IL-13R130Q modulates human airway smooth muscle cell phenotype to contribute to severe asthma

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Research Article

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Abstract

Background

A small percentage of asthma patients experience severe and refractory asthma that responds poorly to standard therapy. However, this percentage is increasing and better treatment options are therefore needed. Our previous study showed that cultured human airway smooth muscle cells treated with IL-13 R130Q, a naturally occurring IL-13 variant, displayed enhanced functional activities compared to wild-type (WT) IL-13. The present study aims to determine whether IL-13 R130Q could modulate the ASMC phenotype to further contribute to severe asthma.

Methods

Human bronchial smooth muscle cells (hBSMCs) were treated with increasing concentrations of IL-13R130Q or WT IL-13 for 24 h. Endogenous expressions of IL-5 and IL-4 were characterized with Enzyme-linked immunosorbent assay analysis. Acetylcholine-induced changes in intracellular calcium concentration were analyzed with the fluorescent probe Fluo-3/AM with flow-cytometric analysis. Cell proliferation was quantified using the CCK-8 kit and cell migration using the Boyden chamber assay.

Results

IL-13 R130Q induced significantly higher levels of proliferation, migration, and contraction, as well as secretion of IL-4 and IL-5 in hBSMCs compared to WT IL-13.

Conclusions

Taken all together, our data indicate that IL-13R130Q may contribute to severe asthma through phenotypic modulation of hBSMCs.

Background

Asthma is a common chronic airway disease that poses a major threat to public health worldwide. Its incidence has been increasing over the past few decades, particularly in children. A small but significant proportion of asthma patients respond poorly to conventional treatment with corticosteroids and bronchodilators, thereby developing more severe diseases with debilitating symptoms that affect their everyday life [1]. However, the specific mechanisms underlying the development of severe asthma that responds poorly to corticosteroids remain unclear.

Interleukin-13 (IL-13) is a T-helper 2 (Th2) type cytokine that has been implicated to play a central role in the pathogenesis of asthma and other allergic diseases. Evidence shows that IL-13 may induce "pro-

asthmatic responses" in airway smooth muscle (ASM) through the phosphorylation of signal transducer and activator of transcription 6 [2]. A previous study has found that the level of IL-13 is elevated in patients with corticosteroid-resistant severe asthma compared to those with corticosteroid-sensitive asthma [3]. Severe asthma can be treated with novel anti-IL-13 monoclonal antibodies. Studies on the effectiveness of anti-IL-13 therapies have reported a reduction in the severity of asthma symptoms, a decrease in the frequency of exacerbations, and an improvement in the patient's lung function [4].

Airway remodeling is one of the main hallmarks of asthma. Changes in structure and phenotype of ASM, including increased proliferation [5], migration towards the epithelium, and secretion of pro-inflammatory mediators [6] are important processes in airway remodeling. The degree of airway remodeling is directly related to airway obstruction [7] and asthma disease severity [8]. Phenotypic modulations of ASM are the most important pathological difference that distinguishes severe asthma from moderate asthma [9]. Evidence suggests that IL-13 has the ability to interact with ASM directly to stimulate eotaxin release, increase ASM contraction by regulating smooth muscle contractile protein expression [10], and increase intracellular $Ca2^{+}$ signaling [11] [12, 13]. IL-13 R130Q is a naturally occurring polymorphism of IL-13 that leads to the replacement of the amino acid arginine at residue 130 with glutamine. This variant has been suggested to lead to higher serum IgE levels [14], increase peripheral blood eosinophil counts [15], and strongly contribute to the susceptibility and severity of asthma [14, 16]. Previous research has shown that asthma patients who are homozygous for the R130Q variant have significantly lower forced expiratory volume in one second (FEV1), higher levels of inflammatory mediators such as IL-13, IL-23, IL-11 and chemokine (C-C motif) ligand 8 (CCL8) in bronchoalveolar lavage, as well as increased thickness of the subepithelial layer in airways compared to wild-type (WT) [17]. The mechanism behind this effect of IL-13 R130Q is believed to be due to the lower affinity of this variant to the IL-13 receptor subunit α2, which in turn leads to increased local IL-13 concentrations, thus augmenting its effect [18].

Although previous research including those from our group [19, 20] has shown that the R130Q variant of IL-13 displays significantly increased functional activity compared with WT IL-13, there have been few reports on the effects of IL-13 R130Q on the phenotype of human bronchial smooth muscle cells (hBSMCs). Thus, the present study aimed to investigate whether the R130Q variant of IL-13 could affect the phenotype of hBSMCs in a way that contributes to more severe asthma.

