Chondrocyte-Specific MicroRNA-140 Regulates Endochondral Bone Development and Targets Dnpep To Modulate Bone Morphogenetic Protein Signaling

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MicroRNAs (miRNAs) play critical roles in a variety of biological processes in diverse organisms, including mammals. In the mouse skeletal system, a global reduction of miRNAs in chondrocytes causes a lethal skeletal dysplasia. However, little is known about the physiological roles of individual miRNAs in chondrocytes. The miRNA-encoding gene, Mir140, is evolutionarily conserved among vertebrates and is abundantly and almost exclusively expressed in chondrocytes. In this paper, we show that loss of Mir140 in mice causes growth defects of endochondral bones, resulting in dwarfism and craniofacial deformities. Endochondral bone development is mildly advanced due to accelerated hypertrophic differentiation of chondrocytes in Mir140-null mice. Comparison of profiles of RNA associated with Argonaute 2 (Ago2) between wild-type and Mir140-null chondrocytes identified Dnpep as a Mir140 target. As expected, Dnpep expression was increased in Mir140-null chondrocytes. Dnpep overexpression showed a mild antagonistic effect on bone morphogenetic protein (BMP) signaling at a position downstream of Smad activation. Mir140-null chondrocytes showed lower-than-normal basal BMP signaling, which was reversed by Dnpep knockdown. These results demonstrate that Mir140 is essential for normal endochondral bone development and suggest that the reduced BMP signaling caused by Dnpep upregulation plays a causal role in the skeletal defects of Mir140-null mice.

MicroRNAs (miRNAs) are endogenously produced, small RNAs that regulate gene expression mainly at the posttranscriptional level. Direct binding of miRNAs to their target RNAs usually suppresses gene expression and facilitates RNA degradation (1, 3, 39). It is suggested that miRNAs act not only as genetic buffers to suppress harmful leaky gene expression that may compromise the robustness of cellular phenotypes but also as drivers of phenotypical diversity (14). miRNAs have been shown to regulate important biological functions in diverse organisms, including mice (33). In mouse chondrocytes, global miRNA deficiency caused by conditional ablation of Dicer, a gene encoding an RNase III that catalyzes miRNA maturation, results in reduced proliferation and accelerated hypertrophic differentiation downstream of the parathyroid hormone-related peptide (PTHrP) signaling pathway, presumably at a level that inhibits premature hypertrophic differentiation (20). Because chondrocytes express several hundred detectable miRNAs, it is unclear which particular miRNAs are responsible for the skeletal defects of Dicer-deficient chondrocytes. Clarifying the physiological roles of individual miRNAs in chondrocytes is critical for us to further understand the miRNA-mediated gene regulatory system in the skeletal system and to ultimately harness this system for treatment of skeletal diseases.

MicroRNA-140 (miR-140) and -140*, encoded by the Mir140 gene, are expressed almost exclusively in chondrocytes (38, 40). Mir140 is evolutionarily conserved among vertebrates, but this gene is not present in invertebrates. These findings suggest that Mir140 confers certain advantages to vertebrate skeletal systems.

A previous study using zebrafish embryos demonstrated that mir140 regulates palate chondrogenesis by inhibiting the expression of platelet-derived growth factor receptor, alpha polypeptide (PDGFRA) (9). Derepression of PDGFRA signaling appears to influence the migration of neural crest-derived mesenchymal cells to the rostral region, which subsequently affects the formation of cartilaginous elements of the head. However, this study provides little insight into the role of Mir140 in chondrocytes, the major cell type expressing this miRNA gene. In order to understand the physiological role of Mir140, we generated Mir140-null mice. Mice lacking Mir140 showed a shortening of endochondral bones, likely due to mildly accelerated chondrocyte differentiation into postmitotic hypertrophic chondrocytes. Furthermore, we have experimentally identified Dnpep as a Mir140 target whose upregulation may play a causal role in the skeletal defect of Mir140-null mice by dampening bone morphogenetic protein (BMP) signaling.

MATERIALS AND METHODS

Generation of Mir140-null mice. The targeting vector was constructed to replace the 55-nucleotide sequence of the Mir140 gene by an FLP recombinant target (FRT)-flanked neomycin-resistant gene cassette (FRT-neo) (Fig. 1A to C). The 4.4-kb-long 5′ homologous arm was PCR amplified using the high-fidelity polymerase PrimeStar (Takara-Mirus) with the primers mir140-5-S, 5′-GAGTCTGGACCTGGGTGGTTTTAG-3′, and mir140-5-AgeII, 5′-GTAAAACCACTGGCAGGACACAGAG-3′. The correct sequence was confirmed.

