reached 80–90% confluence, then reseeded into new culture dishes. When the density of passaged 16HBE cells reached approximately 80–90%, about 2.0 × 10^6 cells per 6-well plates, they were exposed to serum-free medium containing 10 μg/mL HDM extract (Greer Laboratories, United States) and incubated for 24 h [21].

Cell transfection and pharmacological treatment

The siRNAs targeting REDD1 (si-REDD1-1: 5′-CCAGGUGGGCAAAGAACUA-3′, 5′-UAGUUCUUUGCCCACCUGG-3′; si-REDD1-2: 5′-CCUGAGGAUGAACACUUGU-3′, 5′-ACAAGUGUUCAUCCUCAGG-3′; si-REDD1-3: 5′-CGGAGGAAGACACGGCUUA-3′, 5′-UAAGCCGUGUCUUCCUCCG-3′) were synthesized (Tsingke Bio, Beijing, China). 16HBE cells were transfected by these siRNAs using Lipofectamine™ 3000 transfection reagent (Thermo Fisher Scientific) according to the manufacturer's protocol. In brief, cells were seeded into a 6-well plate and cultured overnight. When cell confluence reached 70–80%, transfection was performed. The efficiency was tested 48 h after transfection. The Nrf2 activator (Curcumin, HY-N0005) and the inhibitor (ML385, HY-100523) were purchased from MCE. Curcumin and ML385 were separately diluted in DMSO and PBS before use.

Western blot (WB)

Total protein extracts were obtained by lysing cultured 16HBE cells in RIPA total protein extraction lysis buffer (Bioworld Technology, Nanjing, China). The protein extracts were subjected to SDS-polyacrylamide gel electrophoresis for separation. Then, the separated proteins were transferred onto polyvinylidene fluoride (PVDF) membranes. After blocking, the membranes were probed with primary antibodies and then incubated with secondary antibodies. The bands were visualized by the enhanced chemiluminescence (ECL) method (Amersham Biosciences) and analyzed by ImageJ. The primary antibodies used in the experiments were as follows: REDD1 (1:1000; Abclonal, China), β-actin (1:10000; Wuhan Sanying, Wuhan, China), Nrf2 (1:1000; Abcam, Cambridge, UK), IL-33 (1:1000; Abclonal, China), and HO-1 (1:1000; Abclonal, China).

Quantitative PCR (qPCR)

Total RNA was extracted by lysing cultured 16HBE cells in- Total RNA Extractor Trizol (Sangon Biotech, Shanghai, China). Using 1 μg of RNA in 10 μl of reaction buffer containing 5 × Evo M-MLV RT Master Mix (Accurate Biology, Hunan, China) and random primers, the first strand of complementary DNA was generated by sequentially heating the mixture to 37 ℃ for 15 min and 85 ℃ for 5 s. Real-time PCR was performed using a Platinum SYBR Green Premix Pro Taq HS qPCR Kit (Accurate Biology, Hunan, China). The relative abundance of each gene (IL-6, IL-1β, and TNF-α) was determined by the comparative CT (cycle threshold) value normalized against the CT value of β-actin. The primer sequences used shown as follows: IL-6: forward 5′-ACTCACCTCTTCAGAACGAATTG-3′, reverse 5′-CCATCTTTGGAAGGTTCAGGTTG-3′; IL-1β: forward 5′-ATGATGGCTTATTACAGTGGCAA-3′, reverse 5′- GTCGGAGATTCGTAGCTGGA-3′; TNF-α: forward 5′-TATCCTGGGGGACCCAATGT-3′, reverse 5′-AAAAGAAGGCACAGAGGCCA-3′; β-actin: forward 5′- CTCGCCTTTGCCGATCC-3′, reverse 5′- ATCCTTCTGACCCATGCCC-3′.

Immunofluorescence

Cells were treated as aforementioned and harvested, then fixed with paraformaldehyde. Permeabilization and blocking were done before incubation with primary antibody against IL-33 (dilution 1:100, prepared in 5% BSA), followed by incubation with Alexa Fluor 488-labeled goat anti-rabbit antibody. Cells were then counterstained using DAPI, and imaged on a confocal microscope LSM980.

Statistical analysis

The data were from at least three independent experiments. The difference analysis of bioinformatics is based on the 'limma' package, which is an important foundation. Continuous variables were evaluated for a normal distribution using the Kolmogorov-Smirnov test. Normally distributed variables were presented as the mean ± SD. Non-normally distributed variables are presented as either medians (Median[Minimum-Maximum). The statistical differences were analyzed by one-way analysis of variance (ANOVA) and post-hoc Tukey test.

$P < 0.05$ was considered statistically significant. Statistical analyses were performed using GraphPad Prism 8.0.

Results

REDD1 expression is increased in asthma patients

Based on RNA sequencing data analysis, we discovered that REDD1 gene expression is significantly upregulated in sputum samples from patients with asthma and chronic obstructive pulmonary disease (COPD), with statistically significant differences between groups (Fig. 1A). This finding strongly suggests that REDD1 may play a crucial role in the pathological processes of these respiratory diseases. Further correlation analysis revealed an intriguing phenomenon: in COPD patients, REDD1 expression levels showed a significant positive correlation with type 1 inflammation-related factors (such