Materials And Methods

Reagents

Human IL-5 and IL-4 Enzyme-linked immunosorbent assay (ELISA) kits were supplied by Ray Biotech. Recombinant human IL-13 and R130Q were purchased from PeproTech (London, UK). Cell counting kit-8 and Fluo-3/AM were obtained from Beyotime (Jiangsu, China).

Culture of primary hBSMCs

Primary hBSMCs and cell culture agents were supplied by ScienCell (ScienCell, Carlsbad, CA, USA) and cultured at 37°C in a humidified atmosphere with 5% CO_2 using a smooth muscle cell medium (SMCM; Science Cell) according to the supplier's instruction. The medium was supplemented with 2% fetal bovine serum, 0.5 ng/mL human epidermal growth factor, 5 µg/mL insulin, 2 µg/mL human fibroblast growth factor-basic, 50 µg/mL gentamicin, and 50 ng/mL amphotericin B. Medium change was performed every 2 days. The hBSMCs were passaged when they reached 90-95% confluence. Cells between the third and sixth passage with 80-85% confluence were used for further experiments.

Treatment with IL-13

For the treatment experiments, hBSMCs were first cultured in a serum-free medium for 24 h. The serum-deprived cells were then stimulated with increasing concentrations (1 ng/ml, 10 ng/ml, 50 ng/ml, 100 ng/ml) of recombinant human WT IL-13 or IL-13 R130Q for 24 h. The specific concentration and incubation times were chosen based on previous reports conducted in this area [21, 22] .

Cell proliferation assay

For the cell proliferation assay, hBSMCs were incubated in 96-well plates at a density of $1x10^4$ cells per well with SMCM and treated with IL-13 R130Q or WT IL-13 for 24 h. Then, 10 µl of cell counting kit-8 (CCK-8) solution (Beyotime, Jiangsu, China) was added to each well. The cells were incubated with the solution at 37°C for 2 h. The proliferation activity of each clone was quantified by measuring the absorbance at 450 nm with an enzyme immunoassay analyzer (Bio-Rad, Hercules, CA, USA).

Cell migration assay

Migration of hBSMCs was analyzed using a modified Boyden chamber [23] separated by an 8 mm polycarbonate membrane (Costar, Corning, USA) coated with 0.01% collagen type-I in 0.01N HCl solution (Sigma). The free-floating hBSMCs that detached from the plate with trypsin-EDTA (Invitrogen Canada Inc, Burlington, ON) following 24 h of serum deprivation were washed and resuspended in SMCM at a density of $5*10^4$ cells/ml. The cell suspension was added to the upper chamber of the modified Boyden chamber apparatus. The lower chambers contained IL-13 R130Q or WT IL-13 (1, 10, 50, 100 ng/ml) in the culture medium. After incubation for 24 h at 37°C in a humidified atmosphere with 5% CO_2, hBSMCs that have migrated to the lower side were counted under a phase-contrast microscope (OPTIPHOT, Nikon, Japan). The magnification was 20X and four-five random fields were counted for each chamber.

Intracellular calcium concentration

After pretreatment with WT IL-13 and IL-13R130Q for 24h, cells were incubated at 37°C in a medium containing 5 mM Fluo-3AM (a $Ca2^+$- sensitive fluorescent probe) for 1 h followed by 30 min in extracellular saline. After that, the relative median fluorescent activity was measured with flow cytometry as an indicator of intracellular calcium ($Ca2^+$) concentration.

ELISA for IL-4 and IL-5

Before measurement, hBSMCs were incubated in 6-well cell culture plates at a density of $1x10^5$ cells/cm until 80-85% confluence was achieved and then treated with IL-13 R130Q or WT IL-13 for 24 h after serum deprivation. The concentrations of IL-4 and IL-5 (expressed as picograms per milliliter) in the collected cell culture supernatant were determined by ELISA kits following the manufacturer's instructions without any prior dilution.

Statistical analysis

All values were expressed as mean ± standard deviation (SD). Continuous variables were compared with one-way analysis of variance. A P value of < 0.05 was considered statistically significant.

Results

The effect of IL-13 R130Q and WT IL-13 on the proliferation of hBSMCs

Airway remodeling is characterized by ASM cell hypertrophy (increase in size) and hyperplasia (increase in amount). The latter is caused by cell proliferation and is believed to have a greater contribution to asthma than the former.