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mi140-3-Spe1Hind3, 5′-ACTAGTAAGCTTACTGGAGCACCCTCTGCATCAGAG-3′, and mi140-3-Not1, 5′-GCGGCCGCAGTGAGTAACAGTCT-3′. FRT-neo, a kind gift from Susan Dymecki, was ligated between the homologous arms. A diphtheria toxin fragment A (DT-A)-negative selectable marker was placed outside the 3′ homologous arm. The linearized targeting vector was electroporated into V6.5 embryonic stem (ES) cells (10), selected in the presence of 250 μg/ml G418. Homologous recombination was determined by Southern blot analysis and long PCR using the Phusion polymerase (New England BioLabs). ES cells carrying FRT-neo were subjected to blastocyst injection to generate chimeric mice. F1 mice carrying the mutation were further crossed to flpe transgenic mice (35) to remove FRT-neo. Mice were then backcrossed to the C57BL/6 strain for three generations. The animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) and were performed in accordance with its regulations and guidelines.

**Mouse genotyping.** The FRT-neo-free Mir140-null and wild-type alleles were detected by PCR using the primers Mi140-5S2, 5′-CTTGGTGGCCTCCTGTGCATCGTGTGGCTC-3′, and Mi140-3N, 5′-GAGCTAAACAATTGGGGAACAATC-3′, as 256-bp- and 233-bp-long PCR amplicons, respectively. Primer sequences to genotype Cre transgenic mice (19) and floxed Pdgfra mice (37) were described previously.

**Skeletal preparation and histology.** Alizarin red and Alcian blue staining was performed using a modified McLeod method (25). Carcasses were fixed in 95% ethanol, stained with Alcian blue and Alizarin red, cleared in 1% KOH, and kept in 50% glycerol. For histological analysis, mice were dissected, fixed in 10% formalin, decalcified in 10% EDTA, paraffin processed, cut, and subjected to hematoxylin-eosin staining, *in situ* hybridization, and BrdU staining. *In situ* hybridization was carried out as previously described (28). The probe for Col10a1 was described previously (19).

**BrdU labeling and detection.** For BrdU labeling, 50 μg BrdU/g of body weight was given to mice intraperitoneally 2 h before sacrifice. BrdU was detected using the BrdU staining kit (Invitrogen). The BrdU labeling index was calculated as the ratio of the number of BrdU-positive nuclei over the total number of nuclei in proliferating columnar chondrocytes of the growth plate.

**Primary chondrocyte isolation and culture.** The frontal part of the rib cage was removed from 1.5-day-old mice, rinsed with phosphate-buffered saline (PBS), and incubated in a digestion medium containing Dulbecco modified Eagle medium (DMEM), 10% fetal calf serum (FCS), and 0.2% (approximately 600 U/ml) collagenase type II (Worthington) at room temperature for 10 min. Muscle, soft tissues, and mineralized rib and sternal bones were removed from the rib cage using fine tweezers and surgical scissors under a dissection micro-

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**FIG. 1. Generation of Mir140-null mice.** (A) The Mir140 gene was replaced by an FRT-flanked, neomycin-resistant gene (Neo), which was subsequently removed by crossing mice with Flpe transgenic mice. (B) Southern blot analysis shows that an ES cell clone has both wild-type (Wt) and homologous recombinant (Rec) alleles. (C) PCR genotyping of mice using primers spanning FRT (see the small arrows in panel A). (D) Mir140-null mice show growth retardation in both male (left) and female (right) mice, whereas heterozygotes are indistinguishable from wild-type mice (n > 5). The body weight (BW) of Mir140-null mice was significantly lower than that of wild-type or heterozygous mice (n = 5; P < 0.05, t test) at all data points. (E) Picture of 3-week-old female mice. (F) miR-140 expression in primary rib chondrocytes is not detectable (N.D.) in Mir140-null mice, whereas miR-140 expression is reduced by approximately 50% in heterozygotes. The expression level of the host gene of Mir140, Wwp2, is not affected in Mir140-null mice.
Western blot analysis. Anti-phospho-Smad1/5/8 antibody (Cell Signaling Technology), anti-PDGFRα antibody (Millipore), anti-Dnpep antibody (Protein Tech Group, Inc.), anti-Smad4 antibody (GeneWay Biotech, Inc.), and antiactin antibody (Santa Cruz Biotechnology) were purchased. Western blot analysis was performed according to the standard procedure.

