Published in final edited form as:

Bone. 2016 March ; 84: 57–68. doi:10.1016/j.bone.2015.12.016.

MicroRNA variants as genetic determinants of bone mass

Neha S. Dole, Ph.D.^{a,b} and Anne M. Delany, Ph.D.^a

^aCenter for Molecular Medicine, UCONN Health, Farmington, CT, U.S.A.

Abstract

Single nucleotide polymorphisms (SNPs) are the most abundant genetic variants that contribute to the heritability of bone mass. MicroRNAs (miRNAs, miRs) are key post-transcriptional regulators that modulate the differentiation and function of skeletal cells by targeting multiple genes in same or distinct signaling pathways. SNPs in miRNA genes and miRNA binding sites can alter miRNA abundance and mRNA targeting. This review describes the potential impact of miRNA-related SNPs on skeletal phenotype. Although many associations between SNPs and bone mass have been described, this review is limited to gene variants for which a function has been experimentally validated. SNPs in miRNA genes (miR-SNPs) that impair miRNA processing and alter the abundance of mature miRNA are discussed for miR-146a, miR-125a, miR-196a, miR-149 and miR-27a. SNPs in miRNA targeting sites (miR-TS-SNPs) that alter miRNA binding are described for the bone remodeling genes bone morphogenetic protein receptor 1 (Bmpr1), fibroblast growth factor 2 (Fgf2), osteonectin (Sparg) and histone deacetylase 5 (Hdac5). The review highlights two aspects of miRNA-associated SNPs: the mechanism for altering miRNA mediated gene regulation, and the potential of miR-associated SNPs to alter osteoblast, osteoclast or chondrocyte differentiation and function. Given the polygenic nature of skeletal diseases like osteoporosis and osteoarthritis, validating the function of additional miRNA-associated SNPs has the potential to enhance our understanding of the genetic determinants of bone mass and predisposition to selected skeletal diseases.

Keywords

miRNA; SNP; Osteoporosis; Osteoarthritis; osteoblast; osteoclast

1. Introduction

Bone mass phenotype is a complex and highly heritable trait. Approximately 38 million SNPs have been discovered in the human genome, representing the majority of gene variants [1]. Linkage studies, in conjunction with candidate gene studies, have identified some rare

To whom correspondence should be addressed: Dr. Anne M. Delany, Center for Molecular Medicine, UCONN Health, 263 Farmington Ave, Farmington, CT, 06030, USA, Tel: (860) 679-8730; Fax: (860) 679-1258, adelany@uchc.edu.

^bPresent address: Department of Orthopaedic Surgery, UCSF School of Medicine, San Francisco, CA, U.S.A. nehashashank.dole@ucsf.edu

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

genetic variants with high impact on bone phenotype, most notably variants of low density lipoprotein-related receptor 5 (LRP5). More recently, GWAS (genome wide association study) identified 62 genome-wide significant loci associated with bone mineral density (BMD) [2, 3]. Some of these loci contain candidate genes with known function in pathways important in bone physiology, such as canonical Wnt signaling, the RANK-RANKL-OPG system, and endochondral ossification. However, only chromosomal regions have been determined, and the genes or mechanisms underlying the linkage with BMD are not yet identified. In general, the SNPs identified are common variants with small effect size. Presently, less than 6% of the variance in femoral neck BMD can be explained by the GWAS-identified loci. [4].

It may be relatively straightforward to determine whether SNPs in the protein-coding region of a gene or within intronic areas that affect splicing might cause changes in protein function or abundance, and thereby, phenotype. However, the functional impact of SNPs in untranslated regions (UTRs), promoter/enhancer regions or regulatory RNAs can be more difficult to evaluate. Remarkably, at conserved sites within the human genome, the largest proportion of variants lies within the UTRs [1]. Moreover, a study comparing GWAS-SNP sets found an enrichment of trait-associated variants in 3' UTRs, compared with 5' UTRs or coding sequences (CDS) [5]. The 3' UTR is recognized as a critical control region, with the potential to regulate mRNA stability, translation and localization. Many binding sites for microRNAs (miRNAs, miRs), which are key post-transcriptional regulators, are found in 3' UTRs [6].

