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The contribution of chronic intermittent hypoxia to OSAHS: From the perspective of serum extracellular microvesicle proteins

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ABSTRACT

Objective: Obstructive sleep apnea hypopnea syndrome (OSAHS) is an independent risk factor for many clinical complications. However, how OSAHS cause multiple organ injury and initiate inter-organ communication remains unclear. Moreover, despite it is well-recognized that chronic intermittent hypoxia (CIH) is a main feature of OSAHS, specific contribution of CIH to overall OSAHS-initiated pathological complications remains unclear. This study aimed to use an unbiased proteomic approach to determine whether OSAHS alters protein profiles of serum extracellular microvesicles (SEMVs) and how CIH contributes to such alterations.

Methods: Tandem mass tag (TMT)-labeled quantitative proteomics assay was used to compare the differentially expressed proteins (DEPs) in SEMVs of OSAHS patients and non-OSAHS subjects. To evaluate the contribution of CIH to OSAHS, CIH rodent model was constructed and the same comparative proteomics study was performed in SEMVs from CIH and normoxia rats. The similarity and disparity of DEPs and DEPs-related functions predicted by bioinformatics tools were compared in above-mentioned two models, and several DEPs were selected and further verified by ELISA or Western blotting.

Results: TMT-labeled quantitative proteomics assay unravels 32 DEPs in OSAHS patient SEMVs from a total of 560 human SEMV proteins identified. Four DEPs, namely C-reactive protein (CRP), Haptoglobin (HP),Fibronectin (FN1) and Platelet factor 4 (PF4), were further verified by ELISA and three of them (CRP, FN1 and Hp) showed significant difference in expression level between OSAHS and non-OSAHS groups. In SEMVs of rat CIH model, 121 DEPs out of 723 proteins were identified. By comparing the DEPs identified from the two models, 3 proteins (CRP and FN1 and F13a1) were found identical with the same alteration pattern (CRP was upregulated, FN1 and F13a1 were downregulated) in SEMVs from OSAHS patients and CIH rats, which were further verified by Western blotting. Computational functional analysis further revealed the common and distinct DEP-involved pathways under OSAHS or CIH status.

Conclusions: This study provides the first evidence that OSAHS causes significant alteration in SEMV protein composition, which may contribute to OSAHS-triggered multiple organ injury and organ-to-organ communication. Moreover, we have demonstrated that CIH is the primary contributor for increased inflammatory protein expression in SEMV. As CRP is being increasingly recognized not only as a marker but also a mediator of inflammatory response to tissue injury, increased SEMV CRP in CIH/OSAHS may play an important role in OSAHS-induced tissue injury, suggesting SEMV CRP might be a therapeutic target against OSAHS-related complications.

Abbreviations: AUC, area under the curve; CIH, chronic intermittent hypoxia; CRP, C-reactive protein; DEPs, differentially expressed proteins; FN, fibronectin; HP, haptoglobin; OSAHS, obstructive sleep apnea hypopnea syndrome; PF4, placelet factor 4; ROC, receiver operating characteristic; TMT, tandem mass tag.

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Contents

1. Introduction

Obstructive sleep apnea hypopnea syndrome (OSAHS), affecting up to 2% of middle-aged females and 4% of middle-aged males worldwide [1], is an independent risk factor associated with many clinical complications (i.e., hypertension, stroke, coronary heart disease, diabetes and cancer) [2–6]. Chronic intermittent hypoxia (CIH) which commonly seen as episodes of apnea and hypopnea, appears to be the key property of OSAHS and is considered as the major pathological origin of OSAHS-associated diseases. However, CIH alone is insufficient to explain the occurrence of other conditions in OSASH syndrome, such as negative intrathoracic pressure variation and autonomic nervous system disorder [7,8]. Therefore, systematical and precise dissection of the molecular indexes under OSAHS and CIH conditions is so important as that will help to determine the specific contribution of CIH to OSAHS and its underlying mechanism, facilitate to evaluate the main causes of OSAHS-associated diseases, and subsequently aid to develop more effective and precise diagnosis and treatment in clinical practice. However, comparative study based on CIH and OSAHS status is very limited because it is nearly impossible to obtain clinical specimens from patient with solely CIH status. As an alternative, CIH animal model has been well established through controlling the environmental oxygen concentration. Despite the significant interspecies differences between murine and *Homo sapiens*, CIH animal model is widely used in OSAHS basic research because of the similarity on hypoxia property.

Extracellular microvesicles (EMVs) are nanoscale membrane vesicles released from miscellaneous cells into the extracellular fluid compartments, including serum. They are reported to be involved in cell-to-cell and organ-to-organ communications through selectively carrying RNA and protein cargos from donor cells and delivering them to recipient cells. EMV components therefore may closely reflect the physiological and pathological status of their tissue origins and thus are considered as desirable targets for clinical diagnosis and therapeutics. Serum is the most frequently used liquid biopsy material in clinical diagnosis. The use of serum EMVs' components to reflect physiopathological status is well recognized. However, to our knowledge, there is no previous report on the systematic analysis of protein components of EMVs from OSAHS patients and non-OSAHS subjects. In addition, it is unknown whether protein profile of EMVs from CIH rodent model is similar to that of OSHAS patient, concerning that CIH is one of the hallmark features of OSAHS.

In this study, we profiled the proteins of serum EMVs (SEMVs) from OSAHS patients and non-OSAHS subjects, and compared the differentially expressed SEMV proteins by TMT-labeled quantitative proteomics study. The same strategy was applied to compare the protein composition of SEMVs from rats under CIH or normoxia conditions. The differentially expressed proteins (DEPs) of SEMVs identified from the two models were classified by gene ontology (GO) and a group of functionally significant proteins were selected and verified by Western blotting and ELISA. These proteomics study enabled us to identify some common and unique alterations of SEMV proteins under CIH and OSAHS status, which will help to understand the role of CIH in OSAHS complications and will provide potential diagnostic or therapeutic targets for OSAHS and OSAHS-associated diseases in clinical trial.

2. Methods

2.1. Ethics Statement

The study design was approved by the Medical Ethics Committee of Beijing Anzhen Hospital (2017005). Written informed consent was obtained from all participants.

2.2. Animals

Male SD rats (weighing 250 to 300 g) were exposed to CIH as previous reported [9]. All animal experiments were approved by HKU Animal Experimentation Ethics Committee and in compliance with the Guide for the Care and Use of Laboratory Animals (NIH Publication Eighth Edition, updated 2011).

2.3. Clinical Samples

The moderate or severe OSAHS patients (AHI ≥ 15/h) and control subjects (AHI < 5/h) were enrolled to the research. The detail recruitment standard can be found in Supplementary methods. All fasting blood samples were collected using standard serum draw without anticoagulation. The detailed patient characteristics were presented in Table 1.