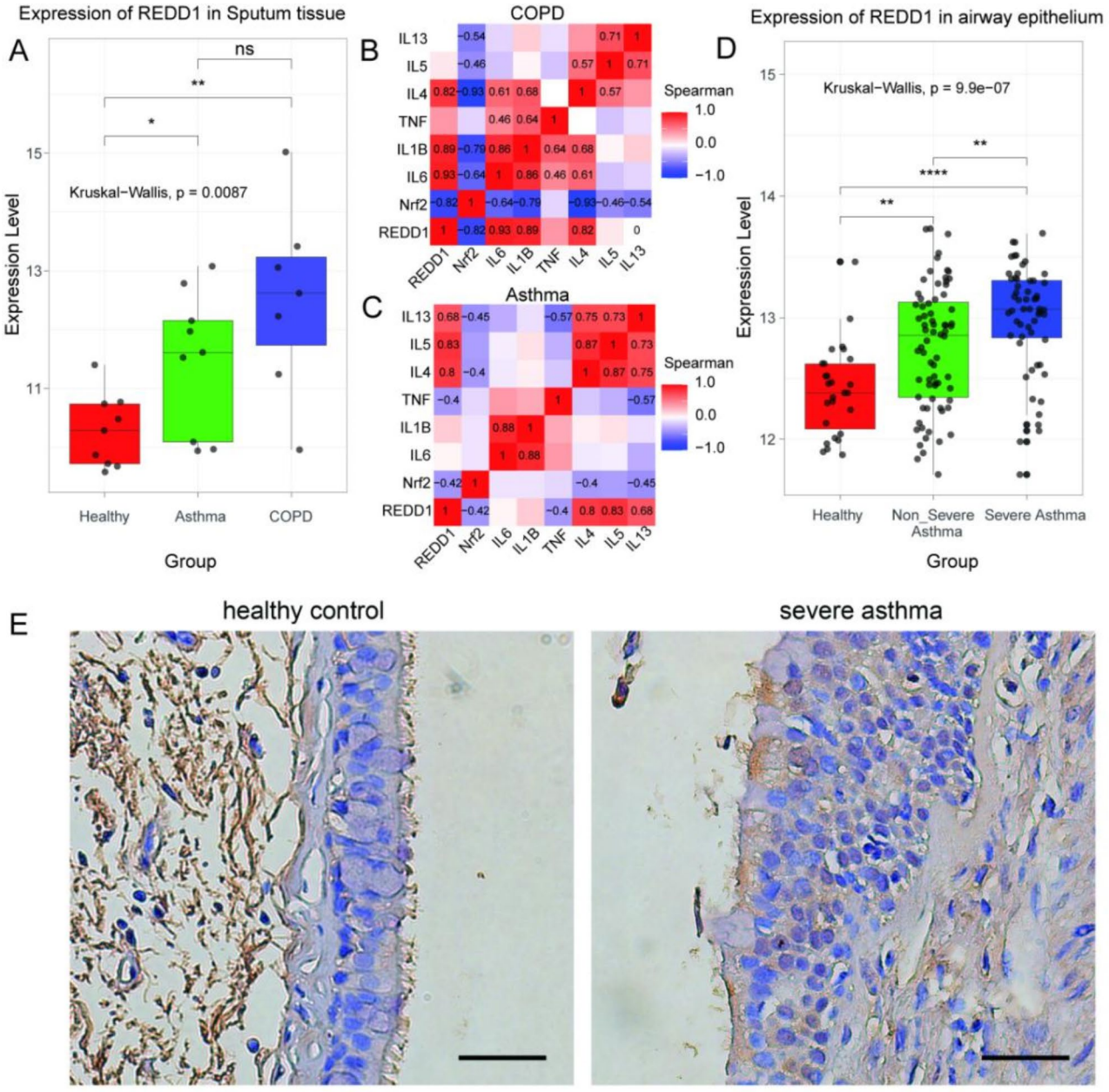

Fig. 1 REDD1 expression is increased in asthma patients. REDD1 is significantly upregulated in respiratory-related diseases, positively correlates with inflammation, and negatively correlates with Nrf2. (**A**) Expression levels of REDD1 in sputum samples from asthma and COPD patients; (**B**) Correlation heatmap of REDD1 with Nrf2 and inflammatory factors in sputum samples from COPD patients; (**C**) Correlation heatmap of REDD1 with Nrf2 and inflammatory factors in sputum samples from asthma patients; (**D**) Expression levels of REDD1 in non-severe and severe asthma patients; (**E**) Representative immunohistochemical staining of REDD1 in the bronchial mucosal of patients with asthma as well as healthy controls. Scale bar, 100 μm

as IL6, TNF, and IL1B) (Fig. 1B); whereas in asthma patients, REDD1 exhibited a marked positive correlation with type 2 inflammation factors (such as IL4, IL5, and IL13) (Fig. 1C). Notably, in both patient groups, REDD1 expression demonstrated a strong negative correlation with the Nrf2 factor (Fig. 1B-C), potentially indicating REDD1's role in regulating antioxidant responses. To further validate these findings, we subsequently analyzed sequencing data from bronchial epithelial cells of asthma patients. The results reaffirmed the significant upregulation of REDD1 in diseased tissues, with its expression levels correlating with asthma severity (Fig. 1D). Mucosal biopsy samples from asthmatic patients and control subjects were obtained for immunohistochemical analysis of REDD1, as can be seen in Fig. 1E, there was almost no positive staining of REDD1 in the bronchial epithelia of healthy controls, but diffused expression was found in the bronchial epithelial of asthma patients. The clinical characteristics for these patients were provided in Table S1. This accumulating evidence strongly supports the hypothesis that REDD1 may play a key role in the pathogenesis of respiratory diseases, particularly asthma and COPD. This discovery not only deepens our understanding of the molecular mechanisms underlying these diseases but also provides an important theoretical basis for future development of therapeutic strategies targeting REDD1.

HDM promotes nuclear-cytoplasmic translocation of IL-33 in 16HBE, but not total protein level of IL33

We evaluated changes of IL-33 protein expression in 16HBE cells before and after HDM stimulation. Across various concentrations of HDM stimulation (0–40 μg/mL), we did not observe any significant changes of whole-cell IL-33 levels in 16HBE cells (Fig. 2A). Subsequently, we selected a concentration of 10 μg/mL HDM to stimulate 16HBE cells for 6, 12, and 24 h to examine the time-dependent effect of HMD on IL-33. Similarly, no statistically significant differences were found (Fig. 2B). These results suggest that HDM did not directly affect the total protein level of IL-33 in 16HBE.