Microarray data accession number. The microarray data are available in the Gene Expression Omnibus (GEO) database under the accession number GSE27177.

RESULTS

Mir140-null mice show defects in endochondral bone growth.

In order to investigate the physiological role of the chondrocyte-specific miRNA gene, Mir140, mice lacking Mir140 were generated by gene targeting (Fig. 1A to C). Mir140-null mice survived postnatally and reached adulthood but showed impaired growth (Fig. 1D and E). The Mir140 gene is present in an intron of the Wwp2 gene, which encodes an E3 ubiquitin ligase. The deletion of the Mir140 gene did not alter the expression level of Wwp2 in primary chondrocytes, whereas miR-140 miRNA was not detectable in Mir140-null mice (Fig. 1F).

As suggested by the postnatal growth retardation of Mir140-null mice, long bones, which grow through endochondral bone formation, were significantly shorter in Mir140-null mice than in control mice (Fig. 2A and B). Another characteristic skeletal phenotype of Mir140-null mice was a defect in the longitudinal growth of the skull (Fig. 2C and D). Like long bones, longitudinal growth of the basal skull is mediated primarily by the growth plate cartilage. The shortening of these bones in the basal skull was detectable even in embryonic stages (Fig. 2E). A sagittal section of the basiphenoidal bone, flanked by bidirectional growth plates, at postnatal day 5.5 (P5.5) showed a reduction in longitudinal length (Fig. 2F).

Mir140 deficiency results in mild acceleration of chondrocyte differentiation and bone development. As in endochondral bones of the skull, mild skeletal abnormalities were observed in endochondral bones in other parts of the body at embryonic and neonatal stages. At birth, we found that talus bone development was consistently advanced in Mir140-null mice, as indicated by its large mineralized area (Fig. 3A and B). Similarly, the initial mineralization of the radius, ulna at embryonic day 14.5 (E14.5), and the hyoid horn at P1.5 was advanced in Mir140-null mice (Fig. 3C and see Fig. 5D). In situ hybridization to detect Coll10a1, a specific marker of hypertrophic chondrocytes, indicated an increase in the size of the hypertrophic region of the talus at P0.5 and tibia at E14.5 in Mir140-null mice (Fig. 3D and E). The size of the hypertrophic region of the tibia was significantly larger in Mir140-null mice (0.473 ± 0.003 mm versus 0.503 ± 0.008 mm) (n = 3 each; P < 0.05, t test) than in normal mice. These findings suggest that initial hypertrophic differentiation and subsequent bone formation occur prematurely in the absence of Mir140.

Premature differentiation of proliferating chondrocytes into postmitotic hypertrophic chondrocytes causes shortening of bones by reducing the number of proliferating chondrocytes, as exemplified by mutant mice lacking parathyroid hormone-related peptide (PTHrP) signaling, a pivotal regulator of chondrocyte differentiation (16, 22). It is therefore possible that mild acceleration of differentiation into postmitotic hypertrophic chondrocytes reduced the net mass of growth plate chon-
Over time, leading to the growth defects observed in Mir140-null mice.

We did not find an alteration in the proliferation of columnar chondrocytes in Mir140-deficient tibiae at P5.5 (Fig. 3F), P21, P14, P3.5, or E17.5 (data not shown). These results suggest that impairment of endochondral bone growth in Mir140-null mice is caused primarily by altered chondrocyte differentiation.

Reciprocal change in the sizes of the resting and columnar zones in the Mir140-null growth plate. In growth plates, chondrocytes in the resting zone proliferate slowly and then differentiate into vigorously proliferating columnar chondrocytes. Changes in cellular differentiation at this step influence the columnar length. Chondrocytes in the resting zone are small and do not form distinct columns, whereas columnar chondrocytes are flat and form orderly columns.

The growth plate morphology at early postnatal stages was relatively normal (data not shown). However, at later stages, when the secondary ossification center is already formed, we found that Mir140-null growth plates showed an increase in the size of the resting zone and a modest decrease in the size of the columnar zone (Fig. 4A to C). This finding suggests a possible inhibition of chondrocyte differentiation from the resting zone into proliferating columnar chondrocytes. We did not find significant shortening of the overall growth plate length in Mir140-null mice.