The proliferative activities of hBSMCs after treatment with WT IL-13 or IL-13 R130Q were studied using a CCK-8 cell proliferation assay. Results showed that both WT IL-13 and IL-13 R130Q promoted hBSMC proliferation in a concentration-dependent manner. IL-13 R130Q displayed a significantly greater effect on proliferative activity than WT IL-13 at concentrations of 10 ng/ml, 50 ng/ml, and 100 ng/ml ($P < 0.05$, Fig. 1). However, the effect of WT IL-13 and IL-13 R130Q was similar at 1 ng/ml ($P > 0.05$, Fig. 1).

The effect of IL-13 R130Q and WT IL-13 on the migration of hBSMCs

The presence of ASM cells outside the smooth muscle cell compartment in the airways due to cell migration has been shown to be associated with asthma [24]. A Boyden chamber assay was utilized to study the effect of WT IL-13 or IL-13 R130Q on hBSMC migration. Results showed that both WT IL-13 (10-100 ng/ml) and IL-13 R130Q induced the migration of hBSMCs. At higher concentrations (50-100 ng/ml), the number of migrated cells was significantly higher after culture with IL-13 R110Q compared to WT IL-13 ($P < 0.01$, Fig. 2).

The effect of IL-13 R130Q and WT IL-13 on the contractility of hBSMCs

An increase in intracellular $Ca2^+$ concentration activates myosin L chain kinase and leads to the contraction of smooth muscle cells [25]. We studied the effect of IL-13R130Q and WT IL-13 on the acetylcholine-induced influx of intracellular $Ca2^+$ by flow cytometry using the fluorescent probe Fluo-3/AM. Results showed that after incubation with increasing concentrations of WT IL-13 or IL-13 R130Q

for 24 h, IL-13 R130Q induced significantly higher levels of intracellular Ca^{2+} increase compared to WT IL-13 over the concentration range 10–100 ng/ml ($P < 0.05$, Fig. 3).

The effect of IL-13 R130Q and WT IL-13 on cytokine secretion

Th2 type cytokines such as IL-4 and IL-5 play major roles in asthma and allergic inflammation. These cytokines can be produced and released by hBSMCs [26]. ELISA of cell culture supernatant was performed to quantify the amount of IL-4 and IL-5 produced by hBSMCs following stimulation with WT IL-13 or IL-13 R130Q for 24 h. Results showed that both WT IL-13 and IL-13 R130Q promoted the release of IL-4 and IL-5 in a concentration-dependent manner within the concentration range 1-100 ng/mL. The secretion of IL-4 and IL-5 was significantly higher with IL-13 R130Q compared to WT IL-13 at the concentrations of 10 ng/mL, 50 ng/ml, and 100 ng/mL ($P < 0.05$, Fig. 4). The greatest differences occurred in the higher concentrations of 50 ng/mL and 100 ng/mL ($P < 0.01$; Fig. 4).

Discussion

To our best knowledge, this is the first study to investigate the effect of WT IL-13 and its variant IL-13 R130Q on the phenotype properties of hBSMCs, including the proliferation, migration, contraction, and secretion of cytokines IL-4 and IL-5. Our results indicate that IL-13R130Q is significantly more active than WT IL-13 in multiple effector assays, which may contribute to more severe asthma. Our results strongly suggest that natural variation in the coding region of IL-13 may be an important genetic determinant of the severity of asthma.

Many genetic polymorphisms of IL-13 exist in humans and have been suggested to strongly contribute to the susceptibility [27] and severity of asthma [16]. For example, the variant IL-13 R130Q has been found to promote the production of IgE [20, 28]. The present study focused on the variant IL13 R130Q, which is formed by the non-conservative replacement of a positively charged arginine (R) with an extra glutamine (Q) at position 130 of IL-13. This has previously been reported to accelerate the development of airway remodeling, airway inflammation, and airway hyperresponsiveness in an asthmatic population [29].

IL-13 mediates its effect via a receptor complex containing the subunit IL-13Rα1, IL-13Rα2 and IL-4Rα. Binding IL-13 to IL-13Rα1 and IL-4Rα initiates downstream signaling but binding IL-13 to IL-13Rα2 does not. The high affinity IL-13 receptor IL-13Rα2 has instead been suggested to be primarily responsible for the local sequestration of IL-13. IL-13 R130Q is a naturally occurring polymorphism of IL-13, and there is no difference in the affinity between R130Q and WT IL-13 to IL-13Rα1 [18]. However, the affinity of IL-13 R130Q and WT IL-13 to IL-13Rα2 is different. IL-13R1310Q has a relatively low affinity for IL-13Rα2 compared to WT IL-13. This leads to less local clearance through IL-13Rα2, which results in more local IL-13 acting through IL-13Rα1 [30].

