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A reciprocal inhibitory relationship between adiponectin and mammalian cytosolic thioredoxin

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Abstract Thioredoxin is a redox protein while adiponectin is an adipokine. Their relationship has been less appreciated. Here we show that in serum of patients with acute exacerbations of chronic obstructive Pulmonary Disease, decreased activity of thioredoxin coexists with increased level of adiponectin as partial pressure of arterial oxygen decreases. From the results with adiponectin-knockout mice and in vitro experiments, we have found a reciprocal inhibitory relationship, by which adiponectin inhibits cytosolic *thioredoxin* (*Trx1*) expression, whereas serum Trx1 influences adiponectin multimerization. The association between extracellular Trx1 and adiponectin attenuates their functions. This relationship is dynamic, and correlated with a body's physiological conditions.

SPECIAL TOPIC: Lipid metabolism and human metabolic disorder

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1 Introduction

Chronic obstructive pulmonary disease (COPD) is a global health problem, involving dysfunction in lungs and bronchial epithelium [1]. Acute exacerbations of COPD (AECOPD) are often associated with increased mortality. However, the efficient prevention and treatment of AECOPD are currently hampered because the detailed mechanism remains largely unclear [2]. Adiponectin is the most abundant adipokine. Recently, the close association was found between adiponectin and COPD [3] as well as AECOPDs [4]. The latter is a common presentation to emergency departments and an important cause of respiratory failure [5]. Serum adiponectin was usually excessive or inappropriate accumulation in COPD patients [6]. As such, an understanding of the mechanism that mediates or controls adiponectin effects on respiratory system is of important therapeutic values.

Human adiponectin circulates in plasma/serum as trimer (LMW), hexamer (MMW) and multimeric (HMW) isoforms [7]. Adiponectin isoforms have differential actions [8]. Mature monomeric adiponectin has a molecular weight about 26 kDa [9]. The HMW and MMW isoforms of adiponectin are composed of disulfide bonded trimer [10]. Thus, redox chemistry may provide a mechanism for regulating adiponectin actions. Indeed, a disulfide-bond A oxidoreductase-like protein (DsbA-L) was identified as a regulator of adiponectin multimerization [11]. DsbA-L contains thioredoxin folds with "Cys-X-X-Cys" motif

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[11], and belongs to thioredoxin superfamily [12], which is a major player in oxidative protein folding. Thioredoxin is a class of small redox proteins present in all living cells [13]. In hepatocellular carcinoma, adiponectin induced apoptosis through differential modulation of thioredoxin proteins [14]. Although Trx1 expression in lung homogenates from COPD patients did not show significant alteration [15], changes of Trx1 in serum and respiratory system of AECOPD patients have not been well characterized.

Presently there are three distinct human thioredoxin proteins: cytosolic thioredoxin (Trx1), mitochondrial Trx (Trx2) and testis specific thioredoxin. The most well-studied is Trx1. In response to inflammation, Trx1 may be secreted into blood circulation [16], where it coexists with adiponectin. In particular, oxidative stress plays important roles in AECOPD [17]. Trx1 and its reductase (TrxR), together with NADPH, form Trx1 system that is important in maintaining cellular redox balance and cell survival [18]. In addition, inflammation is a core feature of AECOPD [19]. Extracellular Trx1 acted as chemoattractant to attract inflammatory cells to the sites of inflammation and mediate release of some inflammatory cytokines [20]. Trx1 induction might ameliorate cigarette smoking-induced lung inflammation and emphysema in mice [21]. These observations led us to speculate a Trx1-based mechanism for adiponectin-related effects on respiratory system.

In this study, AECOPD patients were grouped according to partial pressure of arterial oxygen (PaO_2), because the prevalence of hypoxemia is common in AECOPD patients, but not in COPD patients [22]. This uncovered the inverse correlation between adiponectin and Trx1 in serum of AECOPD patients. Using adiponectin-knockout mice' sera/lung tissue, human bronchial epithelial cells and purified adiponectin/Trx1 as models, we further analyzed the molecular basis that underlies the interaction between adiponectin and Trx1, and found a reciprocal inhibitory relationship between them.

2 Materials and methods

2.1 Materials

Mammalian *TrxR* and recombinant human *Trx1* (*hTrx1*) were prepared as described previously [23, 24]. hTrx1, adiponectin receptor-1 and IL-8 monoclonal antibodies were purchased from Santa Crus Biotechnology, Inc. (CA, USA). Adiponectin monoclonal antibody was from R&D Systems Inc. (MN, USA). Adiponectin ELISA kit was bought from Shanghai BlueGene Biotech Co., Ltd., China. SYBR® Green Real-Time PCR Master Mix was purchased from Toyobo Co., Ltd. (Osaka, Japan). Protein A+G agarose and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) monoclonal antibody were from Beyotime (Shanghai, China). Dylight 488 amine-reactive dye was from PIERCE (USA). Ni Sepharose 6 Fast Flow was from GE Healthcare Life Sciences (USA). A human bronchial epithelial cell line (16-HBE) was provided by Central South University Xiangya Medical Center. All other reagents were purchased from Sigma unless otherwise stated.

Human male serum samples were from the subjects who were treated at the First Hospital of Shanxi Medical University. COPD was defined according to GOLD criteria [25]. To define AECOPD, the following definition was used: deterioration in the respiratory status of a patient with COPD defined clinically by symptoms or signs or biochemically by arterial blood gas analysis. The Institutional Review Board of the Shanxi Medical University approved this study (No. 2011061). All procedures performed in studies were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

The male adiponectin-knockout mice (C57BL/6, n = 4) and normal mice (C57BL/6, n = 3) were used at 12 weeks of age. Adiponectin-knockout mice were kindly offered by Dr. Lianfeng Zhang, Key Laboratory of Human Diseases Comparative Medicine, Ministry of Health; Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China. C57BL/6 (B6) mice were purchased from Beijing University Experimental Animal Center (Beijing, China). All animal experiments were approved by the Animal Care and Use Committee of Beijing, China. All applicable institutional and/or national guidelines for the care and use of animals were followed.

2.2 Cell protein extraction

16-HBE cells were cultured in Dulbecco's modified eagle medium (DMEM, Invitrogen, USA) supplemented with 10 % fetal bovine serum (FBS, SiJiQing China) at 37 °C in an incubator containing 5 % CO_2. Cells were routinely split at a ratio of 1:4. To examine the effect of adiponectin on Trx1, the cell medium was replaced with DMEM without FBS when the cells grew to about 70 % confluence. After another 24 h, the medium was replaced by fresh DMEM plus adiponectin. For harvesting, the cells were washed three times with PBS and scraped with a cell scraper, followed by centrifugation to collect the cells. The latter were resuspended in 50 mmol/L potassium phosphate, 1 mmol/L EDTA, pH 7.5 (PE) buffer containing 1 mmol/L PMSF, and lysed by ultrasonication. Nuclear and cytoplasmic proteins were extracted using commercialized kit (Beyotime, China). Protein concentration in the