**Pdgfra is not a physiologically important target of Mir140 in mouse skeletal development.** It has been shown that miR-140 targets Pdgfra to control palatal skeletogenesis in zebrafish (9). Since Pdgfra is also predicted to be a miR-140 target in mice, we tested whether PDGFRA expression was altered in mouse chondrocytes lacking Mir140. We did not find upregulation of PDGFRA expression was altered in mouse chondrocytes lacking Mir140. We did not find upregulation of PDGFRA expression in Mir140-null primary rib chondrocytes (Fig. 5A), in contrast to results for zebrafish with the miR-140 knockdown. In order to examine the possible causal role of the loss of Mir140-dependent Pdgfra suppression in the skeletal defect of Mir140-null mice, we tested whether genetic deletion of Pdgfra in chondrocytes could rescue the skeletal defect of Mir140-null mice. The Pdgfra gene was ablated in chondrocytes using the Cre-loxP binary system; Cre mice expressing Cre in chondrocytes under the control of a type II collagen promoter.
were crossed to floxed Pdgfra (Pdgfrafl/fl) mice (37). Heterozygous conditional Pdgfra-null (Cre Pdgfrafl/fl) and compound heterozygous (Cre Pdgfrafl/fl Mir140/H11002) mice were indistinguishable from wild-type littermates. These mice were further crossed with Mir140-null mice to generate compound homozygous mutants (Cre Pdgfrafl/fl Mir140/H11002). In the genetic background used in this study, chondrocyte-specific Pdgfra-deficient mice (conditional knockout [cKO]) survived postnatally and reached adulthood. However, Pdgfra cKO mice showed a mild reduction in growth. The body weight of Pdgfra cKO mice at P28 was about 10% less than that of control littermates (11.5 ± 0.4 g versus 13.2 ± 0.3 g), but the mice showed grossly normal appearance. Despite the mild reduction in size, mineralization of the talus at P0.5 was slightly greater in cKO mice and similar to that of Mir140-null mice (Fig. 5B). At P1.5, the shortening of the long bones of Mir140-null mice is not rescued by conditional Pdgfra ablation in chondrocytes. (D) The advanced mineralization of the hyoid horns (arrowheads) of 1.5-day-old Mir140-null mice was even augmented but not rescued in doubly mutant mice.

Mir140 targets Dnpep. To understand the molecular mechanism by which Mir140 regulates endochondral bone development, we sought to identify target transcripts of Mir140 miRNAs. We took advantage of the finding that RNA immunoprecipitation using anti-Argonaute (Ago) antibodies can identify target transcripts of Mir140 miRNAs. We took advantage of the finding that RNA immunoprecipitation using anti-Argonaute (Ago) antibodies can identify target transcripts of Mir140 miRNAs.
We examined genes with relatively high signal intensities by quantitative reverse transcription-PCR (qRT-PCR) and confirmed that the gene encoding an aspartyl aminopeptidase, Dnpep, was enriched in the Ago2-IP fraction of wild-type but not Mir140-null chondrocytes (Fig. 6C). On the other hand, Hmga2 was similarly enriched in the Ago2-IP fractions of both wild-type and Mir140-null chondrocytes. As expected, Dnpep expression was upregulated in Mir140-null chondrocytes (Fig. 6D and E). To further confirm the interaction between miR-140 and Dnpep, we coinjected a miR-140 duplex and Dnpep mRNA into zebrafish embryos. Injection of miR-140 causes defects in palatal skeletogenesis in zebrafish (9). Coinjection of mouse Dnpep mRNA significantly reduced the occurrence of the miR-140-induced palatal defect (Fig. 7A). Interestingly, coinjection of rat Dnpep mRNA also had the same effect. Zebrafish Dnpep lacks strongly predicted miR-140 binding sites, and expression of endogenous zebrafish Dnpep was unchanged 1 and 3 days after miR-140 injection (data not shown), suggesting that miR-140 does not regulate zebrafish Dnpep; therefore, this rescue likely occurred because coinjected Dnpep sequesters miR-140. This result suggests a direct interaction between miR-140 and Dnpep transcripts. Bioinformatics analysis using STarMir (7) identified multiple potential binding sites of miR-140 in the full mouse and rat Dnpep sequence. However, only a single site located in the coding sequence was commonly predicted in mouse and rat Dnpep. This site is located in a 45-nucleotide-long region (designated BD) that contained two potential miR-140 binding sites in mouse Dnpep (Fig. 7B). To test whether the BD sequence could mediate the miR-140–Dnpep interaction, a luciferase reporter plasmid carrying the BD sequence was constructed. Cotransfection with a miR-140 miRNA precursor reduced the luciferase activity of the reporter construct with the BD but not a mutated BD sequence, suggesting that miR-140 suppresses Dnpep expression by binding to BD (Fig. 7C). Furthermore, transfection of the miR-140 precursor into chondrogenic ATDC5 cells reduced Dnpep expression (Fig. 7D).