Airway remodeling, hyperresponsiveness, and inflammation are all hallmarks of asthma, and ASM is involved in all three processes. Excessive contraction of ASM in both central and peripheral airways, known as airway hyperresponsiveness, causes airway narrowing that gives rise to symptoms such as

wheezing, coughing, and in severe cases even respiratory failure and death [31]. ASM proliferation and migration contribute to the airway remodeling process and increased ASM mass is associated with decreased lung function in asthma. ASM cells are also known to express a wide range of cell surface molecules and receptors and secrete inflammatory mediators such as cytokines [32]. ASM is therefore considered to be an additional biomarker that could facilitate the diagnostic process of asthma [33].

Alterations in ASM phenotype may play an important role in the pathogenesis of severe asthma. IL-13 has been linked to all of the phenotype changes of ASM. There is some controversy regarding whether IL-13 affects ASM proliferation. Some studies indicate that IL-13 enhances hBSMC proliferation [34], while other studies found no effect. One study even reported that IL-13 inhibited hBSMC proliferation [35]. In this study, IL-13R130Q was found to be more active than WT IL-13 in promoting the production of IL-4 and IL-5 from ASM, and also increased ASM migration, proliferation, and intracellular $Ca2^{+}$ concentration, leading to increased ASM contractility. The increase in ASM contractility seen in the present study is in line with previous reports. These reports have shown that IL-13 can significantly increase smooth muscle cell contractility by upregulating RhoA protein expression [36], and increase intracellular $Ca2^{+}$ concentration by promoting the release of $Ca2^{+}$ from ASM and store-operated $Ca2^{+}$ entry into the cell [12].

During the process of asthma pathogenesis, ASM migrates from the smooth muscle layer to the subcutaneous layer and as the disease progresses, the distance between the ASM layer and the epithelial layer is gradually shortened (Pepe C, Foley S, 2005). Studies have shown that IL-13 promotes smooth muscle cell migration via Src-kinase and leukotriene-dependent pathways (Parameswaran K, Radford K, 2007). Changes in the contractile morphology of smooth muscle play an important role in promoting non-specific hypersensitivity reactions in asthmatic airways.

In vitro studies have shown that cytokines such as IL-5, IL-1ß, IL-13, TNF-α can induce non-specific airway hyperresponsiveness through G protein-coupled receptors to regulate the contractile phenotype of smooth muscle [37–39]. IL-5 is a Th2 type cytokine and a therapeutic target for the treatment of severe asthma. It is crucial in the survival, migration, and activation of mast cells and eosinophils and therefore serves as an indicator of eosinophilic asthma [40]. A previous study found that serum IL-5 and IL-13 consistently predicted blood eosinophilia in adult asthma patients [41]. Treatment of cultured ASM cells with IL-13 upregulated mRNA expression and the release of IL-5 protein from the treated ASM cells [42].

Conclusions

Our results strongly suggest that the naturally occurring IL-13 variant IL-13 R130Q may contribute to more severe asthma by modulation of hBSMC phenotype. Natural variation in the coding region of IL-13 may be an important genetic determinant of the severity of asthma.

Abbreviations

HBSMCs

Bronchial smooth muscle cells
WT
Wild-type
IL-13
Interleukin-13
Th2
T-helper 2
ASM
Airway smooth muscle
FEV1
Forced expiratory volume in one second
CCL8
Chemokine (C-C motif) ligand 8
ELISA
Enzyme-linked immunosorbent assay
SMCM
Smooth muscle cell medium.

Declarations

Author contributions

Jianhua Zhang and Yixiao Bao are the co-corresponding authors, have contributed to the conception and design of the study, the analysis and interpretation of data, revising the article, and the final approval of the version to be published. Yafang He and Qin Pan, as co-first authors, have jointly carried out the study, analysis and interpretated the data, and wrote the first draft of this paper. Luanluan Li has contributed to the partial experimental technical support to this study. Quanhua Liu has contributed partial to the conception and design of this study.

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Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests

