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MicroRNAs in Cartilage Development and Dysplasia

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Abstract

Small regulatory microRNAs (miRNAs) post-transcriptionally suppress gene expression. MiRNAs expressed in skeletal progenitor cells and chondrocytes regulate diverse aspects of cellular function and thus skeletal development. In this review, we discuss the role of miRNAs in skeletal development, particularly focusing on those whose physiological roles were revealed *in vivo*. Deregulation of miRNAs is found in multiple acquired diseases such as cancer; however congenital diseases caused by mutations in miRNA genes are very rare. Among those are mutations in miR-140 and miR-17~92 miRNAs which cause skeletal dysplasias. We also discuss pathological mechanisms underlining these skeletal dysplasias.

Keywords

cartilage development; chondrocytes; skeletal dysplasia; microRNAs; miR-140; miR-17~92

1. Introduction

Epigenetic regulation encompasses various types of gene regulatory mechanisms that do not involve changes in DNA sequences. It is mediated by mechanisms such as DNA methylation, histone modification, and regulatory noncoding RNAs including microRNAs (miRNAs) [1, 2]. Epigenetic regulation is vital to multiple essential biological processes [2, 3]. For example, histone modification and DNA methylation are two main mechanisms of chromatin remodeling that regulate gene transcription as well as DNA repair and replication [4, 5]. MiRNAs are regarded as another layer of epigenetic regulation, and constitute a large family of approximately 17–25 nucleotide-long single-stranded RNAs that regulate more than 60% of protein-coding genes in the human genome at the post-translational level [6–8].

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Deregulation of miRNAs is reported in many human diseases including skeletal dysplasia and cartilage diseases [9–13].

For the vast majority of miRNAs, their biogenesis in mammals is a two-step process. The process begins in the nucleus where miRNAs are transcribed as a long primary transcript (pri-miRNA) that contains intramolecular hairpin loops formed via self-complementary nucleotide sequences [14]. The microprocessor complex, comprised of DGCR8 and the RNase III endonuclease, Drosha, carries out nuclear cleavage of pri-miRNAs into small hairpin pre-miRNAs. Subsequently, these small hairpin pre-miRNAs are transported into the cytoplasm by Exportin-5 and Ran-GTP [15]. Pre-miRNA maturation in the cytoplasm is further executed by the RNase III endonuclease, Dicer which cleaves pre-miRNAs to remove the loop structure leaving double-stranded miRNAs (miRNA-5p and miRNA-3p from the 5' and 3' strand, respectively). One of the strands is then selected and loaded onto the RNA induced silencing complex (RISC) where Argonaute protein (Ago)-bound miRNAs interact with their target RNAs [16, 17]. A miRNA and its target RNA interact mainly through a short complementary sequence located at nucleotides 2–8 of the 5' end of a miRNA, called “seed” [18]. MiRNA binding suppresses translation of target RNAs and subsequently induces their degradation [7, 19, 20].

MiRNAs are differentially expressed depending on tissue and cell types. Multiple miRNAs are expressed in chondrocytes where they play key roles in diverse aspects of cartilage development, physiology, and pathology [21]. In this review we focus on several miRNAs whose physiological and/or pathological significance was demonstrated in skeletal development.

2. MicroRNAs in cartilage development and homeostasis

Bone and cartilage are the two main tissues that provide the structural framework to the skeleton. Cartilage is composed of chondrocytes and extracellular matrix whose major components include type II collagens and proteoglycans such as aggrecan [22, 23]. Three major types of cartilage, *i.e.*, elastic cartilage, hyaline cartilage, and fibrocartilage, are distinguished by relative amounts of collagen and proteoglycan [24]. In addition to being an important structural part of the skeleton, cartilage also serves as a temporary template for mineralized bone to drive bone development and growth of the skeleton. During this process, called endochondral bone formation, mesenchymal cells first differentiate into chondrocytes to form a cartilaginous template, which is subsequently replaced by mineralized bone. This hyaline cartilage, the growth plate, located in the metaphysis of a long bone drives longitudinal bone growth until it closes [25, 26]. Chondrocytes in the growth plate undergo sequential differentiation steps; slowly proliferating resting chondrocytes differentiate into proliferating chondrocytes that form orderly columns, and then differentiate into post-mitotic hypertrophic chondrocytes. Hypertrophic chondrocytes differentiate further, mineralize, and mostly die to be replaced by bone tissue [27]. These processes are regulated by multiple mechanisms including signaling molecules, such as BMPs, FGFs, Indian hedgehog (Ihh), and PTHrP, transcription factors including Sox9 and Mef2, and epigenetic regulators including miRNAs [21, 27, 28].