To investigate whether HDM could alter the subcellular distribution of IL-33, we extracted cytoplasmic and nuclear proteins from 16HBE cells treated with 10 μg/mL HDM. The results showed that after 6 h of stimulation, nuclear IL-33 level significantly decreased (Fig. 2C), while the cytoplasm level markedly increased (Fig. 2D). Similar results were found 12 h post HDM treatment, though the expression pattern did not differ significantly from that at 6th hour. These data suggest that HDM induces the translocation of IL-33 from the nucleus to the cytoplasm, a process that starts in the early stages of exposure and persists over time. This sustained nuclear-cytoplasmic translocation of IL-33 not only reveals its significant pathophysiological role in the development and progression of asthma but also highlights its value as a potential therapeutic target.

HDM induces IL-33 secretion and increases expression of REDD1 and Nrf2 in 16HBE cells

Western blot analysis revealed a time-dependent increase in REDD1 and Nrf2 protein levels (Fig. 3A). At the same time, we examined IL-33 levels in the cell culture supernatant and found a significant time-dependent increase (Fig. 3B). These results indicate that HDM can induce upregulation of REDD1 and Nrf2 in 16HBE cells and promote the secretion of the inflammatory cytokine IL-33.

Knocking down REDD1 alleviates HDM-induced IL-33 release and inflammatory responses in 16HBE cells

We designed and constructed siRNA plasmids targeting REDD1 (siREDD1-1, siREDD1-2, and siREDD1-3) and transfected them into HDM-treated 16HBE cells. REDD1 expression was subsequently assessed using WB assay. Results demonstrated reduced REDD1 expression in all transfected cells, with siREDD1-2 and siREDD1-3 exhibiting more pronounced suppression compared to siREDD1-1 (Fig. 4A, B). Based on these findings, siREDD1-2 was selected for subsequent experiments. Additionally, we found that the siREDD1 group showed significantly reduced levels of IL-33 in the cell culture supernatant, as well as decreased mRNA expression of TNF-α, IL-6, and IL-1β (Fig. 4C, D). These results suggest that silencing REDD1 could attenuate HDM-induced IL-33 release and inflammatory responses in airway epithelial cells.

Knocking down REDD1 enhances Nrf2 signaling in HDM-treated 16HBE cells

To elucidate the potential mechanism underlying the protective effect of REDD1 knockdown in HDM-induced 16HBE cells, we detected the expression of Nrf2. As shown in Fig. 5, the protein levels of Nrf2 and its target gene HO-1 significantly increased after REDD1 knockdown. This evidence suggests that REDD1 may regulate cellular antioxidant and anti-inflammatory responses by inhibiting the Nrf2 signaling pathway.

Nrf2 can negatively regulate the nuclear cytoplasmic translocation and release of IL-33, as well as inflammatory response in HDM -treated 16HBE

To further elucidate the role of the Nrf2 signaling pathway in regulating HDM-induced inflammatory responses, we administered an Nrf2 activator (Curcumin, 20 μM) to HDM-induced 16HBE cells. We observed that Nrf2 protein level was significantly elevated by Curcumin, while the expression of IL-33 protein in the cell culture supernatant was markedly reduced (Fig. 6A).