Acknowledgements

Not applicable

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Figures

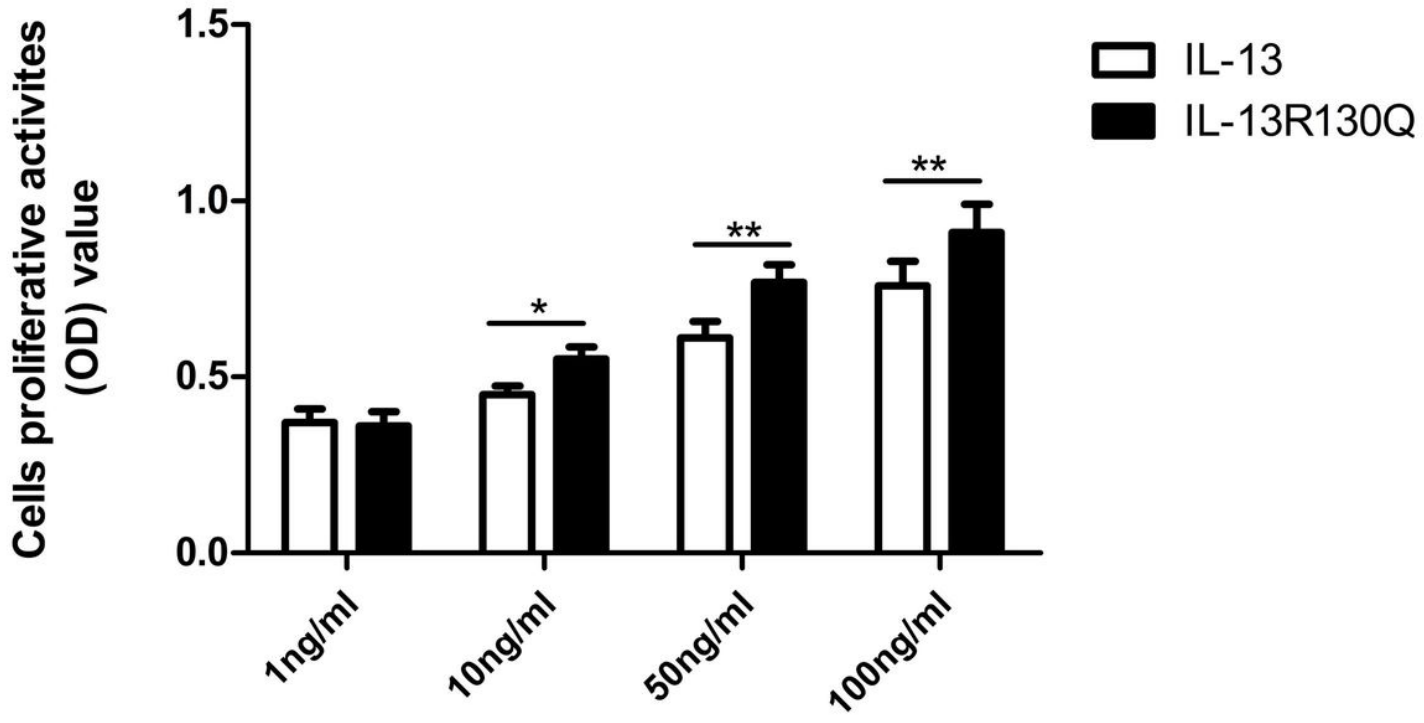
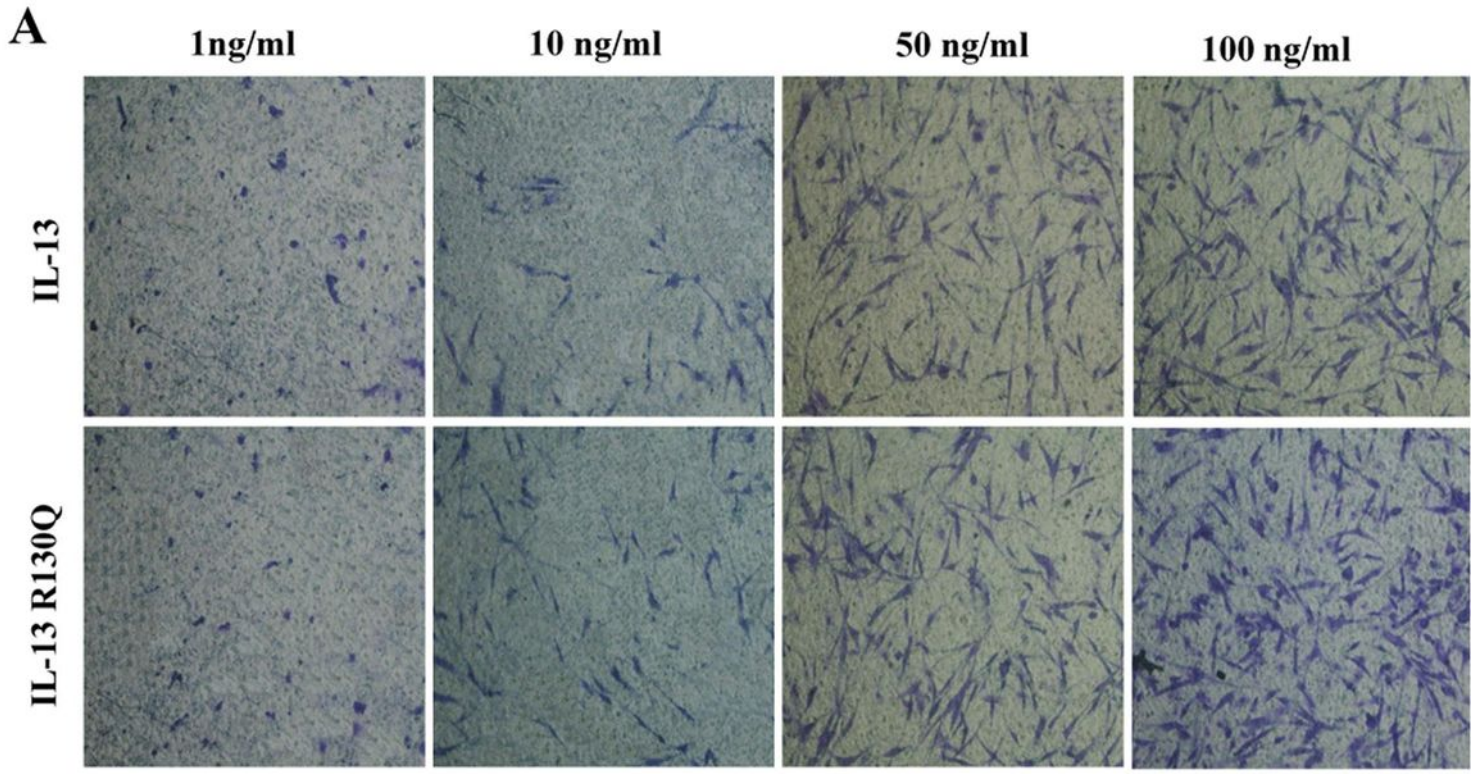