2.1 Consequences of disruption of miRNA processing

The significance of miRNAs for animal development was demonstrated by a study where loss of Dicer, which eliminated most miRNAs, caused early embryonic lethality in mice [29]. Dicer is also essential for normal skeletal development; conditional Dicer ablation in chondrocytes in mice results in significant growth defects and premature death. Dicer deficiency reduced chondrocyte proliferation and accelerated differentiation into postmitotic hypertrophic chondrocytes [30]. Deletion of Drosha also reduces the number of most miRNAs by blocking the first step of miRNA processing, and conditional Drosha ablation in articular chondrocytes of postnatal mice resulted in pathological changes including increased cell death and mild osteoarthritis [31]. These findings emphasize the importance of miRNAs in chondrocyte proliferation, differentiation, and survival.

The role and molecular function of individual miRNAs in cells of the skeletal lineage have been extensively studied in various experimental systems, and these findings have been described in a large body of literature. Most of these studies were conducted *in vitro* using different types of cells, such as mesenchymal stromal cells and cell lines. Although these studies substantially increased our understanding of the roles and functions of miRNAs at cellular and molecular levels, their physiologic significance is yet to be determined. Therefore, in this review we focus on a relatively limited number of studies that have demonstrated the physiological significance of individual miRNAs in skeletal development using *in vivo* animal models or human genetics approaches.

2.2 MiR-140

Several individual miRNAs have been shown to play important roles in cartilage development and homeostasis. MiR-140 is abundantly and relatively specifically expressed in chondrocytes [32, 33]. MiR-140 is encoded in an intronic region of the gene encoding ubiquitin E3 ligase, Wwp2 [34], and its expression appears to be regulated in both *Wwp2* transcription-dependent [35] and independent manner [36]. The robust expression of *Wwp2* and miR-140 in chondrocytes appears to be mediated by the associated super enhancer [11]. Sox9, the master transcription factor essential for chondrocyte differentiation and survival, is an important regulator of miR-140 expression [34]. In *Sox9* mutant zebrafish, miR-140 (primir-140) expression was substantially decreased. Moreover, *SOX9* siRNA transfection into human chondrocytic cells inhibited the expression of miR-140 in a dose-dependent manner, suggesting that the regulation of miR-140 by *Sox9* is conserved [37]. While Sox9 regulates miR-140 expression, the converse may also be true [38]. In human mesenchymal stem cells (hMSCs), miR-140-5p targets Ras-related protein Ral-A (RALA) that suppresses SOX9 expression. MiR-140-5p inhibition impairs chondrocyte differentiation and is associated with downregulation of SOX9 [38], suggesting that SOX9 and miR-140 may form a positive feedback loop to enhance the chondrocyte phenotype. This positive feedback may be limited to the stage of initial chondrogenic differentiation of mesenchymal progenitor cells, as Sox9 expression was not significantly altered in mature miR-140-null chondrocytes [39].

The role of MiR-140 was studied in different experimental settings [40]. Depletion of miR-140 in zebrafish using antisense oligonucleotides significantly decreased the size of the pharyngeal cartilage, while overexpression of miR-140 increased it [34]. In mice, miR-140-