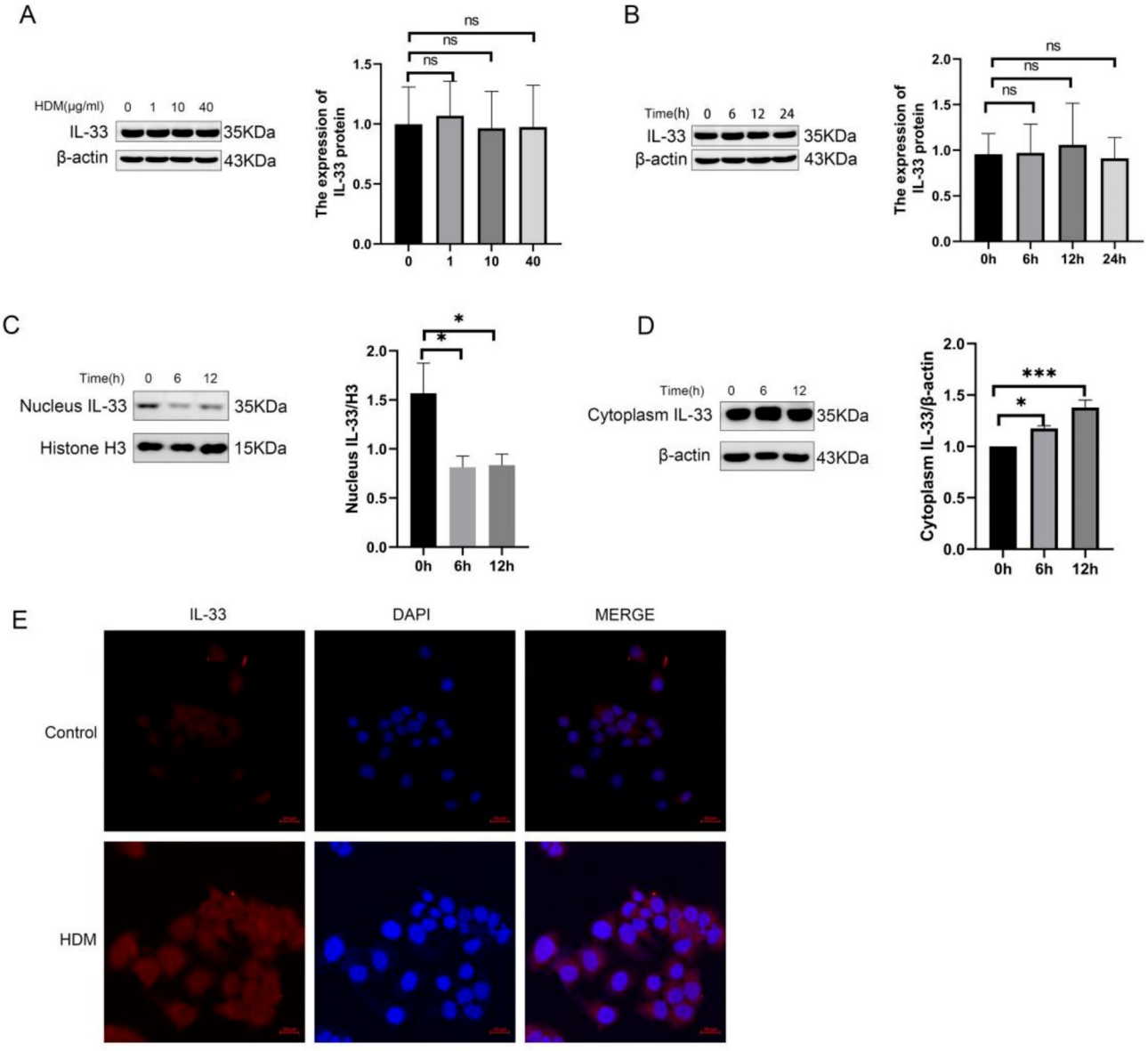

Fig. 2 HDM promotes nuclear-cytoplasmic translocation of IL-33 in 16HBE. **(A-B)** Total IL-33 protein levels measured by Western blot across different HDM concentrations (0–40 μg/mL) and time points (0, 6, 12, 24 h). **(C-D)** Changes in nuclear and cytoplasmic IL-33 protein levels in 16HBE cells stimulated with HDM (10 μg/mL) at different time points (0 h, 6 h, 12 h), detected by Western blot. (**E**) Immunofluorescence detection of IL-33 expression and distribution in 16HBE cells after 12 h of HDM (10 μg/mL) stimulation.*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Additionally, qPCR analysis confirmed that the mRNA expression levels of IL-6, TNF-α, and IL-1β were also significantly decreased after Curcumin treatment (Fig. 6B). Conversely, when we added an Nrf2 inhibitor (ML385, 5 μM) to HDM-induced 16HBE cells, we observed opposite effects. Ml385 significantly reduced Nrf2 protein expression, increased the level of IL-33 in the cell culture supernatant (Fig. 6C), and elevated the mRNA levels of IL-6, TNF-α, and IL-1β (Fig. 6D). Immunofluorescence of IL-33 revealed similar findings (Fig. 6E). After adding the Nrf2 activator to HDM-induced 16HBE cells, IL-33 distribution decreased in the cytoplasm while increased in the nucleus. Conversely, when the Nrf2 inhibitor was added, IL-33 distribution significantly increased in the cytoplasm and decreased in the nucleus. These suggested that Nrf2 can negatively regulate the nuclear cytoplasmic translocation and release of IL-33, and downregulate inflammatory cytokine expression in HDM-treated 16HBE cells.

Knocking down REDD1 regulates IL-33 release through activating Nrf2

To verify whether REDD1 knockdown inhibits IL-33 release by upregulating Nrf2 activity, we applied the Nrf2

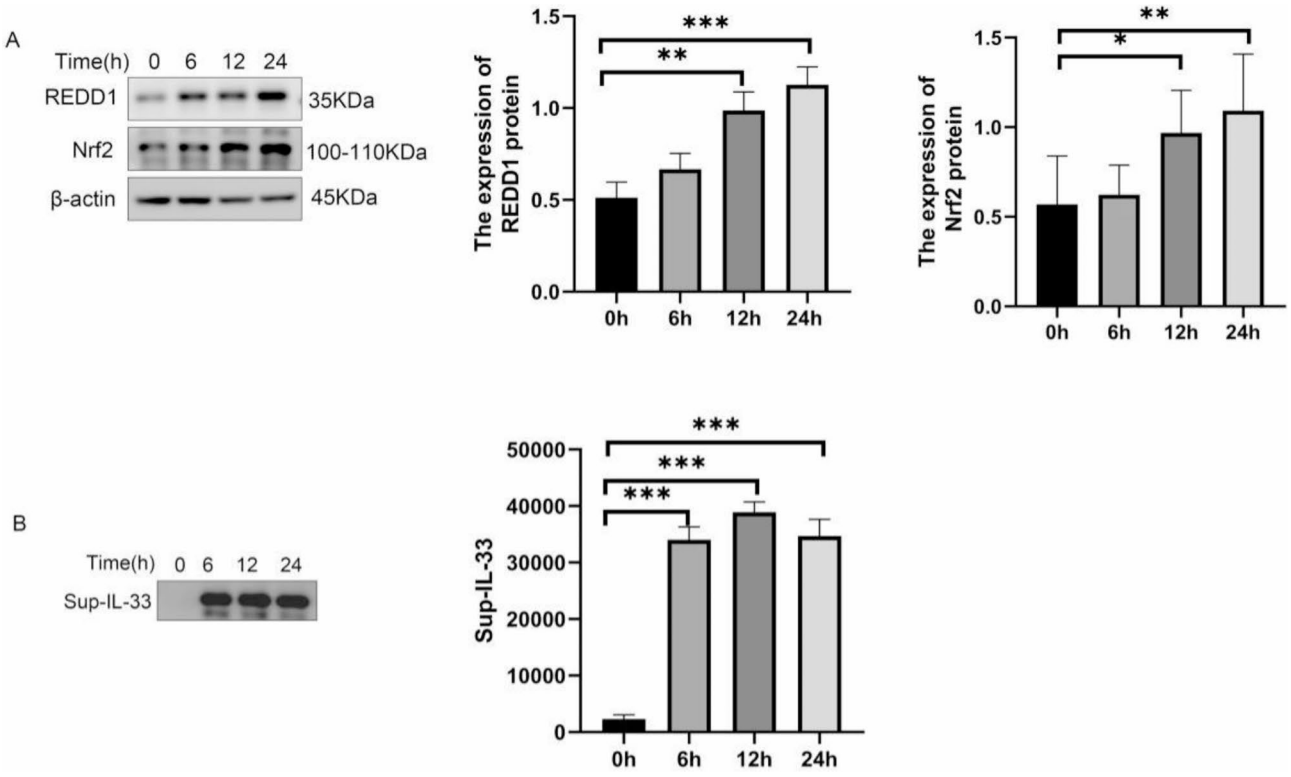

Fig. 3 HDM induces IL-33 secretion and increases expression of REDD1 and Nrf2 in 16HBE cells. **(A)** Detection of REDD1 and Nrf2 protein levels by Western blot in 16HBE cells stimulated with HDM (10 μg/mL) for 0 to 24 h; **(B)** Detection of IL-33 protein levels in the supernatant by western blot after stimulation of 16HBE cells with HDM (10 μg/mL) for 0 to 24 h. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

