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Unique roles of microRNA140 and its host gene WWP2 in cartilage biology

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MicroRNAs (miRNAs) constitute a large family of approximately 21 nucleotide-long, non-coding RNAs that have emerged as key post-transcriptional regulators of gene expression[1-5]. In mammals, miRNAs are predicted to regulate approximately 30% of protein-coding genes[5]. miRNAs play key roles in many cellular processes by regulating gene expression through translational repression or mRNA degradation. As their roles are revealed, miRNAs have become a major focus in biology[5,6]. Among known miRNAs, miRNA140 (miR-140) is strongly expressed in cartilage of developing zebrafish and mice[7,8]. miR-140 is encoded in an intronic region of the ubiquitin E3 ligase, *WWP2*. These genes are likely transcribed as a single primary transcript. The miR-140 gene is highly conserved among vertebrates including zebrafish, mice and humans; however, it is not present in invertebrates. This suggests that miR-140 plays an important role in vertebral skeletal development. We have reported that miR-140 regulates PDGF signaling and thereby modulates palatogenesis in zebrafish[9]. However, the role of miR-140 in the regulation of axial and appendicular skeletons is still unknown. In addition, the role of the host gene of miR-140, *WWP2*, in the skeletal system is unknown.

We investigated the biological roles of miR-140 in zebrafish and mammalian cell lines. First, we performed *in situ* hybridization to examine the expression of miR-140 in zebrafish. The expression of miR140 was strongly localized in cartilage, suggesting that miR-140 plays an important role in cartilage development. Then, the function of miR-140 was investigated *in vivo* using loss-of-function and gain-of-function approaches. In zebrafish, depletion of miR-140 using anti-sense oligonucleotides (Morpholinos: MOs) significantly decreased the size of the pharyngeal cartilage. This phenotype was rescued when miR140 duplex was co-injected. When miR-140 was over-expressed, the size of the pharyngeal cartilage was significantly increased. However, we do not yet know the mechanism by which miR-140 regulates facial cartilage development. Based on the expression pattern of miR-140 in zebrafish cartilage, we hypothesized that the transcription factor Sox9, a master regulator of cartilage development, might regulate miR-140 expression. Analysis of Sox9 mutant zebrafish revealed that the expression of the primary transcript for miR-140 was lost in the pharyngeal arch. When Sox9 was knocked down in human chondrocytic cells using siRNAs, the expression of miR-140 primary transcripts was decreased. These observations suggest that Sox9 is upstream of miR-140 expression. We are currently undertaking experiments to determine whether Sox9 directly regulates miR-140 expression, or whether it lies further upstream in the expression pathway.

We also investigated the expression and function of *WWP2* in zebrafish. *WWP2* was strongly expressed in cartilage, a result consistent with the notion that *WWP2* and miR-140 are derived from a common transcript. Knock-down of *WWP2* by MOs caused a fusion of palate and trabeculae, a phenotype different from that of miR-140 deficiency. These results indicate that miR-140 and its host gene *WWP2* have distinct roles in regulating cartilage development.

Our long-term goal is to better understand the physiological roles of miR-140 and *WWP2* during skeletogenesis using zebrafish and mammalian cell lines, and to extend these studies to other model organisms.

The authors have no conflict of interest.

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