deficiency reduced cell proliferation, delayed differentiation of resting chondrocytes to proliferating chondrocytes, and mildly accelerated hypertrophic chondrocyte differentiation in the growth plate, which led to shortening of endochondral bones, dwarfism, and craniofacial malformations [41, 42]. MiR-140-null mice also develop spontaneous osteoarthritis. At 3 months of age, miR-140-null mice start developing signs of early osteoarthritis, such as progressive proteoglycan loss and cartilage fibrillation [42]. Adamts-5, an enzyme involved in degradation of articular cartilage in osteoarthritis, was found to be directly regulated by miR-140, suggesting that miR-140 regulates both endochondral bone development and articular cartilage homeostasis [42, 43]. The precise mechanism by which miR-140 regulates longitudinal endochondral bone growth is still unknown. However genetic interaction studies suggest that miR-140 is an important component of the PTHrP/HDAC4/MEF2 axis that controls growth plate chondrocyte differentiation. While miR-140 is not the primary regulator of chondrocyte differentiation, it provides robustness to this process by modulating the PTHrP/HDAC4/MEF2 pathway [39]. In addition, miR-140 has been shown to target many other genes, such as *Pdgfra*, *43SP1*, *Rala*, *Igfbp1*, *MMP-13*, and *Smad3*, although the physiologic significance of miR-140 regulation of these target genes in skeletal development remains to be determined [21, 27, 40].

2.3 Let-7 family miRNAs

As in other somatic cells, let-7 miRNAs are the most abundant miRNAs in chondrocytes. Major targets of let-7 miRNAs include *Ras* [44], *Hmga2* [45, 46], and *Igf2bp's* [47] that regulate cell proliferation. Our study suggests that while miR-140 mainly regulates chondrocyte differentiation, let-7 family miRNAs regulate chondrocyte proliferation in the growth plate [48]. In this study, let-7 miRNAs were suppressed in the developing limb mesenchyme and growth plate by overexpressing the RNA binding protein, lin-28 homolog A (Lin28a), which inhibits let-7 miRNA processing via binding to the loop region of pri- and pre-let-7 miRNAs [49]. Lin28a overexpression in the limb mesenchyme caused limb overgrowth, while in growth plate chondrocytes, it decreased chondrocyte proliferation, increased cell death, and caused a modest growth defect via upregulation of let-7 target genes [48]. Lin28a overexpression in the joint primordium stimulated Ras/MAPK signaling and increased the size of articular cartilage [50]. Although these results should be interpreted with caution since Lin28a has additional functions other than inhibition of let-7 biogenesis, these findings suggest that the let-7 miRNAs' regulatory role of cell proliferation is dependent on the cellular context.

2.4 MiR-17~92 cluster miRNAs

The mir-17~92 cluster gene (*MIR17HG* in humans and *Mirc1* in mice) produces a single polycistronic transcript that yields 6 mature miRNAs, including miR-17, miR-18a, miR-19a, miR-20a, miR-19b, and miR-92a [51]. Together with two paralogous miRNA genes, miR-106b-25 and miR-106a-363, miR-17~92 family miRNAs consist of 15 miRNAs that belong to 4 distinct seed groups [52]. MiR-17~92 cluster miRNAs are oncogenic miRNAs. Their amplification or aberrant elevation are frequently observed in different types of tumors [52]. MiR-17~92 miRNAs were originally identified as miRNAs induced by MYC, an oncogenic transcription factor overexpressed in approximately half of human cancers [52].

In addition to MYC, additional transcription factors regulate their expression including E2F transcription factors [53]. Mir-17~92's major targets include *Pten*, a negative regulator of PI3K/Akt signaling [54], Bim, an antiapoptotic factor, and cyclin-dependent kinase inhibitors [51, 53, 55]. Thus, miR-17~92 miRNAs appear to be ones of downstream effectors of these transcription factors regulating cell cycle progression.

The miR-17~92 miRNAs are abundantly expressed in mesenchymal cells, but their expression decreases with cell differentiation [56, 57]. MiR-17~92 miRNAs are essential for normal animal development promoting both cell proliferation and cell survival [51]. Knockout mice missing the entire miR-17~92 gene as well as individual seed-groups miRNAs were generated and analyzed [51, 58]. Deletion of the miR-17~92 gene in mice results in prenatal lethality with multiple developmental defects, including a ventricular septal defect and lung hypoplasia [51]. Compound mutants of the miR-17~92 and miR-106b-25 genes with or without miR106a-363 deletion showed a more severe phenotype than single miR-17~92 deletion, causing earlier embryonic lethality. Mice missing only miR-17 seed group miRNAs of the miR-17~92 cluster gene developed skeletal abnormalities including brachydactyly, dwarfism, and homeotic transformation of the axial skeleton, whereas mutant mice missing other seed group members did not show overt skeletal abnormalities other than a modest weight reduction, demonstrating the pivotal role of miR-17 seed group miRNAs in skeletal development [58]. Another study described craniofacial phenotypes in mice missing miR-17~92 and/or miR-106b-25. The authors proposed that miR-17~92 modulates expression of T-box transcriptional regulators essential during midface development and that miR-17~92 expression is regulated by Bmp-signaling and AP-2 α , a transcription factor important for craniofacial development [59].

