中文題目：在人類支氣管上皮細胞中，缺氧誘導因子 HIF1α與叉頭框轉錄因子 FOXA2 共定位於細胞核內並下調 FOXA2 所調控的神經內分泌多肽α-和β-CGRP 基因及緊密連接蛋白基因 *TJP1* 和 *TJP2* 的表現，然而卻顯著上調第二型發炎細胞激素 *IL5*、*IL13* 和 *IL33* 的表現

英文題目：Hypoxia-Inducible Factor HIF1α Co-localizes with the Forkhead Box Transcription Factor FOXA2 and Down-regulates FOXA2-Mediated Expression of Neuroendocrine Peptide α- and β-CGRP Genes and the Tight Junction Protein Genes *TJP1* and *TJP2*, whereas Up-regulates the Type 2 Inflammatory Cytokines *IL5*, *IL13* and *IL33* in Human Bronchial Epithelial Cells

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Background: Our recent study has reported that human bronchial epithelial cells (HBECs) cultured under both consecutive hypoxia and intermittent hypoxia-reoxygenation (H/R) display significantly increased expression of the mucin protein and gene MUC5AC, which is associated with increased expression of the hypoxia-inducible factor HIF1α under both hypoxia and H/R. Notably, previous studies have shown that MUC5AC expression is up-regulated by Th2 inflammatory cytokines including IL-5 and IL-13, and down-regulated by the forkhead box transcription factor FOXA2. To further decipher the molecular mechanisms underlying the regulatory interplay between hypoxia, HIF1α, FOXA2, and Th2 inflammatory cytokines in human airway epithelium, it is of interest to study the effects of consecutive hypoxia, intermittent H/R, and changes of HIF1α expression levels on the expression of Th2 inflammatory cytokines, FOXA2, and the downstream target genes of FOXA2, including the neuroendocrine cell marker calcitonin gene-related peptides α- and β-CGRP (encoded by *CALCA* and *CALCB* genes, respectively), and the epithelial barrier tight junction proteins ZO-1 and ZO-2 (encoded by *TJP1* and *TJP2* genes, respectively).

Method: The normal (NHBECs) and COPD-diseased (DHBECs) human bronchial epithelial cells were each derived from three distinct age-matched Caucasian donors and were all obtained from the Lonza Biotechnology Company in the U.S.A.. The HBECs were either transfected with a scrambled siRNA or empty cDNA vector, or transfected with *HIF1A* siRNA or *HIF1A*-overexpressing cDNA vector, followed by air-liquid interface (ALI) culturing under normoxia (21% O_2) for 3 days for cell proliferation, and subsequently cultured in the differentiation medium consecutively under 21% O_2 for another 18 days, or under 24/24-hour cycles of intermittent hypoxia-reoxygenation (H/R) (i.e., 1% O_2 and 21% O_2 alternately) for 18 days in total, or cultured consecutively under 1% O_2 for 9 days in total, followed by returning to 21% O_2 for another 9 days in total. Total mRNAs were then extracted from NHBECs and DHBECs cultured under different oxygen tensions, followed by microarray analyses, qPCR analyses and immunofluorescence staining.

Results: Both consecutive hypoxia and intermittent H/R significantly down-regulated expression of both α- and β-CGRP genes *CALCA* and *CALCB* as well as their upstream activators, the transient

receptor potential vanilloid type 1 gene *TRPV1* and the forkhead box gene *FOXA2*, and also the tight junction protein genes *TJP1* and *TJP2*, whereas up-regulated the type 2 inflammatory cytokine genes *IL5*, *IL13* and *IL33*, in both NHBECs and DHBECs. Transfection with *HIF1A* siRNA into HBECs at the beginning of the ALI culturing was sufficient to up-regulate *CALCA*, *CALCB*, *TRPV1*, *TJP1* and *TJP2* mRNA expression to the levels comparable with the expression levels under normoxia, while down-regulating Th2 cytokine genes *IL5*, *IL13* and *IL33* to the expression levels comparable to those under normoxia. Interestingly, transfection with *HIF1A*-overexpressing cDNA vector into HBECs cultured under normoxia was sufficient to significantly decrease the mRNA levels of *CALCA*, *CALCB*, *TRPV1*, *TJP1* and *TJP2* and significantly increase the mRNA levels of *IL5*, *IL13* and *IL33*. Nonetheless, it is noteworthy that neither transfection with *HIF1A* siRNA nor transfection with *HIF1A* cDNA significantly altered the mRNA or protein level of FOXA2, indicating that FOXA2 expression is not subject to regulation by HIF1α.

Notably, our immunofluorescence staining analyses indicated that CGRP immunostaining signals were detected in HIF1α$^-$/FOXA2$^+$ cells only, whereas MUC5AC immunostaining signals were detected in HIF1α$^+$/FOXA2$^-$ cells only, in agreement with the respectively positive and negative regulation of the CGRP and MUC5AC genes by FOXA2, and in contrary the respectively negative and positive regulation of the CGRP and MUC5AC genes by HIF1α.

Conclusion: Taken together, our study shows for the first time that HIF1α in human airway epithelial cells is capable of down-regulating *TRPV1*, *CALCA*, *CALCB*, *TJP1* and *TJP2* mRNA expression, and may regulate gene expression of the Th2 inflammatory cytokines, including the pro-tumorigenic cytokine IL-33, either directly or indirectly via CGRP inhibition. Down-regulation of the tight junction proteins *TJP1* (ZO-1) and *TJP2* (ZO-2) expression by HIF1α antagonizes the anti-EMT (epithelial-to-mesenchymal transition) effect of FOXA2 and may facilitate tumor metastasis. It remains to be studied whether HIF1α and FOXA2 mutually inhibits the transactivation activity of each other in the airway epithelium.