inhibitor ML385 to REDD1-knockdowned cells and compared the relationship between Nrf2 activity and IL-33 release. Results showed that in HDM-induced 16HBE cells with REDD1 knocked down, Nrf2 expression was significantly elevated, indicating enhanced Nrf2 activity (Fig. 7A), while IL-33 release was decreased (Fig. 7B). However, after ML385 treatment, Nrf2 expression was markedly reduced, suggesting inhibition of Nrf2 activity (Fig. 7A), and IL-33 release was significantly increased (Fig. 7B). These findings suggested that REDD1 knockdown regulates IL-33 release through activation of the Nrf2 signaling pathway.

Discussion

In this study, we found that REDD1 expression is upregulated in the airway epithelial cells of asthmatic patients, and demonstrated in vitro that REDD1 mediates HDM-induced nuclear-to-cytoplasmic translocation and secretion of IL-33, which partly depends on inhibition of the Nrf2 pathway.

The airway epithelial cell plays a fundamental role in the initiation and perpetuation of type 2 allergic airway inflammation in asthma [22]. It can produce a range of mediators to promote and regulate the immune response, such as IL-33, which can trigger and facilitate type 2 inflammation by activating ST2, a receptor expressed in a variety of immune cells including mast cells and type 2 innate lymphoid cells(ILC2) [23, 24]. Although IL-33 is constitutively expressed and stored within the nucleus [25], it can also translocate into the cytoplasm and be released to the extracellular milieu either passively (which occurs upon cell death) or actively [26, 27]. Unfortunately, little is known about how it is mobilized and released extracellularly, especially the active pathway. Like most IL-1 family members, IL-33 lacks an N-terminal signal peptide and therefore bypasses the classical secretory pathway composed of the endoplasmic reticulum and Golgi apparatus [28]. Dysregulation of several signalings have been shown to be associated with IL-33 release from the airway epithelium. Du J et al. found that PTRF positively regulates IL-33 expression and its uncontrolled release from bronchial epithelial cells [29]. In agreement with the findings of Chen W et al. [30], in this study, we also demonstrated allergen-induced nuclear-cytoplasmic translocation and secretion of IL-33 in 16HBE cells, confirming the critical role of IL-33 for driving allergic airway inflammation in asthma. Therefore, elucidating the upstream mechanisms that orchestrate IL-33 secretion would improve our understanding of the disease.

REDD1 is mainly localized in cytosol and mitochondria, and widely expressed in healthy human tissues