Figure 1

Effect of WT IL-13 and IL-13 R130Q on hBSMCs proliferation. The hBSMCs were cultured with the indicated concentration of IL-13 or IL-13 R130Q (1, 10, 50, 100 ng/ml) for 24 h. The proliferative activities were studied with a CCK-8 cell proliferation assay. Data is presented as mean ± SD. $N \geq 3$. $^{*}P < 0.05$ vs IL-13 (WT) group, $^{**}P < 0.01$ vs IL-13 (WT) group.



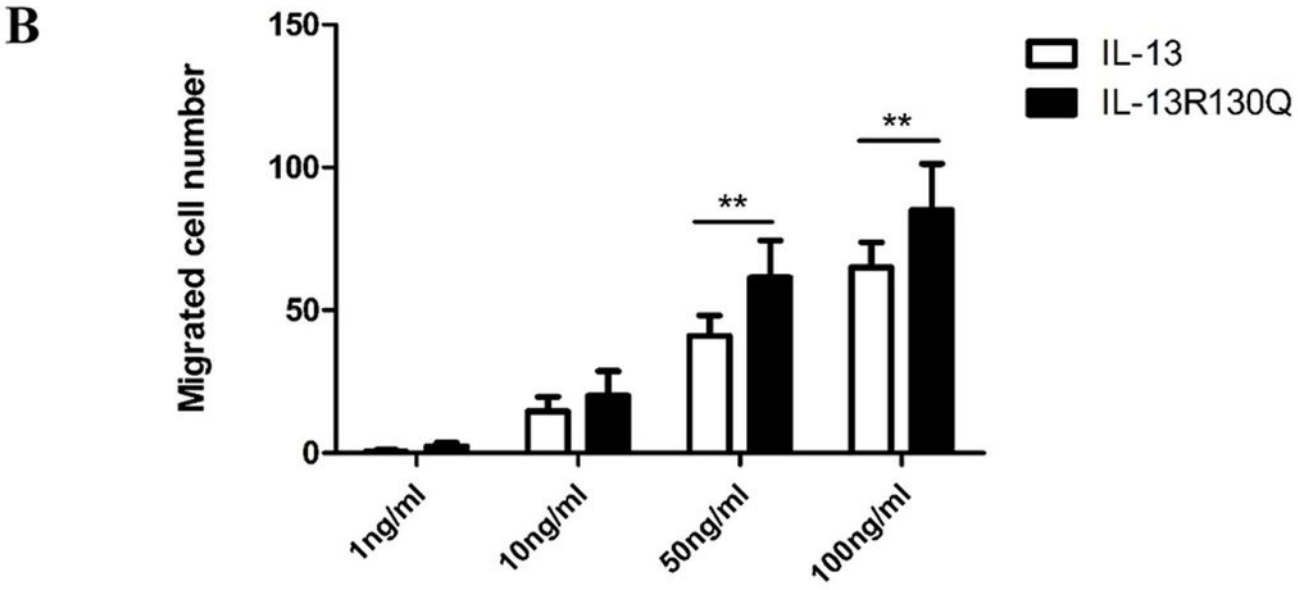


Figure 2

Effect of WT IL-13 and IL-13 R130Q on hBSMC migration. In a Boyden chamber, hBSMCs were seeded in the upper chamber, and WT IL-13 or IL-13 R130Q (1, 10, 50, 100 ng/ml) was added in the