

The Role of Hypoxia-inducible H19-derived miR-675 in Promoting Chondrocyte Function

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Introduction

MicroRNAs are endogenous regulators of gene expression whose function may be as important as transcription factors. We have previously found H19, a non-coding RNA which functions as a primary microRNA to be both highly expressed and hypoxia-inducible in healthy primary human chondrocytes (Lafont et al, 2008). We now investigate its function.

Materials and Methods

Using real-time PCR-based assays, both primary microRNA transcript H19, and the subsequently derived mature microRNA – miR-675 were measured in isolated normal (non-diseased) primary human articular chondrocytes (HACs). Experiments with HACs were performed both in 20% oxygen and the more physiological 1% oxygen tension. Both inhibition (using specific siRNA and antisense oligonucleotides) and overexpression (using microRNA mimics) studies were performed in HACs.

Results

Expression levels of primary microRNA transcript H19 were comparable to the most abundant (and important) matrix genes Col2a1 and Aggrecan in healthy primary HACs. RNA interference experiments showed that, like these matrix genes H19 was hypoxia-inducible specifically through HIF-2a, but not HIF-1a. In addition, again similarly to these cartilage-specific matrix genes, H19 hypoxic induction was largely abolished by depletion of transcription factor SOX9. MiR-675, the mature microRNA which derives from H19 was also hypoxia-inducible and SOX9-dependent in HACs. Finally, depletion of miR-675 significantly reduced hypoxic induction of cartilage matrix gene, Col2a1 (P<0.02).

Discussion

We have identified a specific microRNA (miR-675) in healthy primary human articular chondrocytes which is highly expressed, SOX9-dependent and which regulates expression of the most important cartilage matrix gene, Col2a1. Thus this potentially represents a new mechanism regulating cartilage matrix expression.

References

Lafont J.E., Talma S., Hopfgarten C. & Murphy C.L. (2008) Hypoxia promotes the differentiated human articular chondrocyte phenotype through SOX9-dependent and -independent pathways. *J. Biol. Chem.* 283, 4778-4786.