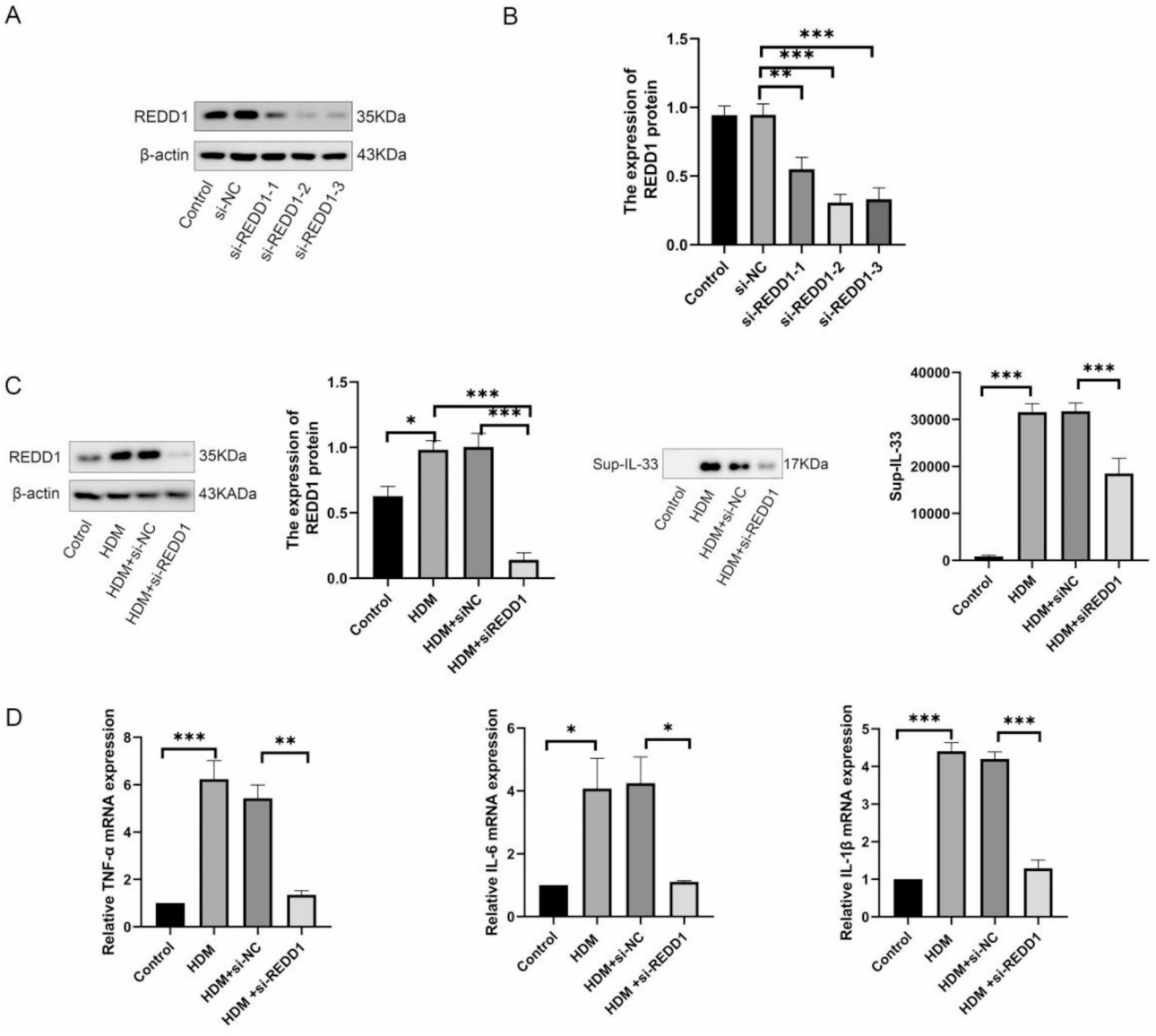

Fig. 4 Knocking down REDD1 alleviates HDM-induced IL-33 release and inflammatory responses in 16HBE cells. **(A-B)** Expression of REDD1 in 16HBE cells transfected with control, si-NC, si-REDD1-1, si-REDD1-2, or si-REDD1-3, detected by Western blot. **(C)** The effect of reduced REDD1 expression on IL-33 protein levels in the cell supernatant, determined by Western blot. **(D)** The effect of reduced REDD1 expression on TNF-α, IL-6, and IL-1β mRNA levels, quantified by qPCR. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

[14]. Various pathological and physiological stress conditions, including metabolic imbalance, hypoxia, inflammation, stress-related hormones, and aging can upregulate REDD1 [31]. Increased expression of REDD1 has been found in immune, endothelial and epithelial cells of experimental mice exposed to lipopolysaccharides (LPS), cigarette smoke condensate, oxazolone, viruses, and etc [19, 32, 33]. A number of studies have demonstrated that knockdown or knockout of REDD1 offered protection against injury, apoptosis, oxidative stress and inflammation both in vivo and in vitro, suggesting that REDD1 is a potent inflammatory mediator. Recently, Gress C and colleagues reported that REDD1 expression was significantly elevated in ILC2 cells of asthma patients [34, 35]. In line with these, here we found that asthma patients had higher expression of REDD1 in the airway epithelial cells than the healthy volunteers. Significant upregulation of REDD1 was also observed in in vitro cultured bronchial epithelia treated with HDM. Silencing REDD1 not only dramatically inhibited HDM-induced IL-33 secretion, but also suppressed overexpression of inflammatory cytokines including IL-6, TNF-α, and IL-1β. These indicate that REDD1 mediates epithelial IL-33 secretion and contributes to allergen-induced airway inflammation in asthma.Reduced expression of

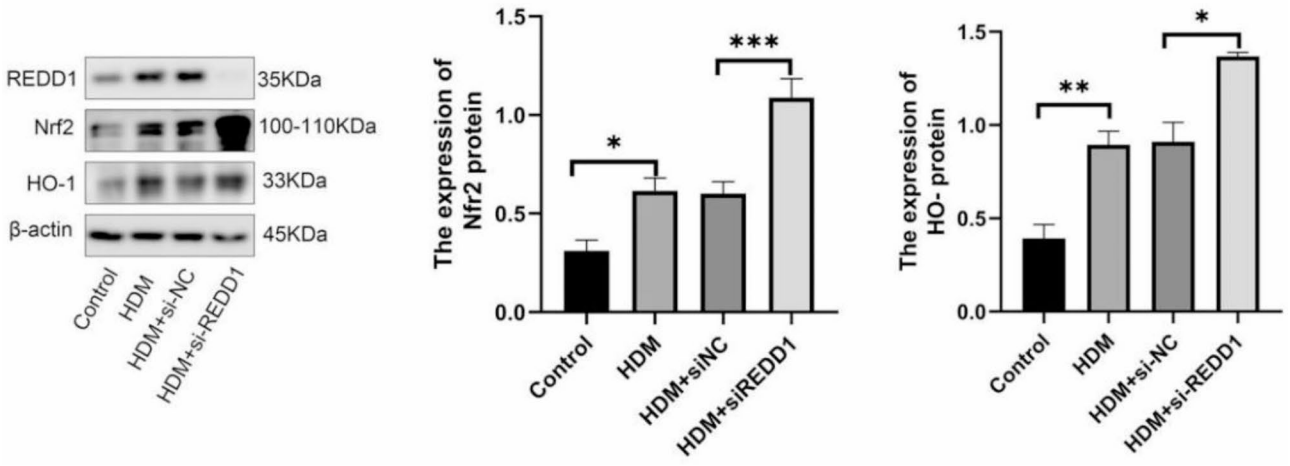

Fig. 5 Knocking down REDD1 enhances Nrf2 signaling in HDM-treated 16HBE cells. The effect of 24-hour incubation with HDM (10 μg/mL) on Nrf2 and HO-1 protein levels in 16HBE cells with reduced REDD1 expression, determined by Western blot.*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

REDD1 can prevent high glucose-induced podocyte apoptosis, oxidative stress, and inflammatory injury, and it has been observed in various models that knockout of REDD1 can attenuate inflammatory responses to stimuli such as LPS and cigarette smoke, reducing resulting tissue damage [17, 36, 37].

