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House dust mite extract induces growth factor expression in nasal mucosa by activating the PI3K/Akt/HIF-1α pathway

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ABSTRACT

Growing evidence suggests that hypoxia-inducible factor-α (HIF-1α) plays an important role in the progression of allergic airway inflammation and remodeling. However, the biochemical mechanisms leading to the activation of HIF-1α and the effects of HIF-1α on the expression of growth factors, including vascular endothelial growth factor (VEGF), transforming growth factor-β1 (TGF-β1), and fibroblast growth factor-2 (FGF-2), in allergic nasal inflammation are not clear. We examined the relationship between HIF-1α activation and production of VEGF, TGF-β1, and FGF-2 in primary cultured nasal epithelial cells (NECs) after stimulation with house dust mite (HDM) extract. Moreover, we evaluated the importance of phosphoinositide3-kinase(PI3K)/Akt signaling in HDM-induced production of these growth factors *in vitro* and in the nasal mucosa of a murine model of allergic rhinitis (AR). Our results indicate HDM extract induced the expression of VEGF, TGF-β1, and FGF-2 by activating the PI3K/Akt/HIF-1α pathway in human primary cultured NECs and in the nasal mucosa of a murine model. HIF-1α regulated the expression of VEGF, TGF-β1, and FGF-2 in the nasal mucosa through direct and indirect pathways, which suggested that targeting the HIF-1α pathway could be a novel therapeutic approach for reducing nasal airway inflammation and remodeling in AR.

1. Introduction

The house dust mite (HDM) is the most common indoor aeroallergen; exposure to HDM has been shown to be an independent risk factor for the development of allergic respiratory diseases [1]. HDM particles may contribute to or amplify immune and inflammatory pathological reactions or the course of allergic reactions [2], although the molecular mechanisms by which this occurs are not fully understood.

Growing evidence suggests that hypoxia-inducible factor 1(HIF-1)α expression is increased after allergic challenge in the lung and nasal mucosa and that it plays an important role in allergic airway inflammatory responses [3–6]. As a major contributor to the inflammatory process and key mediator of angiogenesis and vascular permeability [7], HIF-1α can promote the expression of growth factors, including vascular endothelial growth factor (VEGF), transforming growth factor-β1 (TGF-β1), and fibroblast growth factor-2 (FGF-2) [7–9]. These growth factors have been demonstrated to be strong stimulators of inflammation and remodeling in allergic airway diseases [4,10–13]. Furthermore, some studies have indicated that the accumulation/activation of HIF-1α protein during allergic inflammatory reactions is associated with phosphoinositide3-kinase(PI3K)/Akt signaling in the lungs [4,14–16]. However, the biochemical mechanisms leading to the activation of HIF-1α and the effects of HIF-1α on the expression of VEGF, TGF-β1, and FGF-2 in allergic rhinitis (AR) are not clear.

In view of findings showing a stimulatory role for HIF-1α in the upregulation of VEGF, TGF-β1, and FGF-2 expression in airway inflammation and remodeling, an investigation of the mechanisms underlying the interaction between HIF-1α and HDM-induced production of the growth factors VEGF, TGF-β1, and FGF-2 in response to allergen exposure in the nasal mucosa is crucial for understanding the pathogenesis of AR. Therefore, we examined this relationship in primary cultured nasal epithelial cells (NECs). We hypothesized that induction of specific growth factors by HDM in NECs might involve activation of the PI3K/Akt/HIF-1α pathway. Subsequently, we also investigated the effects of HDM exposure on the nasal mucosa in a murine model of AR.

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2. Materials and methods

2.1. Primary nasal epithelial cell culture

The nasal mucosa of the inferior turbinate was obtained from patients who underwent partial inferior turbinectomy because of obstructive sleep apnea-hypopnea syndrome. Primary NECs were isolated according to a previously reported method [17]. For subsequent experiments, the primary NECs were used at passage 2–3. This work is performed in accordance with the Declaration of Helsinki. All patients provided written informed consent for the collection of samples and subsequent analysis. The study was approved by the ethics committee of Nanjing Medical University.

2.2. Cell stimulation, shRNA transfection, and drug treatment

NECs at 80–90% confluence were washed with PBS (37 °C, pH 7.4), and fresh culture medium; purified HDM whole-body extract (0–1 μg/ml) (Greer Laboratories, Lenoir, NC,USA) was added to the cultures. Cells were incubated at 37 °C for 0–24 h.