The mechanism by which miR-17 miRNAs regulate skeletogenesis was investigated by gene expression analysis; Gene Ontology (GO) analysis using tail bud RNA-seq datasets of miR-17 seed group miRNA mutant mice suggested that miR-17 group miRNAs may regulate many aspects of skeletal development, as significantly enriched GO terms with their associated genes included mesoderm formation (Bmp4 and Bmpr2), specification (Tbx6), segmentation (Notch2, Jag1, Dll3, Dll1), axial patterning (Gdf11, Hoxa5, Hoxa6, Hoxb4), and limb development (Tbx3, Bmp4) [58].

These *in vivo* findings revealed that miR-17~92 miRNAs, let-7 miRNAs, and miR-140 coordinately regulate skeletal development in a stage-specific manner (Fig. 1).

2.5 Other miRNAs

Mouse genetic models have provided evidence for involvement of other miRNAs in skeletal development. The Dnm3os gene, encoding two miRNAs, miR-199a-2 and miR-214, is located in the 14th intron of the mouse dynamin-3 (Dnm3) gene and is transcribed from the opposite strand of Dnm3 [60]. In a study where a lacZ gene was introduced into the Dnm3os locus to disrupt its expression, Dnm3os(lacZ/lacZ) homozygous mice exhibited various skeletal abnormalities such as growth retardation, osteopenia, craniofacial hypoplasia, and defects in dorsal neural arches and spinous processes of the vertebrae, and most of the pups died shortly after birth [61]. Another recent study, where miR-199a-2 was specifically ablated, demonstrated that miR-199a-2 loss in mice causes a phenotype similar to human

Rett syndrome including the marked body size reduction [62]. In contrast, miR-214 knockout mice have no overt skeletal abnormalities [63]. Considering that mice with miR-199a-2 single deletion do not exhibit perinatal lethality unlike Dnm3os knockout mice, miR-214 and miR-199a-2 may have overlapping roles. The mechanism by which these miRNAs control animal development is unclear. However, since it was reported that miR-199a-3p and miR-193b are linked to accelerated aging of chondrocytes in humans [64], it is possible that loss of Dnm3os-encoded miRNAs directly regulate chondrocyte behavior and thus endochondral bone development. In fact, the possible role of miR-214 in skeletal development was investigated by its overexpression; miR-214 overexpression decreases chondrocyte differentiation by targeting *Atf4*. Because *Atf4* ablation in mice (*Atf4*^{-/-}) reduces chondrocyte proliferation and increases hypertrophic chondrocytes possibly through downregulation of *Ihh* expression [65], miR-214 might indirectly fine-tune the balance of chondrocyte proliferation and differentiation. This notion is reinforced by another animal model; miR-214 overexpression impeded cranial cartilage formation in zebrafish and elicited concomitant downregulation of *Atf4*, *Col2a1*, and *Sox9*, supporting its regulatory role on *Atf4* expression and cartilage development [66].

By miRNA profiling of femoral growth plates of 12-day-old mice comparing different zones of the growth plate, miR-322 and miR-503 were found to be upregulated in differentiated chondrocytes. The *Mirc24* gene, encoding miR-322, miR-351, and miR-503, was ablated in mice in a tissue specific manner. Most mice lacking *Mirc24* in the cartilaginous tissue died shortly after birth likely due to tracheal growth restriction and respiratory failure. The authors found that miR-322 altered ERK signaling and suggested that miR-322 might fine-tune chondrocyte differentiation via modulation of MAPK signaling during endochondral bone formation, although *Mirc24*-deficient long bones have not been characterized [67]. Another study identified miR-503 as one of miRNAs differentially expressed between the proliferating and hypertrophic zones of juvenile rats. MiR-503 along with two other proliferating zone-specific miRNAs were regulated by PTHrP signaling, and inhibition of these miRNAs decreased proliferation of primary epiphyseal chondrocytes and increased hypertrophic differentiation [68].