Various signaling pathways are engaged in REDD1-mediated inflammatory responses in respiratory diseases. One of these is Nrf2, a transcription factor that exerts powerful anti-oxidative and anti-inflammatory functions [38]. Evidence showed that REDD1 deficiency or inhibition can enhance Nrf2 activity, thereby improving cellular antioxidant capacity [39–41], indicating a close relationship between Nrf2 and REDD1. In the current study, we observed that knocking down REDD1 upregulated Nrf2 expression in the airway epithelia, supporting the interplay between REDD1 and Nrf2. To further elucidate this relationship, we employed Nrf2 activator and inhibitor in our study to investigate the regulatory effects of Nrf2 on IL-33 and other pro-inflammatory mediators. The results showed that Nrf2 activator significantly dampened HDM-induced nuclear-cytoplasmic translocation and extracellular secretion of IL-33 as well as inflammatory response in the airway epithelia while Nrf2 antagonist strengthened these effects. This implied that Nrf2 is a negative regulator of HDM-induced airway epithelial IL-33 translocation and release. Our results not only confirmed a significant regulatory relationship between Nrf2 activity and key inflammatory factors such as IL-6, TNF-α, and IL-1β, but also revealed a close association with IL-33 nuclear-cytoplasmic translocation. Collectively, these findings strongly suggest that REDD1 and Nrf2 play crucial roles in the pathogenesis of asthma, with their interaction likely serving as a key regulatory node in airway inflammatory responses.

There are several limitations of this study. While we conducted validations at multiple levels (patient data, tissue samples, and cellular models), the primary mechanistic investigations were concentrated on in vitro cell models. These models may not fully replicate the complex pathophysiological environment in vivo. Moreover, the number and diversity of patient samples used in the study may be limited. Differences among patients of various ages, genders, ethnicities, or asthma phenotypes may exist. Although our study provides a theoretical basis for new treatment strategies, the translation from basic research to clinical application still faces numerous challenges.

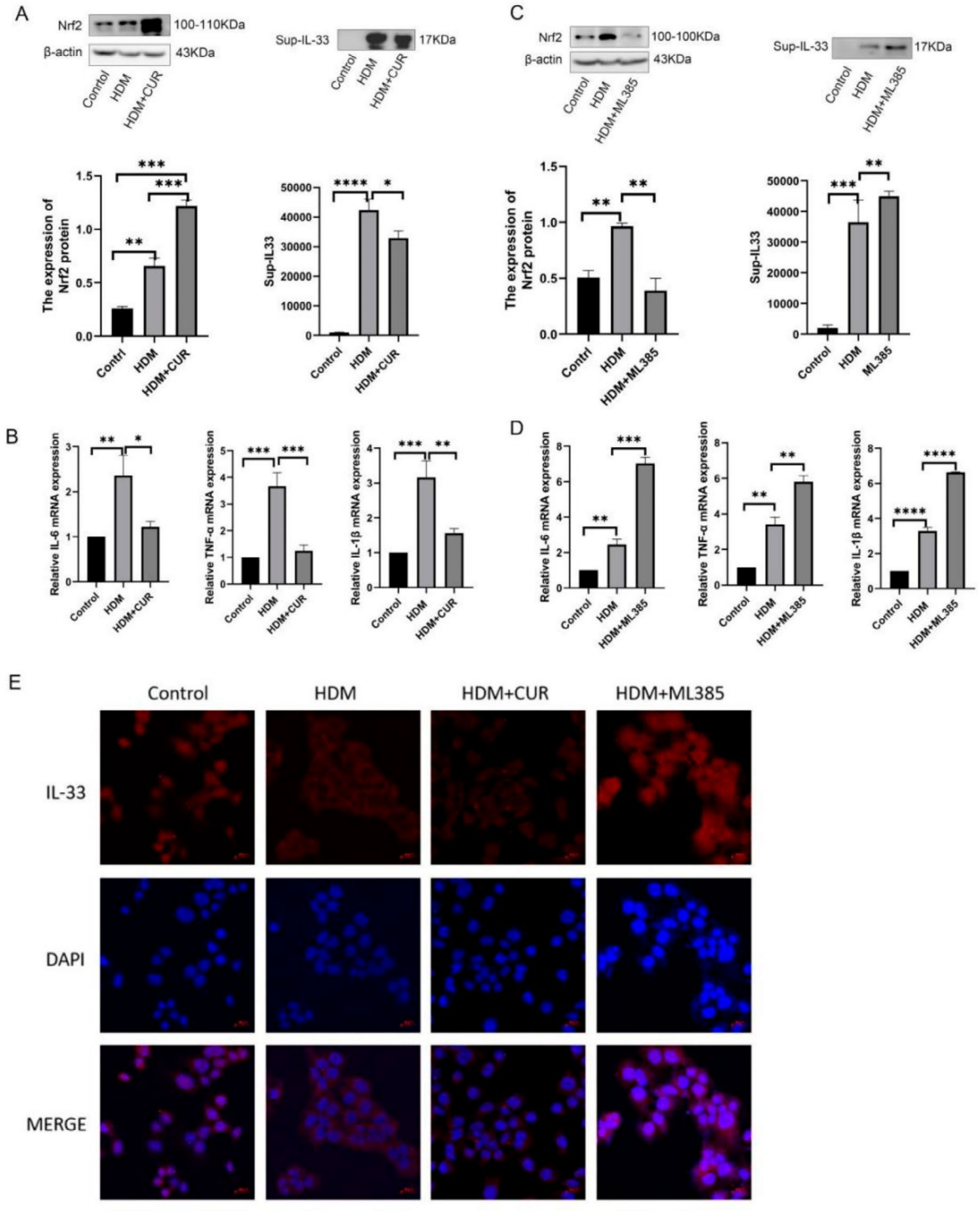

Fig. 6 Nrf2 can negatively regulate the nuclear cytoplasmic translocation and release of IL33, as well as inflammatory response in HDM -treated 16HBE. **(A)** Pre-treatment of 16HBE cells with 20 μM curcumin for 4 h, followed by stimulation with 10 μg/mL HDM for 20 h. Nrf2 protein expression was detected by Western blot. **(B)** The expression of pro-inflammatory cytokines IL-6, TNF-α, and IL-1β in 16HBE cells treated with HDM before and after the use of curcumin was quantified by qPCR. **(C)** Pre-treatment of 16HBE cells with 5 μM of the Nrf2 inhibitor ML385 for 2 h, followed by stimulation with 10 μg/mL HDM for 22 h. Nrf2 protein expression was detected by Western blot. **(D)** The expression of pro-inflammatory cytokines IL-6, TNF-α, and IL-1β in 16HBE cells treated with HDM before and after the use of ML385 was quantified by qPCR. **(E)** Pre-treatment of 16HBE cells with the Nrf2 activator curcumin and the Nrf2 inhibitor ML385, followed by stimulation with HDM (10 μg/mL), and detection of changes in the expression and distribution of IL-33 in 16HBE cells by immunofluorescence.*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

