Intermittent Hypoxia Accelerates Adipogenic Differentiation In Human Subcutaneous Preadipocytes In Vitro

Y. Wang[1], S. C. Yeung[1], M. Y.K. Lee[1], J. C. Mak[1], M. S. Ip[1, 2]

[1]The University of Hong Kong, Hong Kong, Hong Kong, [2]

Corresponding author's email: judithmak@hku.hk

Rationale: Obstructive sleep apnea (OSA), characterized by intermittent hypoxia (IH), is highly associated with obesity. Depot-specific adipogenic differentiation, an important physiological mechanism in maintaining adipose tissue homeostasis, could be regulated by intracellular transcriptional factors, extracellular signaling pathways and inflammation in obesity. However, the impact of IH on adipogeneisis is unclear. This study aims at investigating the pathologic role of IH during the adipogenic differentiation process in human subcutaneous preadipocytes in vitro.

Methods: Human subcutaneous preadipocytes (HPAs) underwent 6 differentiation cycles into mature adipocytes. Each differentiation cycle consisted of two sequential procedures of differentiation (for 3 days) and maintenance (for 2 days). During each 3-day differentiation, HPAs were subjected to IH (IH; 1% for 10 min and 21% for 5 min per cycle; 5% CO_2) or intermittent normoxia (IN; 21% O_2 and 5% CO_2) treatment. The degree of differentiation was investigated using Oil-Red-O staining at different cycles. RNA samples were extracted at Cycle 2, 4 and 6 of differentiation to determine the expression levels of FABP4, a marker of differentiated pre-adipocytes, and of CEBP, an adipogenic transcriptional factor, using real time PCR. Proteins were extracted from undifferentiated HPAs with or without IH for the analysis of adipogenic insulin-like growth factor 1(IGF-1)/AKT pathway using Western blot analysis. Conditioned media were also collected from each cycle of differentiation to detect pro-inflammatory markers, interleukin (IL)-6 and monocyte chemoattractant protein (MCP)-1, and anti-inflammatory marker adiponectin using ELISA.

Results: Oil-Red-O staining of accumulated oil droplets after the induction of differentiation indicated that IH facilitated the accumulation of oil droplets. During differentiation, IH induced elevation of FABP4 mRNA expression level and prevented the down-regulation of CEBPδ mRNA expression level in HPAs, compared to cells in the same cycle of differentiation exposed to IN. In undifferentiated HPAs, IH activated IGF-1/AKT pathway via the up-regulation of IGF-1 receptor expression and Akt (ser473) phosphorylation. Compared to IN, IH also induced increase in levels of IL-6 and MCP-1, and attenuation of adiponectin in the conditioned media during the process of differentiation.

Conclusion: IH could promote the adipogenic differentiation of HPAs via regulating transcriptional factor CEBPδ, IGF-1/AKT pathway and inflammation. This suggests a potential role of IH in the modulation of obesity.

This abstract is funded by: Mok hing Yiu Chair Professor in Respiratory Medicine Endowment Fund and Stanley Ho Alumni Challenge Matching Grant