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Adipokine adiponectin is a potential protector to human bronchial epithelial cell for regulating proliferation, wound repair and apoptosis: Comparison with leptin and resistin

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ABSTRACT

Epidemiological data indicate an increasing incidence of asthma in the obese individuals recent decades, while very little is known about the possible association between them. Here, we compared the roles of adipocyte-derived factors, including leptin, adiponectin and resistin on proliferation, wound repair and apoptosis in human bronchial epithelial cells (HBECs) which play an important role in the pathogenesis of asthma. The results showed that exogenous globular adiponectin (gAd) promoted proliferation, cell-cycle and wound repair of HBECs. This effect may be relevant to Ca^{2+}/calmodulin signal pathway. Besides, gAd inhibited apoptosis induced by ozone and release of lactate dehydrogenase (LDH) of HBECs via regulated adipoR1 and reactive oxygen species. No effects of leptin or resistin on proliferation, wound repair and apoptosis of HBECs were detectable. These data indicate that airway epithelium is the direct target of gAd which plays an important role in protecting HBECs from mechanical or oxidant injuries and may have therapeutic implications in the treatment of asthma.

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1. Introduction

Obesity and asthma have become the momentous public health problems for the past decades. The increasing prevalence of overweight and obesity and their association with asthma have generated a great interest in investigating the potential mechanisms linking obesity and asthma [25]. Nowadays, adipocyte-secreted factors, leptin, adiponectin and resistin, have been thought to contribute to several obesity-related diseases and aroused much attention of people. Most of the investigations regarding these so-called adipokines mainly focus on fatty acid oxidation, glucose metabolism and insulin sensitivity.

Leptin plays a key role in appetite and body weight regulation. It always increases in proportion to BMI and has a number of proinflammatory effects on cells involved in innate and adaptive immunity. Adiponectin is a 30-kDa protein which usually decreases in obese rodents and humans [12]. In mammalian plasma, both the full-length form of adiponectin (fAd) and the globular domain (gAd) have been detected [3]. The effects of adiponectin are mediated by two receptor isoforms (AdipoR1 and AdipoR2) as well as T-cadherin. The globular domain of adiponectin (gAd), which has stronger biological activity, has a high affinity with the AdipoR1 receptor [31]. Adiponectin's primary effects are on energy metabolism and antidiabetic in nature. It also has many anti-inflammatory effects [20]. Resistin belongs to the RELM/FIZZ family of four cysteine-rich secretory proteins that share homology with resistin. Studies in animals suggest that members of the RELM/FIZZ family have important pro-inflammatory and remodeling roles [5].

Some clues showed that these adipokines may also be involved in asthma. Multiple studies have investigated the relationship between serum levels of these adipokines and the presence of asthma. Leptin was higher secreted as a predictive factor for asthma in children [9,10], especially in girl [22]. Study in adults by Sood et al. found that leptin increased in asthmatics, more in women [27]. Adiponectin was lower in female asthmatics [26] and significantly correlated with AHR induced by exercise challenge in children with asthma [1]. In a study of Kim et al., resistin may play a negative predictive role in asthma. Adiponectin and leptin showed close associations with pulmonary function and may have disease-modifying effects in children with asthma [18]. The data are sometimes conflicting in some studies which do not appear to be a strong relationship between serum levels of leptin, adiponectin or resistin and the presence of asthma [14–17]. However, serum levels may not accurately reflect levels in the lungs; it holds good such that local biological effects of these adipokines need to be considered.

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A recent report indicated that increasing BMI was associated with increased BAL leptin and was marginally and inversely associated with BAL adiponectin [13]. It is also confirmed increased expression of resistin homologue FIZZ1 measured from BAL fluid and lung tissues from OVA-challenged mice by using western blotting [32]. However, the exact targets and mechanisms the adipokines act in lungs are still unclear. Airway epithelium is the interface between internal environment and external environment of our bodies. It acts not only as mechanical barrier but also possesses abundant biological function. Airway mucosa's microscopic examination showed the apoptosis of human bronchial epithelial cells (HBECs) increased in asthma patients, and the asthmatic epithelium responded inappropriately to challenges [11], which led to dysfunction of homeostasis in airway and finally airway hyperresponsiveness associated diseases such as asthma. Bruno et al. showed that bronchial epithelium expresses leptin and leptin receptor and the expression of leptin and leptin receptor is altered in severe asthma [2]. Daniele analyzed the adiponectin pathophysiological conditions and found that oligomerization state of adiponectin is altered in lung of COPD and the presence of AdipoRs is a lower expression of AdipoR2 compared to AdipoR1 [3]. Resistin homologue RELM-beta immunoreactivity, which is colocalized with airway epithelial cells, increased with the severity of asthma [7]. It is likely to predict that the adipokines may have an influence on biological function of airway epithelium which may offer partial explanations in the relationship between obesity and asthma, while very little related investigations have been taken.

The present study was carried out to examine whether the adipokines act on proliferation, mechanical wound repair or ozone-induced apoptosis in HBECs and its possible mechanism.