Transfection of primary NECs with HIF-1α short hairpin RNA (shRNA) was performed 36 h before HDM (1 μg/ml) stimulation. The HIF-1α shRNA, scrambled sequence (control shRNA), and adenoviral vectors were synthesized by Hanbio (Shanghai, China). The HIF-1α shRNA was generated using oligonucleotide primers: forward 5′-AATTCGCCGGCCGCTGGAGACACAATCATATCTCGAGA-TATGATTGTGTCTCCACGGTTTTTTG-3′ and reverse 5′-GATC-CAAAAAACCGCTGGAGACACAATCATATCTCGAGA-TATGATTGTGTCTCCAGCGGCCGGCG-3′. Titers of recombinant adenoviruses expressing HIF-1α shRNA (ad-HIFshRNA) or control shRNA (ad-HIFshRNA-neg) were measured by Green Fluorescent Protein(GFP) expression after virus collection and purification. The NECs were seeded into six-well plates and infected at a multiplicity of infection (MOI) of 50 with the ad-HIFshRNA or ad-HIFshRNA-neg. After infection, the cells were incubated for 24 h and then harvested.

The PI3K-specific inhibitor, LY294002 (Sigma–Aldrich, St. Louis, MO, USA) was dissolved in dimethyl sulfoxide (DMSO) before use. Varying doses of LY294002 (0, 5, 10, 20 μM) or vehicle (DMSO, <0.1%) were added to the cultures 3 h before HDM stimulation. The NECs were then stimulated with HDM (1 μg/ml) for 24 h. No cell cytotoxicity due to LY294002 at the doses used in this study was observed based on Trypan blue dye exclusion.

2.3. Western blot analysis

Western blot analyses were performed as previously described with some modifications [6]. We used monoclonal mouse anti-HIF-1α and anti-p-Akt (Novus Biologicals, Littleton, CO, USA), rabbit polyclonal anti-VEGF, anti-TGF-β1 and anti-FGF-2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The relative quantities of protein were determined by using a densitometer.

2.4. ELISA

Levels of VEGF, TGF-β1, and FGF-2 in the supernatant were determined by enzyme-linked immunosorbent assay (ELISA) using commercially available solid-phase sandwich ELISA kits (R&D Systems Inc., Minneapolis, MN, USA).

2.5. Animals and experimental protocol

Six-week-old healthy male BALB/c mice (20–30 g) were obtained from the Experimental Animal Center of Nanjing Medical University (Nanjing, China). The sensitization and antigen challenge of mice were performed as previously described [18] with some modifications. Briefly, mice were immunized by intraperitoneal injection of 100 μg purified HDM whole-body extract (Greer Laboratories) and 1 mg aluminum hydroxide (Sigma–Aldrich). The sensitization was repeated three times at weekly intervals (days 1, 8, and 15), followed by daily intranasal instillations of HDM solution (25 μg HDM protein extract) into the nostrils on days 22–29 (challenge).

Mice were divided into four groups consisting of 10 mice each, including 1) negative control group: saline-challenged mice with vehicle treatment (SAL + VEH); 2) positive control group: HDM-challenged mice with vehicle treatment (HDM + VEH); 3) LY294 group: HDM-challenged mice with LY294002 treatment (OVA + LY294); 4) 2ME2 group: HDM-challenged mice with 2-methoxyestradiol (2ME2) treatment (OVA + 2ME2). Along with sensitization and challenge, LY294002 (7.5 mg/kg body weight/day, dissolved in DMSO, Sigma–Aldrich), 2ME2 (30 mg/kg body weight/day, dissolved in DMSO, Sigma–Aldrich), or vehicle (DMSO) in 40 μl saline was given by intranasal instillation 2 h before each intranasal challenge. All of the animal exams were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals with the approval of the Institutional Animal Care and Use Committee of Nanjing Medical University (Nanjing, China).

2.6. Histological analysis

Histological analysis was performed as described in our previous report [6]. Five high-power fields (magnification, 400 ×) were randomly selected from each section and infiltrating eosinophils were counted. The number of infiltrating eosinophils for each animal was expressed as the mean value from the five fields.

2.7. Statistical analysis

Data were analyzed using SPSS19.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA followed by Dunnett's test was used to determine significant differences between treatment groups. Significance was set at $p < 0.05$.

3. Results

3.1. HDM stimulation increased VEGF, TGF-β1, and FGF-2 proteins in the supernatants of primary cultured NECs

To understand the effects of HDM extract on the production of VEGF, TGF-β1, and FGF-2 in primary cultured NECs, growth factor levels were measured in response to different doses of HDM (0–1 μg/ml) at different time points (0–24 h). Our findings demonstrated dose- and time-dependent release of growth factors in response to HDM stimulation. The levels of VEGF, TGF-β1, and FGF-2 showed the most prominent elevation after stimulation with HDM (1 μg/ml) for 24 h.

3.2. HDM induced growth factor expression via the HIF-1α pathway

To understand the molecular mechanisms underlying the induction of VEGF, TGF-β1, and FGF-2 expression, we evaluated HIF-1α activation in primary cultured NECs. The expression of HIF-1α protein in NECs was assessed by western blotting after stimulation with different doses of HDM (0–1 μg/ml) for 24 h. As illustrated in Fig. 1A, HIF-1α immunostaining was weak in the untreated NECs, but was increased significantly after stimulation with the higher concentrations of HDM. Densitometric analysis also showed that HDM stimulation significantly increased the levels of HIF-1α