2. SNPs and miRNAs

There are increasing reports of SNPs that can interfere with miRNA function. Such polymorphisms can occur in the miRNA targeting site of a mRNA (miR-TS-SNPs) or in the miRNA genes themselves (miR-SNPs) [7-9]. miR-TS-SNPs present at or near a miRNA binding site in a protein coding gene could modulate miRNA function by creating or eliminating a miRNA binding site in that target mRNA [10]. The functional impact of a miR-TS-SNP on phenotype largely depends on whether the corresponding miRNA is expressed in a particular tissue, as well as the expression of other possibly compensatory miRNAs. For example, miRs and their targets can be expressed in a tissue specific manner, and a miR-TS-SNP might only be of functional consequence if the targeted mRNA is expressed in the same tissue. These considerations could provide some basis for understanding the tissue-restricted phenotype associated with some common SNPs [5].

MicroRNA biogenesis is an intricate process; multiple steps are involved in generating a mature miRNA from its primary (pri-) miRNA transcript. These include the cleavage of pri-miRNA by DGCR8/Drosha microprocessor complex, to yield a shorter hairpin, the precursor miRNA (pre-miRNA) [11-14]. Pre-miRNAs are actively exported from the nucleus to the cytoplasm and further processed by the Dicer/TRBP complex into roughly symmetric miRNA duplexes. This duplex is loaded onto the Argonaute (Ago) protein of the RNA induced silencing complex (RISC), unwound, and processed into a single stranded mature miRNA [15, 16]. Guide strand selection from the miRNA duplex is governed by thermodynamics and the remaining passenger strand is often degraded. However, in some

cases, both strands of duplex can be loaded into the RISC as functional miRNAs [17, 18]. Thus, SNPs in miRNA genes (miR-SNPs) have the potential to alter miRNA processing, guide strand selection, or the mature miRNA, itself. Since a single miRNA could target hundreds of genes and regulate multiple pathways, a polymorphism in a miRNA gene leading to functional impairment could impact all regulatory pathways involving that miRNA. It is estimated that only approximately 10% of human miRNA genes contain SNPs; and SNPs in the region corresponding to the mature miRNA are the most rare (<1%) [8, 19]. Thus, there appears to be considerable selective constraint on the transmittance of miR-SNPs.

3. Approach for this review

In terms of trying to understand the function of mutations or polymorphisms in miRNA genes and targets, the field of cancer biology has been in the forefront [20]. Similar work in the field of skeletal biology is in its infancy. Fortunately, some miRNAs and mRNA targets important in malignancy are also important in bone or cartilage. In this review, we have included studies performed in the context of cancer and other diseases to infer the function of miRNA gene and target polymorphisms in skeletal biology. Although long non-coding RNAs (lncRNAs) that are frequently targeted by miRNAs, also contain SNPs and can impact skeletal physiology, there is relatively little known about the function of lncRNAs in bone [21-23]. Consequently, in reviewing the published data on sequence variants in miRNAs and miRNA targets, the following discussion will be restricted to protein coding genes and miRNAs that could have an impact on the skeleton. Moreover, many studies have associated miRNA-SNP variants with a particular phenotype, although few studies have identified a potential mechanism involved. Therefore, this review will also be restricted to work demonstrating molecular mechanisms and validated SNP function. Lastly, each miRNA has many possible regulatory interactions with targets. Modeling all regulatory circuits for the selected miRNAs presented below is beyond the scope of this review. Rather, we have limited our models to the miRNAs, targets and signaling pathways discussed in each section.

4. miR-SNPs: SNPs in miRNA genes altering their biogenesis and function

MicroRNAs regulate skeletal development and homeostasis by targeting multiple genes and pathways [24-26]. Thus, SNPs that alter expression and/or function of a single miRNA could represent variants with larger effects. SNPs that compromise miRNA expression levels can occur in the miRNA gene promoter (miR-P-SNPs), or in the body of the miRNA gene (miR-SNP) (Figures 1 and 2). miR-P-SNPs modulate miRNA expression by affecting transcription factor binding sites in one of the following ways: (1) abolishing an existing transcription factor binding site (TFBS) or (2) creating a new TFBS or (3) altering the binding affinity of TFBS [27]. Reportedly, databases using bioinformatics to predict TFBS in miRNA promoters (i.e. miRGen 2.0) and the potential for a SNP to affect transcriptional regulation of a miRNA gene (i.e. dPORE-miRNA) have estimated that ~20,000 SNPs prevail in human miRNA promoters [28, 29]. Despite the elaborate predictions, however, information on the impact of miR-P-SNPs on disease risk is limited and the mechanism by which miR-P-SNPs affect miRNA gene regulation is known for only a few cases. In