2. Materials and methods

2.1. Reagents

Dulbecco's modified eagle medium (DMEM), trypsogen and fetal bovine serum (FBS) were obtained from Gibco Laboratories (Grand Island, NY, USA). Primer synthesis of RT-PCR was provided by Tangent Company (China), reverse transcription kit (Invitrogen, USA). PCR mix was provided by Aldly Company (China). Immunohistochemical detection kit was from Zhongshan Goldenbridge Biotechnology. Leptin, gAd and resistin recombination proteins were purchased from Prospec Corporation (Israel). Rabbit anti-adipoR1 antibodies with or without FITC were from Abcam (Cambridge, UK). Fluo-3 AM for Ca^{2+} and DCFH-DA probes for ROS were from Beyotime, China. MTT (Sigma, USA), annexin V/PI apoptosis kit (Becton, Dickinson and Company, USA), LDH kit (Nanjing Jiancheng Bioengineering Institute, China) were prepared.

2.2. Cell culture and ozone stress in vitro

An immortalized human bronchial epithelial cell line (16HBE14o-cells) provided by Professor Gruenert of the University of California at San Francisco was maintained in a mixture medium of DMEM supplemented 10% heat inactivated fetal bovine serum (FBS) and incubated at 37 °C in 5% CO_2.

In ozone stress assay, when 16HBE14o-cells reach about 80% of confluence in culture dishes, cells were stressed with ozone (1.5 ppm) for 2 h with or without pre-incubation by adipokines for 12 h, then cells were harvested and washed twice in PBS.

2.3. Cell proliferation and cell cycle analysis

MTT (3-[4,5-dimethylthiazolyl-2]-2,5-diphenyltetrazolium bromide) is cleaved by mitochondrial dehydrogenases to formazan crystals in metabolically active cells and this method is used to detect viable cells. MTT diluted 10 times from a stock solution of 5 mg/ml was added to the 96-well plate. The cells were then incubated for 4 h and solubilized in DMSO. Absorption was measured and the difference between the sample wavelength (490 nm) and the reference wavelength (650 nm) was calculated. The proportion of viable cells was expressed as percentage of control.

16HBE14o-cells were performed with a flow cytometer (Cytomics TM FC 500, USA). After treatment with trypsin, the 16HBE14o-cells were centrifuged at 1000 rpm for 5 min, washed with phosphate-buffered saline, and resuspended in phosphate-buffered saline containing 40 μg/ml propidium iodide, 1 mg/ml RNase, and 0.1% Triton X-100 in a dark room. The DNA contents of the cells were then analyzed using a flow cytometer with a single excitation 488 nm of 15 mW Ar laser. The propidium iodide signal was detected by FL3 sensor at 605–635 nm.

2.4. Measurement of intracellular Ca^{2+}

16HBE14o-cells grow in a 96-well plate labeled by fluorescence enzyme were loaded with 5 μM Fluo-3/AM in DMEM medium for 60 min at 37 °C and then stimulated by adiponectin for different time. The following fluorescence filters were used for Fluo-3: excitation laser of 488 nm and emission filter of 525 nm. Changes in fluorescence intensity of Fluo-3 that reflect calcium changes were captured by Thermo Scientific Varioskan Flash in numerical value and data and plotted over time.

2.5. Wound repair assay

The wound repair procedure was performed as described previously [15,29]. A circular wound (~2.5 mm^2) was made in the confluent 16HBE14o-cell monolayer using a 10 μl pipette tip (5 wounds per well). The wounds were imaged at several time points in 24 h by video microscopy (Olympus Company, Japan). Corresponding wound areas were determined using BI-2000 image immunohistochemical analysis system and the remaining wound areas were calculated as a percentage of area at time 0. In order to determine the effects and the signal pathway of gAd in mediating wound repair of 16HBE14o-cells, cells were pretreated with calmodulin inhibitor W7 (10^{-5} M) 1 h prior to addition of adipokines..

2.6. Apoptosis assay

Apoptosis of 16HBE14o-cells was assessed using the apoptosis assay kit. Cells were seeded in a 6-well plate and grown to 80% confluent. Cells were pretreated with or without adipokines for 12 h before stimulated with 1.5 ppm ozone for 2 h. Then cells were harvested and resuspended in binding buffer. FITC-annexin V was added and incubated for 15 min at 37 °C. PI solution was added at room temperature for 30 min before the test by FC500 flow cytometer.

2.7. Measurement of intracellular ROS and LDH release

We used DCFH-DA Molecular Probes to measure the intracellular ROS according to the manufacturer's protocol with a few modifications. Briefly, after the proper treatments, cells were washed with PBS and incubated in pre-warmed PBS containing the probes in a final working concentration of 5 μM for 30 min at 37 °C. Intracellular ROS fluorescence intensity (488 nm/518 nm) was detected by flow cytometry and laser scanning con-focal microscope.

Levels of LDH in the culture media were determined, which can be used as a marker of cellular lysis. After gAd pretreatment and

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