Mice missing the *Mirc33* gene encoding miR-29a and miR-29b-1, show growth retardation and develop kyphosis starting around 4 months of age. Additional deletion of the miR-29b-2 and miR-29c (*Mirc29*) caused greater growth defects and premature death. The mutant mice have diverse systemic defects in adipose, nervous, ocular, and urinary systems, and therefore, it is not clear whether loss of miR-29a/b-1 directly affects cells of skeletal tissues [69, 70], although its direct role in skeletal cells was suggested in articular chondrocytes, as miR-29b-3p, upregulated in osteoarthritis, negatively regulated Smad, NFκB, and canonical WNT signaling pathways [71].

In addition to the “*in vivo*” studies discussed above, a plethora of studies investigating roles of miRNAs in chondrogenesis and cartilage homeostasis in various experimental systems have been published. These studies are summarized well in recent review articles [1, 72, 73]. Many miRNAs were demonstrated to regulate chondrogenesis *in vitro* [74–76]. For example, in a study where hMSC were subjected to transforming growth factor-β (TGF-β)-driven chondrogenesis, miRNA analysis revealed that among 1349 probed miRNAs, 553

were expressed and 169 were significantly regulated during chondrogenesis. Levels of miR-23b, miR-140, miR-181, and miR-210 positively correlated with successful chondrocyte formation [77]. Many more *in vitro* findings await *in vivo* validation. Many miRNAs identified as chondrogenic *in vitro* have been studied as potential targets for osteoarthritis treatment. For example, treatment with miR-210-overexpressing lentiviruses decreased inflammation in a rat osteoarthritis model by suppressing death receptor 6 (DR6, TNFRSF21) expression and thus inhibiting NF- κ B signaling [78]. In another study, intra-articular administration of miR-210 facilitated healing of the damaged white zone meniscus in rats through promotion of collagen type II production by meniscus cells and upregulation of VEGF and FGF2 production by synovial cells [79].

3. MicroRNAs in skeletal dysplasia

Deregulation of miRNAs is found in a variety of diseases including cancer. However, only a few congenital diseases have been reported to be associated with mutations in miRNA genes or miRNA target regions. Among those, mutations in genes encoding miR-140 and miR-17~92 miRNAs have been reported to cause skeletal dysplasia in humans. In this section we discuss pathological mechanisms underlining these skeletal dysplasias.

3.1 MiR-17~92 cluster miRNAs and Feingold syndrome

The miR-17~92 cluster gene (*Mir17HG* in humans, *Mirc1* in mice) was the first miRNA-coding gene of which mutations were found to cause skeletal developmental defects in humans. Its deletion leads to a brachysyndactyly syndrome (Feingold syndrome type 2) [13, 80], whereas its duplications are associated with different skeletal phenotypes; one family shows skeletal overgrowth with polydactyly [81], while another study reported a case of a 9-year-old boy with developmental delay, short stature, mild macrocephaly, hypertelorism, brachydactyly, and clinodactyly [82]. The reason for the phenotypic inconsistency is unclear; it could be explained by possible differences in genetic and non-genetic factors specific to each case including the miR-17~92 expression level.

Feingold syndrome is a skeletal dysplasia, characterized by short stature, brachysyndactyly, microcephaly, facial dysmorphism, gastrointestinal atresias, and a mild-to-moderate learning disability [83, 84]. The majority of cases are caused by loss-of-function mutations of *MYCN* (type 1) [85]. Another causative mutation recently identified was the deletions of *MIR17HG* that encodes miR-17~92 cluster miRNAs (Feingold syndrome type 2) [13]. Several key features of patients with Feingold syndrome were reproduced in mice with targeted deletion of the miR-17~92 cluster gene, confirming that Feingold syndrome type 2 results from haploinsufficiency of the miR-17~92 gene [13].