lower chamber. Migrated cells were counted under a light microscope (20X). Data is presented as mean ± SD. $N \geq 3$. $^{**}P < 0.01$ vs IL-13 (WT) group.

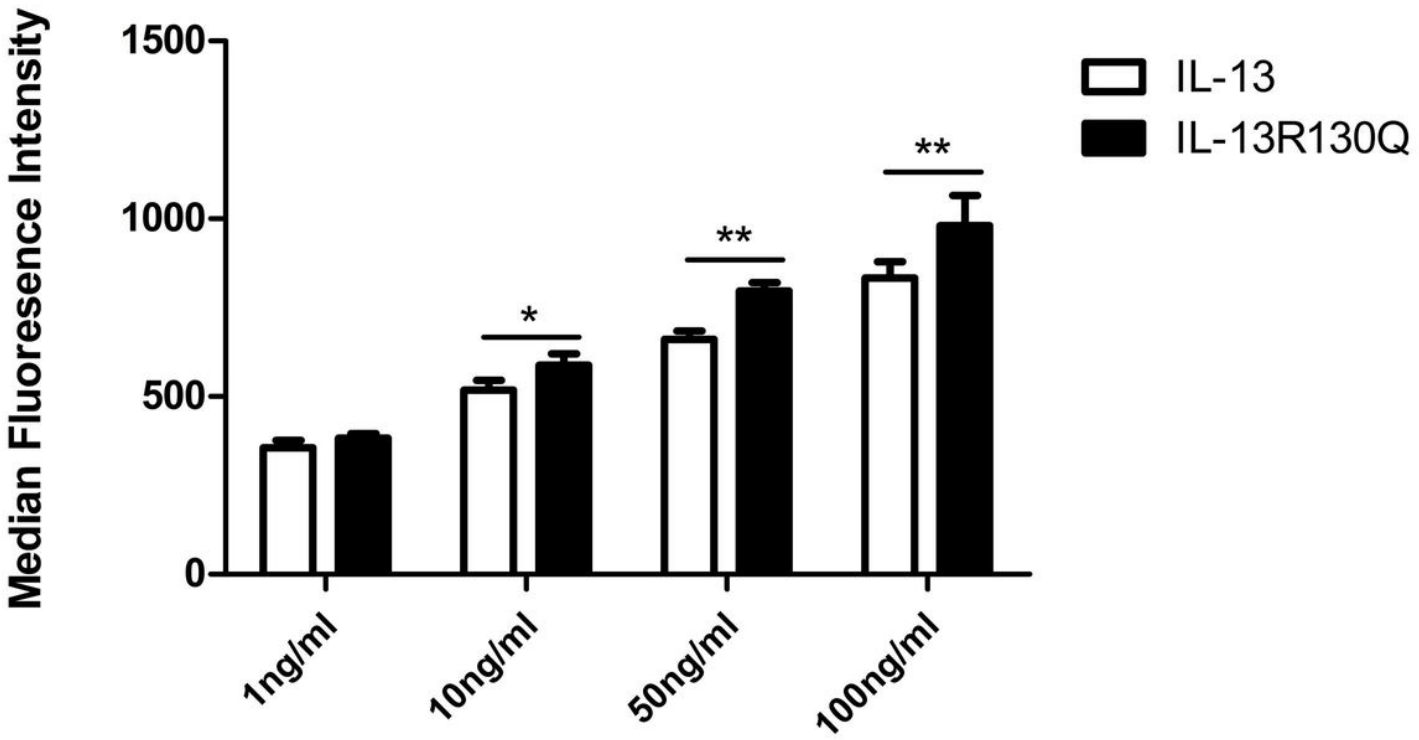


Figure 3

Effect of WT IL-13 and IL-13R130Q on hBSMC contractility. The hBSMCs were treated with the indicated concentration of WT IL-13 or IL-13R130Q (1, 10, 50, and 100 ng/mL) for 24 h. The intracellular Ca^{2+} concentration was measured by flow cytometry with a Fluo-3/AM calcium fluorescent probe. Data is presented as mean ± SD. $N \geq 3$. $^{*}P < 0.05$ vs IL-13 (WT) group, $^{**}P < 0.01$ vs IL-13 (WT) group.