Dnpep antagonizes BMP signaling downstream of Smad activation. Dnpep has been suggested to be involved in intracellular peptide metabolism, but its role in vivo is not known. In order to understand the possible role of Dnpep in vivo, first, we overexpressed Dnpep in zebrafish embryos. The majority of the injected embryos showed grossly normal development. However, we found that 4.2 to 6.3% of Dnpep-injected embryos showed dorsalization, whereas dorsalization was never observed in green fluorescent protein (GFP)-injected embryos (Fig. 8A and B). Dorso-ventral patterning in zebrafish embryos is controlled by BMP and Wnt signaling (36). Since dorsalized, Dnpep-injected embryos maintained anterior head structures, whose formation is antagonized by Wnt signaling, we speculated that dorsalization caused by Dnpep overexpression was due to reduced BMP signaling, rather than increased Wnt signaling. To test this possibility, we coinjected Bmp2b and Dnpep into zebrafish embryos to test whether Dnpep could antagonize the ventralization effect caused by Bmp2b overexpression (Fig. 8C and D). Dnpep coinjection caused a mild but significant inhibitory effect in the ventralization induced by Bmp2b. This result further supports the antagonistic effect of Dnpep in BMP signaling. In order to test whether this effect is conserved in mammalian cells, we overexpressed Dnpep along...
with a BMP reporter construct carrying the BMP-responsive element of the Id1 gene (18). Dnpep overexpression in BMP-responsive C2C12 cells showed significantly reduced responses to BMP-2 (Fig. 9A). Since Dnpep was upregulated in Mir140-null chondrocytes, we tested the BMP activity of Mir140-null primary rib chondrocytes. Primary rib chondrocytes were transfected with the BMP reporter construct and treated with BMP-2. We found that the reporter activity was consistently lower in Mir140-null chondrocytes than in the control (Fig. 9B) at any BMP concentration. However, upon stimulation with BMP-2, the reporter activity increased in a dose-dependent manner in both control and Mir140-null chondrocytes. Mir140-null chondrocytes showed a normal response in the phosphorylation of Smad1/5/8 upon BMP treatment, an immediate consequence of BMP receptor activation, and a normal level of the Smad1/5/8 binding partner, Smad4 (Fig. 9C and D). These results suggest that BMP-dependent gene transcription is compromised in Mir140-null chondrocytes at a position downstream of Smad activation. Next, we tested whether overexpression of Dnpep reduces the BMP reporter activity in primary chondrocytes. Primary chondrocytes were cotransfected with reporter constructs and a Dnpep expression vector and cultured in the presence of 50 ng/ml of BMP-2. Dnpep overexpression reduced the basal BMP reporter activity both in control and in Mir140-null chondrocytes (Fig. 9E). Conversely, Dnpep knockdown using siRNA increased BMP reporter activity in Mir140-null chondrocytes (Fig. 9F). These results show that Dnpep upregulation negatively regulates BMP signaling in Mir140-null chondrocytes.