Since expression of miR-17~92 miRNAs is regulated by MYC transcription factors, it was postulated that Feingold syndrome types 1 and 2 were caused by a common molecular mechanism. However, our recent study using conditional knockout mouse models demonstrated that loss of miR-17~92 miRNAs causes molecular consequences distinct from those of *Mycn* loss [12]. Reduced miR-17~92 miRNAs upregulated TGF- β signaling, likely due to the derepression of *Tgfb2* by the loss of miR-17 seed group miRNAs, whereas *Mycn* deficiency had no effect on TGF- β signaling but downregulated PI3K signaling in limb

mesenchymal cells. Genetic or chemical inhibition of TGF- β signaling rescued the skeletal defects caused by miR-17~92 miRNA deficiency; however, the phenotype of *Mycn* deficient limbs was not rescued by TGF- β inhibition but was partially rescued by haploinsufficiency of *Pten* that encodes a negative regulator of PI3K signaling. Thus, despite the phenotypic similarity between Feingold syndrome type 1 and 2, distinct molecular mechanisms underlie the pathoetiology of these conditions [12].

A predicted miR-17-5p binding site and an overlapping miR-19-3p binding site in the *TGFBR2* 3' UTR are conserved in humans, mice and other vertebrates. Regulation of TGF- β signaling by miR-17~92 miRNAs was also reported in different settings [86, 87]. Therefore, in Feingold syndrome type 2 mouse models, the upregulation of TGF- β signaling reduces mesenchymal proliferation at the limb bud stage, which results in underdeveloped skeletal elements. However, chondrocyte-specific miR-17~92 deletion suggested that the direct impact of miR-17~92 deficiency on growth plate chondrocytes appears to be limited.

3.2 MiR-140 variant in a new skeletal dysplasia

We recently reported a novel autosomal dominant human skeletal dysplasia characterized by short stature with short limbs, small hands and feet, and midface hypoplasia. This skeletal dysplasia is distinct from other short stature skeletal dysplasia entities by its unique radiographic finding where the size of the epiphyseal ossification is disproportionally small. Patients in a Swedish pedigree and an unrelated case in Israel were found to have the same clinical features and an identical single nucleotide substitution (A > G) at the second nucleotide of the miR-140-5p-coding sequence (the first nucleotide of the seed) [11]. Analysis of the family members revealed that this substitution occurred *de novo* in probands of these families. Along with the abnormal vertebral shape, this condition was named spondyloepiphyseal dysplasia MIR140 (SED, MIR140 type) [88].

Since miR-140 is perfectly conserved in vertebrates, we generated knock-in mice with the same nucleotide substitution. Mutant mice show skeletal abnormalities similar to those of the patients, providing evidence for the causal role of this variant. Interestingly, homozygous mutant mice with this substitution (miR-140G/G) showed a cartilage phenotype distinct from that of miR-140-null (miR140-/-) mice [11, 41]. Both miR-140G/G and miR-140-/- mice are smaller and have shorter noses than wild type littermates, but only miR-140G/G and miR-140G/+ mice show delayed secondary ossification of the long bones, delayed cartilage differentiation of other cartilaginous elements of the skeleton with decreased expression of *Col10a1*, a marker of hypertrophic chondrocytes [11]. The delay in chondrocyte differentiation in the epiphysis appears to inhibit proper growth of epiphyses, what results in the characteristic epiphyseal dysplasia in the patients and mutant mice.

Small RNA-seq analysis using primary rib chondrocytes revealed that the mutant miR-140-5p (miR-140-5pG) as well as miR-140-3p were normally processed and expressed in mutant mice, while gene expression profiles were substantially different among wild type, miR-140G/G, and miR-140-/- mice. While target genes of wild type miR-140 were upregulated in both miR-140G/G and miR-140-/- chondrocytes, predicted mutant miR-140-5p target genes were downregulated. These findings suggest that this mutation

causes not only loss of function of wild type miR-140-5p but also gain of function of the mutant miR-140-5p.

Interestingly, gene sets most prominently downregulated in miR-140G/G, but not miR-140-/- chondrocytes, belong to the hypoxia inducible factor 1 (HIF1) pathway, which is essential for chondrocyte survival and is a major regulator of energy metabolism. The mutant miR-140-5p appears to bind to a conserved binding site in the coding sequence of Hif1a to downregulate it. Since Hif1a is essential for chondrocyte survival and regulates their function [89], it is possible that deregulation of the HIF1 pathway contributes to the gain-of-new function effect (Fig. 2).