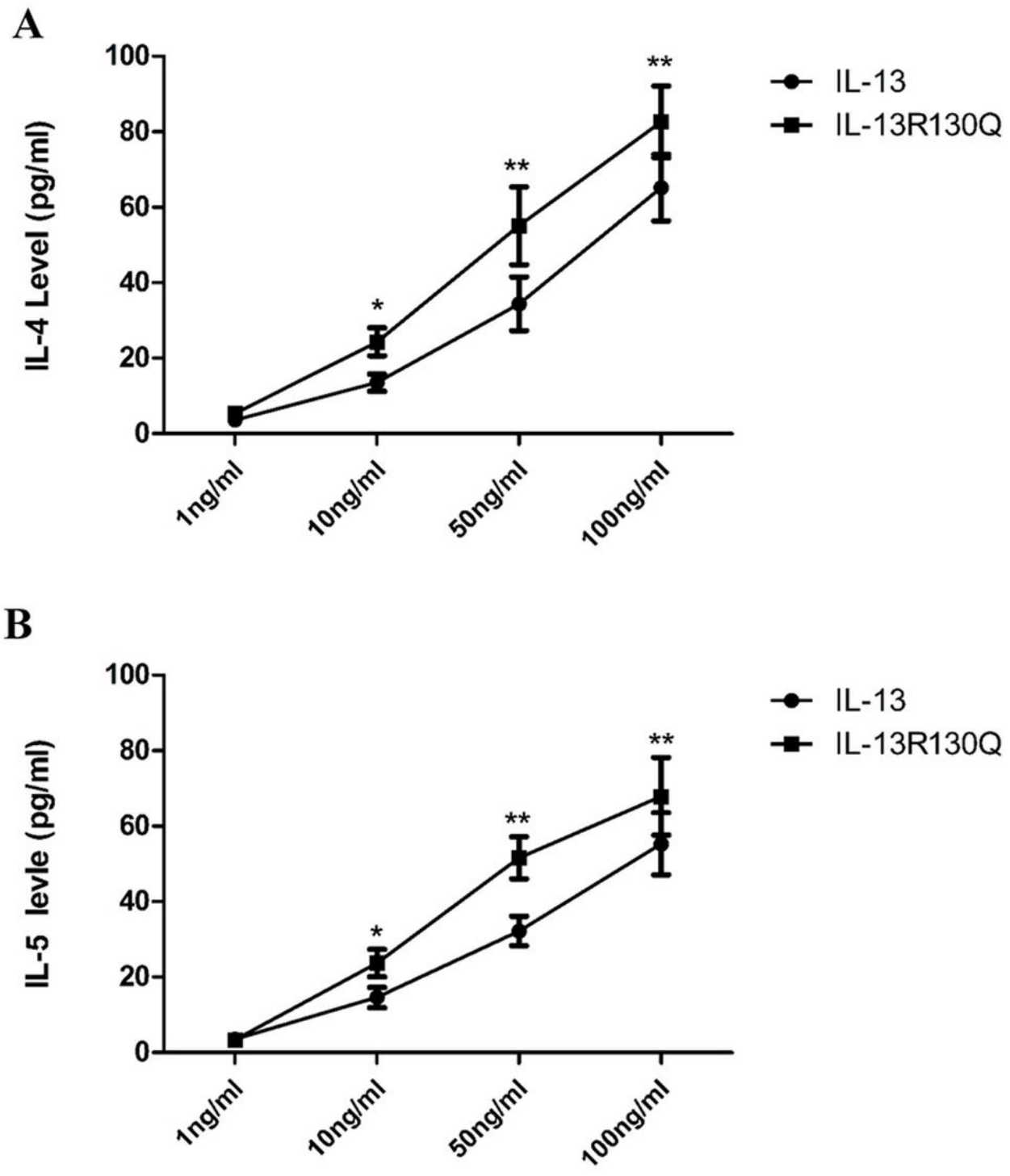


Figure 4

Effect of WT IL-13 and IL-13 R130Qon the secretion of IL-4 and IL-5 in cultured hBSMCs. Cell culture supernatants were collected from hBSMCs treated with increasing concentrations of IL-13 or IL-13 R130Q (1, 10, 50, and 100 ng/mL) for 24 h. Following treatment, the concentrations of IL-4 and IL-5 were measured by ELISA. Data is presented as mean ± SD. N ≥ 3. * $P < 0.05$ vs IL-13 (WT) group, ** $P < 0.01$ vs IL-13 (WT) group.