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Hif-αs fine-tune Sox9-dependent Extracellular Matrix Production in Chondrocytes

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INTRODUCTION: An avascular milieu is a hallmark feature of articular cartilage and therefore the expression of chondrocyte-specific matrix protein is orchestrated under hypoxic condition. Physiological responses under hypoxia are regulated by the ? subunits of hypoxia inducible factors: Hif-1? and Hif-2? (EPAS1). In chondrocytes, Sox9 has an essential role in regulation of type II collagen (Col2a1) and aggrecan (Agc1). Interestingly, expression level of COL2A1 in human osteoarthric articular chondrocytes is not well correlated with Sox9 expression, suggesting that the presence of modulators of Sox9 transcriptional activity. Here, we hypothesized that Hif-?s may regulate Sox9-dependent transcription of the extracellular matrix genes in chondrocytes in response to different oxygen tensions. We aimed to investigate the subcellular expression patterns of Hif-?s and correlate it to the expression levels of Sox9, Col2a1 and Agc1 in chondrocytes cultured at different oxygen tensions. We further investigated how each of the Hif-?s modulate the Sox9-dependent regulation on Col2a1 and Agc1 in chondrocytes using reporter assays.

METHODS: Mouse chondroprogenitor cells ATDC5 cells were cultured for 3 days in DMEM/F12 media supplemented with 10% fetal bovine serum at different oxygen tensions: extreme hypoxia (1% O2), intermediate hypoxia (5% O2) or ambient O2 tension (21% O2). Subcellular expression patterns of Hif-1?, Hif-2?,and Sox9 in ATDC5 cells cultured under different oxygen tensions were identified by immunofluorescence. Quantification of fluorescence intensity across the cell body was performed using ImageJ software and Western blot to confirm the expression levels of each of the Hif-?s in the nuclei and cytosol. Quantitative RT-PCR was exploited to determine the relative expression levels of Sox9, Col2a1 and Agc1. Promoter assays were utilized to test the effects of HIF-? subunits over-expression along with Sox9 on activities of luciferase, which is under the control of multimerized 48bp cis-acting element of Col2a1 or A1 element of Agc1 in ATDC5 cells. Data for relative gene expression and promoter activities was expressed in mean ± standard deviation and statistical significance was assessed by Student愠 t-test.

RESULTS: Expressions of Hif-1? and Hif-2? were only detected in the cell nuclei of ATDC5 cells cultured under hypoxia (5% O2 and 1% O2), but not for normoxia (21% O2). Interestingly, Hif-2? expression was diminished in the cell nuclei of ATDC5 cells

cultured at extreme hypoxia (1% O2) versus intermediate hypoxia (5% O2) (Fig. 1A). On the other hand, hypoxia (1% O2 or 5% O2) induced the expression of Col2a1 and Agc1 in comparison with normoxia (21% O2). In contrast, Sox9 remained at a similar expression level in ATDC5 cells cultured under all oxygen tensions (Fig. 1B). In the luciferase-based promoter assay, Sox9 demonstrated a dominant transcriptional role in the activation of transcription of Col2a1 and Agc1 in ATDC5 cells over HIF-? subunits. Overt synergistic up-regulation of Col2a1 and Agc1 (Fig. 1C) reporter activities was observed when both HIF-1? and Sox9 expression were induced. Strikingly, HIF-2? inhibited the Sox9-dependent transcriptional up-regulation of Col2a1 and Agc1 (Fig. 1C) reporter activities.

DISCUSSION: Our findings indicate an enhancement of Col2a1 and Agc1, but a constant Sox9 expression in ATDC5 cells when cultured under hypoxia versus normoxia, suggesting different oxygen tensions could modulate extracellular matrix production in chondrocytes. We show that Hif-1? is a positive modulator, whereas Hif-2? acts as a negative modulator for Sox9-dependent transcription of Col2a1 and Agc1. Interestingly, extreme hypoxia (1% O2) favours the induction of Hif-1? in the nuclei, but diminishes the nuclei expression of Hif-2? in ATDC5 cells. These findings are consistent with the strong positive regulatory role of hypoxia in chondrogenic differentiation. Taken together, our study demonstrates a direct manipulation of Sox9 transcriptional activity by oxygen tension through an orchestration with different Hif-? subunits.

SIGNIFICANCE: Our study illustrates how oxygen tension fine-tunes extracellular matrix production in chondrocytes, thereby support an essential role of oxygen homeostasis in the maintenance of cartilage function. Precise control of the Hif-? activities may have an unidentified potential in cartilage engineering.