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Downregulation of Sfrp5 in insulin resistant rats promotes macrophage-mediated pulmonary inflammation through activation of Wnt5a/JNK1 signaling

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ABSTRACT

Background: Insulin resistance (IR), a common co-morbidity of chronic obstructive pulmonary disease (COPD), aggravates airway inflammation in COPD patients, but its mechanism is unclear. Sfrp5, a novel anti-inflammatory adipocytokine, inhibits macrophage-mediated inflammation of adipose tissue and abrogates IR. However, few studies have been conducted on the regulatory role of Sfrp5 in lung inflammation.

Methods: In the present study, 30 SD rats were divided into two groups: the normal food (NF) group and the high-fat diet (HFD) group. Oral glucose tolerance test (OGTT) and insulin release test were performed to assess whether a successful IR rat model was established. The expression of Sfrp5 and key downstream moleculars of Wnt5a/JNKl signaling was detected. Lung tissue pathomorphology and macrophage activation were observed. In addition, we counted the number of inflammatory cells and measured inflammatory cytokines in bronchoalveolar lavage fluid (BALF). *In vitro*, rat lung macrophages were isolated and treated with Wnt5a, Sfrp5, and/or JNK inhibitor SP600125. JNK activity and inflammatory cytokines expression were examined.

Results: We found that in a rat model of IR, Sfrp5 expression of lung tissue was downregulated, while the Wnt5a/JNKl pathway was activated and the lung inflammatory response was enhanced. Meanwhile, Sfrp5 significantly suppressed Wnt5a/JNKl-induced macrophage activation.

Conclusions: Collectively, IR reduces Sfrp5 expression of lung tissue and activates the Wnt5a/JNK1 pathway, promoting macrophage activation and contributing to the lung's inflammatory response. In contrast, Sfrp5 suppresses the inflammatory response by inhibiting the Wnt5a/JNKl pathway, which could be a target of treatment of COPD.

1. Introduction

Chronic obstructive pulmonary disease (COPD), a chronic airway inflammatory response, is a preventable and treatable airway inflammatory disease characterized by continuous airflow limitation [1]. COPD has become one of the main causes of pulmonary disease-related mortality worldwide and is projected to be the third leading cause of death by 2020 [2]. Aside from the cardinal symptoms of COPD, many patients also present with systemic inflammation and metabolic abnormalities including insulin resistance (IR) [3].

IR is the central feature of metabolic syndrome. Growing evidence suggests that IR is involved in the development and progression of COPD. For example, compared to healthy controls, COPD patients are found to have a higher risk of IR; fasting insulin and inflammatory cytokines, including IL-6 and TNF-α, levels are also elevated in COPD patients [4,5]. Moreover, IR significantly elevates the relative mortality risk and long-term hospitalization rates of

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patients with acute exacerbation of chronic obstructive pulmonary disease [6]. Based on previous studies, an endless cycle of inflammation-induced IR, worsening metabolic abnormality, and airway inflammation exacerbation occurs in COPD patients. However, the exact associated mechanism between lung airway inflammation and IR remains to be thoroughly explored.

Sfrp5 (secreted frizzled-related protein-5), a recently identified anti-inflammatory adipocytokine, is an important member of the secreted Frizzled-related protein (SFRPs) family, which has been implicated in diverse extracellular regulatory processes, including tumorigenesis, embryonic development, obesity, and diabetes [7]. Members of the SFRPs family share a common characteristic: a Frizzled-related cysteine-rich domain (CRD) that is highly homologous to Wingless-type (Wnt) receptor frizzled proteins. Consequently, SFRPs downregulate Wnt signaling pathway by competitively binding Wnt ligands with Frizzled protein receptor [8]. Sfrp5 has been shown to be highly expressed in adipocytes and significantly downregulated in obesity and type II diabetes, which induces the development of IR and alters the inflammatory response of adipose tissue macrophages [9–12]. Additionally, Sfrp5 suppresses the activation of c-Jun N-terminal kinase 1 (JNK1) through inhibition of its downstream noncanonical signaling pathway, enhancing insulin sensitivity and ameliorating inflammatory states [13,14].

Despite the plethora of studies conducted on Sfrp5, there are virtually few studies available regarding the molecular mechanism of Sfrp5 in inflammatory lung disease, and its role in the interaction between comorbid IR and airway inflammation remains unclear. A few studies have shown that Wnt5a/JNKl signaling pathway may be effectively activated in the development and progression of chronic airway inflammation in asthma [15]. In the present study, we investigated the potential regulatory role of Sfrp5 in airway inflammation by establishing a rat model of IR. We found that IR negatively regulated Sfrp5 expression and activated the Wnt5a/JNK1 pathway, which further induced airway inflammation. Additionally, administration of Sfrp5 played an anti-inflammatory role in pulmonary macrophages via impairment of the Wnt5a/JNK1 pathway. Taken together, we tentatively demonstrated the relationship between IR and airway inflammation and assessed the potential role of Sfrp5 in lung airway inflammation stimulated by IR. Our findings may be helpful for the clinical treatment of COPD.