Another noteworthy finding is the observation that this mutant miR-140 had unusually strong suppressive effects on its predicted target genes. Because miRNA-target RNA interactions are believed to be selected for robust biological effects through the course of evolution, it is rare that newly emerged, unconserved miRNAs, such as this mutant miR-140, show strong regulatory effects. We found that the mutant miR-140 seed binding sequence overlaps the consensus binding sequence of the RNA-binding protein, Ybx1, that stabilizes RNA. Experimental evidence supported that the mutant miR-140 and Ybx1 compete for overlapping binding sites. Therefore, this unusually strong suppressive effect of the mutant miRNA can be partly explained by the competitive antagonism against another RNA regulatory mechanism by the mutant miR-140 in chondrocytes [11].

3.3 RMRP-S1 and RMRP-S2 and cartilage-hair hypoplasia

Human cartilage-hair hypoplasia (CHH) is a rare autosomal recessive syndrome characterized by short-limbed dwarfism due to metaphyseal dysplasia and various systemic abnormalities including fine sparse light-colored hypoplastic hair and immune deficiency [90].

CHH is caused by point mutations in the non-coding RNA, RMRP, a 268-nt non-coding RNA component of mitochondrial RNA processing endoribonuclease (RNase MRP) that processes mitochondrial ribosomal RNAs. Because diverse mutations were found in patients, it is considered that loss of function of the endoribonuclease activity is the major cause for the disease. Recently it was found that RMRP gives rise to two short (~20 nt) RNAs (RMRP-S1 and RMRP-S2) that function as miRNAs. Since several disease-causing mutations are mapped to RMRP-S1 and -S2, it is possible that alteration of these miRNAs contributes to the pleiotropic phenotype of CHH. It was found that over 900 genes were significantly regulated by RMRP-S1 and -S2 and that 90% of these genes contained target sites in the 3' UTR. Pathway analysis revealed that many RMRP-S1/S2-regulated genes were involved in skeletal development, hair follicle development and hematopoiesis, suggesting physiologic roles of RMRP-S1 and -S2 [91].

4. Conclusion

MiRNAs are vital regulators of chondrocyte differentiation, cartilage development and homeostasis. The essential regulatory function of miRNAs in skeletal development is emphasized by genetic studies in experimental animals and humans. Since the majority of

studies on roles of individual miRNAs in cartilage were conducted *in vitro*, further *in vivo* experiments should follow to determine the physiologic significance of these miRNAs in skeletal development and disease.

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Highlights

MiRNAs regulate diverse aspects of chondrocyte function and chondrogenesis.

MiR-140 and let-7 miRNAs regulate differentiation and proliferation of chondrocytes.

Mutations in miR-140 and miR-17~92 miRNAs cause skeletal dysplasias in humans.

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Figure. 1.

MiRNAs regulate skeletal development.

Mycn regulated miR-17~92 miRNAs are abundantly expressed in osteochondroprogenitor cells and promote progenitor cell proliferation by suppressing TGF- β signaling. Let-7 miRNAs, inhibited by Lin28 RNA binding proteins, differentially regulate cell proliferation of progenitor cells and chondrocytes via, at least in part, modulation of Ras/MAPK signaling. Sox9 regulates robust and chondrocyte-specific expression of miR-140, which is essential for normal chondrocyte differentiation and proliferation. The precise molecular mechanism of miR-140-mediated regulation is still unclear.

Figure. 2.

Molecular mechanism of SED MIR140 type.

The C > G substitution at the first seed sequence of miR-140-5p simultaneously causes loss of function (LOF) of wildtype miR-140-5p and gain of new function (GOF) due to the mutant miR-140-5p-G. Mice carrying this mutation show a cartilage phenotype distinct from that of mir-140 knockout mice. At the molecular level, the mutant miR-140-5p-G shows unusually strong suppressive effects on novel targets leading to the downregulation of Hif1a-regulated genes, including *Hif1a* itself. Thus, both LOF of wildtype miR-140-5p and GOF of the mutant miR-140-5p likely contribute to the pathogenesis of SED MIR140 type.