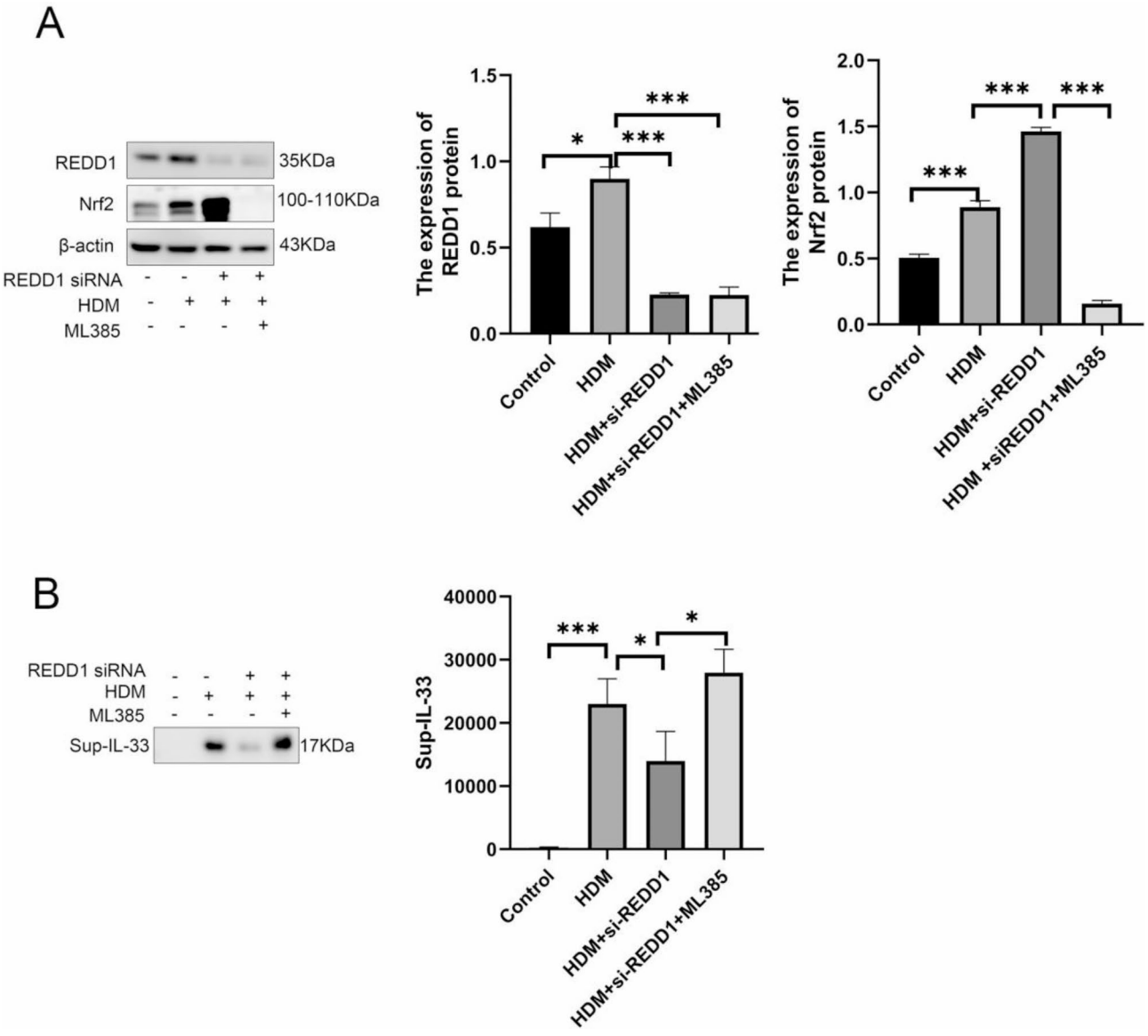

Fig. 7 Knocking down REDD1 regulates IL-33 release through activating Nrf2. **(A-B)** After knockdown of REDD1 with small molecule inhibitors, cells were pretreated with ML385 followed by stimulation with HDM, and the expression of IL-33 in the supernatant was detected by Western blot.*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Conclusion

This study reveals the crucial regulatory role of the REDD1-Nrf2-IL-33 axis in the pathological process of bronchial asthma. REDD1 modulates the expression of IL-33 and other inflammatory factors through the Nrf2 signaling pathway, thereby influencing the onset and progression of asthma. This evidence not only deepens our understanding of the molecular mechanisms of asthma but also provides a theoretical basis for proteins in REDD1-Nrf2-IL-33 axis as a potential diagnostic biomarker and therapeutic target, offering promise for improved clinical management of asthma.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12931-025-03119-7.

Supplementary Material 1

Supplementary Material 2

Author contributions

T.L. wrote the original manuscript, conducted experiments, and analyzed data; WT.J., YX.G., and LC.C. conducted experiments, analyzed data, and visualized the results; C.L., and DD.Z. validated the results; X.L. and YH.L. contributed to the study conception and design. All authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethical approval
was obtained from the Ethics Committee of Zhongshan People's Hospital (approval number: K2020-14).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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