2. Materials and methods

2.1. Animal model

Thirty 7-week-old weaned, clean grade male Sprague Dawley (SD) rats weighing at 60–80 g were purchased from B&K Universal AB (Sollentuna, Sweden). All animals used in the present study were approved by Ethics Committee of the Sixth People's Hospital Affiliated to Shanghai Jiao Tong University (Shanghai, China). Thirty rats were given 3 days to adapt to the new environment, then randomly divided into 2 groups of 15 animals: normal food (NF) group (fed a normal food consisting of 60% carbohydrate, 22% protein, 10% fat and 8% other nutrients) and high-fat diet (HFD) group (fed a high-fat diet composed of 44% carbohydrate, 22% protein, 38% fat, and 5% other nutrients). All animals were housed in groups of six in cages maintained at constant temperature (22 ± 5 °C) and relative humidity ($50 \pm 5\%$) with a light–dark cycle (14:8 h). Rats were supplied food and water for 20 weeks. At the end of experiment, body weight and fat content of all rats were measured.

2.2. Oral glucose tolerance tests

After 20 weeks, rats from the two groups fasted for 12 h, then a baseline blood draw from the lateral tail vein was collected to measure plasma fasting glucose and insulin content. Oral glucose tolerance test (OGTT) was performed as previous reported [16]. Briefly, we treated the rats by gavage with 50% glucose solution (2 g/kg body weight) and harvested blood samples from the tail vein using heparinized Eppendorf tubes at 0, 30, 60, 90, and 120 min after glucose administration. Blood glucose levels were tested using an Accu-Check Active glucometer (Roche). Blood insulin levels were measured by the RIA (radioimmunoassay) method according to the kit instruction.

2.3. Measurement of mRNA levels

The mRNA levels of Sfrp5, Wnt5a, TNF-α, and IL-6 were measured by quantitative real-time PCR using the ABI PRISM 7500 sequence detection system (Applied BioSystems, USA). Briefly, total RNA was extracted from lung tissues using a trizol reagent (Invitrogen) performed according to the manufacturer's protocol. The isolated RNA was applied to synthesize cDNA via reverse transcription using a reverse transcription kit (Promega). The prepared cDNA served as the template for PCR amplification. PCR was performed using the following thermal-cycles: denaturation at 95 °C for 1 min, 45 cycles at 95 °C for 30 s, 62 °C for 30 s, 72 °C for 1 min, extension at 72 °C for 10 min, and finally 4 °C to end the reaction. GAPDH was used as the internal reference. Each sample was measured for three times independently.

2.4. Western blot analysis

Tissues or cells were homogenized in ice-cold RIPA lysis buffer containing protease inhibitors (Roche, Guangzhou, China) for 30 min, then centrifugated at 4 °C to separate the lysates. The measurement of protein concentration and purity were accomplished by BCA protein assay kit (Solarbio, Beijing, China). Next, 30 μg of protein was transferred to a PVDF membrane (Millipore, Bedford, MA, USA) and blocked with non-fat milk at RT for 2 h. The membrane was washed with PBS for three times, then incubated with primary polyclonal antibodies for Sfrp5 (Abcam), Wnt5a, phosphorylated c-jun N-terminal kinase (JNK) and p-JNK, cJUN and phosphorylated c-JUN (p-cJUN) (Santa Cruz). The secondary antibodies were conjugated with horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and incubated for immunoblotting analysis. Signal detection was measured using an ECL Western blotting detection kit (Amersham Biosciences, USA). The expression of the target gene was represented relative to β-actin (Sigma).

2.5. Preparation of serum and BALF

The rats were anaesthetised by ip 1% pentobarbital sodium (1 ml/kg). Venous blood samples (5 ml) were collected from the inferior vena cava. Serum was isolated by centrifugation at 3000 r/min for 5 min and stored at −80 °C. The left lung was washed with 15 ml saline four times at 37 °C after the trachea and both lungs were exposed, and the lavage fluid was collected and all anaesthetised rats were euthanized by exsanguination. The obtained bronchoalveolar lavage fluid (BALF) was filtrated by a single layer, sterile 200-mesh cytoscreener and centrifugated at 1500 r/min (4 °C) for 5 min. Supernatant was harvested and stored at −80 °C for later use. Serum level of ALT (alanine aminotransferase) and bilirubin, markers of liver function, were tested in the serum of NF and HFD rats.

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