**DISCUSSION**

We have shown that loss of the chondrocyte-specific miRNA gene, Mir140, in mice causes defects in endochondral bone development. Since there were no overt changes in the proliferation of Mir140-null growth plate chondrocytes, the growth defects observed in Mir140-null mice are likely caused by the acceleration of chondrocyte differentiation into postmitotic hypertrophic chondrocytes. Although the acceleration of hypertrophic differentiation is relatively mild, causing few detectable histological changes, it is possible that, over time, these defects negatively influence the production of the net chondrocyte mass, leading to a visible reduction in the skeletal size of Mir140-null mice. A recent study describing separately generated Mir140-null mice reports a reduction in the proliferation and shortening of the growth plates (27). In this study, we did not find significant reductions in chondrocyte proliferation or growth plate length at multiple stages of development. The reason for these disparities is not clear. We backcrossed mice to the C57BL/6 strain for three generations, and it is possible...
that the difference in the genetic background may influence the phenotype. We found a relative increase in the size of the resting zone and a mild decrease in the size of the columnar zone in 3-week-old Mir140-null mice. This finding suggests a possible inhibition of resting chondrocyte differentiation into columnar chondrocytes. Stimulation of chondrocyte differentiation at this step increases the length of the columnar zone (21). Therefore, it is likely that inhibition of this step would cause a reduction in proliferating columnar chondrocytes. As with acceleration of hypertrophic differentiation, inhibition of this step would also decrease the number of proliferating chondrocytes, resulting in the shortening of endochondral bones.

The skeletal abnormalities of Mir140-null mice differ from those reported in zebrafish with a miR-140 knockdown (9). Mice lacking Mir140 show a shortening of the palate rather than the elongation observed in zebrafish with miR-140 knocked down. The zebrafish study demonstrated that miR-140 suppressed the expression of PDGFRA, which regulates the migration of neural crest-derived mesenchymal cells to the rostral region. Although Pdgfra is also predicted to be a target of mir-140 in mice, we did not find significant alterations in PDGFRA expression in Mir140-null chondrocytes. In addition, genetic ablation of Pdgfra did not lessen the skeletal defect of Mir140-null mice. These data demonstrate that, unlike in zebrafish palatal development, Pdgfra is not a physiologically important target in mouse chondrocytes. This finding underscores the possibility that a miRNA may have substantially different sets of target transcripts and thus may play different physiological roles, dependent on animal species and cell types.

Identification of miRNA targets has been a challenge. Bioinformatics approaches have offered only limited solutions. Since bioinformatics approaches rely solely on base pairing of miRNAs and target RNAs, they do not take into account particular cellular contexts, such as expression levels of target RNAs and miRNAs. Therefore, to identify physiologically meaningful targets of miRNAs, it is necessary to experimentally examine miRNA-RNA interactions under specific conditions. A few methods have been developed to experimentally identify miRNA targets. Based on the fact that miRNAs bind to target RNAs through base pairing, miRNA-target RNA complexes were successfully isolated by pulldown assays.
using biotin- or digoxigenin-tagged miRNAs (15, 31). However, these methods may not reveal physiologically relevant interactions because tagged RNAs need to be exogenously added. In this study, we performed RNA immunoprecipitation using anti-Ago2 antibody (Ago2-IP) to isolate RNAs regulated by endogenous miRNAs in chondrocytes. Since Mir140 is one of the most abundant miRNAs in chondrocytes, we reasoned that Mir140 target RNAs should be enriched in the Ago2-IP fraction along with targets of other miRNAs expressed in chondrocytes and, therefore, that comparing profiles of Ago2-associated RNA between wild-type and Mir140-null chondrocytes would reveal target transcripts regulated mainly by Mir140. We found only 44 genes (data not shown), including Dnpep, that satisfied the screening criteria. Since many RNAs can be regulated by multiple miRNAs, it is likely that this strategy can identify only RNAs whose expression is regulated exclusively by Mir140. This screening did not find previously reported targets of mir-140, such as Pdgfra (9), Adams5 (27), Hdad4 (38), or Cxcl12 (30). It is possible that these genes may also be regulated by other miRNAs, and therefore, loss of mir-140 alone does not significantly reduce the interaction with Ago2.

Coinjection of both mouse and rat Dnpep transcripts suppressed the miR-140-induced palatal defect in zebrafish, but the only commonly predicted binding sequence was found in the coding sequence. It has been increasingly appreciated that miRNAs bind sequences beyond the 3′ untranslated region of genes (6). Since this short sequence was able to reduce reporter activity in a miR-140-dependent manner, it is likely that Dnpep expression is regulated by miR-140, at least in part, through this sequence.

Dnpep encodes an aspartyl aminopeptidase that catalyzes the sequential removal of amino acids from the unblocked N termini of peptides and proteins. Although Dnpep is expressed broadly, its physiological role in mammalian cells is poorly understood. It is speculated that aspartyl aminopeptidases are involved in protein and peptide metabolism and possibly in protein and peptide metabolism and possibly in the BMP reporter activity.