contrast, miR-SNPs affect miRNA processing by altering the RNA secondary structure and decreasing the stability of either pri- or pre-miRNA. Alternatively, SNPs can lead to increased stability of the passenger strand, which could then function as another guide strand (Figure 2A and B). In this case, both guide and passenger strands will be loaded into the RISC, to target a distinct set of mRNAs [7]. Lastly, SNPs that compromise miRNA function most commonly occur in the seed-binding region of the miRNA (Figure 2C).

In this section, we will highlight SNPs in miRNAs that are important in the skeleton, and how these SNPs modulate miRNA transcription, processing or function.

4.1 miR-146a

4.1.1 miR-146 function—The human miR-146 family consists of 2 distinct isoforms, miR-146a and -146b, which are expressed from human chromosomes 5q33.3 and 10q24, respectively [30]. miR-146a is expressed in articular cartilage and femur, as well as in non-skeletal tissues including prostate, liver, breast, and hematopoietic cells [31-36]. An NF- κ B binding motif near the transcription start site of miR-146a has been recognized, and factors including TNF- α , lipopolysaccharide or IL-1 β (interleukin-1 β) can induce miR-146a expression [34, 37]. In turn, miR-146a limits the inflammatory response by targeting TRAF6 (TNF receptor associated factor 6) and IRAK1 (IL1 receptor associated kinase 1), which play a role in activating NF- κ B signaling [34]. In osteoclasts, TRAF6 and IRAK1 mediate IL-1 β induced activation of NF- κ B signaling, to promote osteoclast survival and activity [38, 39].

miR-146a has been linked to the pathogenesis of osteoarthritis (OA), a disease characterized by the progressive degeneration of articular cartilage, primarily in response to mechanical injury [37, 40, 41]. Studies have reported high levels of miR-146a in synovial fluid during the early stages of OA, whereas in the later stages miR-146a levels decline [40-42].

miR-146a can target Smad4, which might suppress TGF- β mediated chondrocyte survival [40, 41]. In the later stage of OA, lower levels of miR-146a may contribute to increased TRAF6 and IRAK1 levels, and IL-1 β mediated NF- κ B signaling. NF- κ B signaling promotes the expression of cartilage-degrading enzymes, including MMP13 and ADAMTS5, while it suppresses expression of cartilage matrix such as aggrecan and type II collagen [37] (Figure 3).

miR-146a also plays a crucial role in regulating bone remodeling. It functions as a positive regulator in osteoblasts, by driving commitment of mesenchymal stem cells (MSCs) to an osteogenic rather than a chondrogenic fate [43, 44]. By targeting Smad2/3, miR-146a regulates TGF- β signaling and indirectly downregulates expression of chondrocytic marker Sox9, and upregulates Runx2 [37, 40, 45]. In osteoclasts, miR-146a levels increase during differentiation, and miR-146a has been shown to inhibit both TNF- α and RANKL induced osteoclast differentiation by mechanisms likely to include the targeting of TRAF6 and IRAK1, as well as other mRNAs [46, 47].

4.1.2 miR-146 SNPs—The miR-146a gene contains SNPs in the promoter region as well as in the pre-miRNA. miR-P-SNP rs57095329 is located within 1 kb of the miR-146a transcription start site and was first identified as strongly associated with risk of systemic

lupus erythematosus (SLE), a chronic autoimmune disorder affecting multiple organ systems [27]. This disease association was later validated in a meta-analysis of GWAS that included cohorts of European and Asian descent [48]. The studies in SLE linked the risk-associated minor allele variant rs57095329-G (A>G, minor allele frequency (MAF) 0.14 in 1000 Genomes Project) with reduced miR-146a promoter activity, leading to low mature miR-146a levels. The risk allele was shown to reduce the binding affinity of Ets1 to the miR-146a promoter, providing a potential mechanism for decreased miR-146a levels [27]. In bone, Ets1 is also known to interact with Cbfa1/Runx2, a quintessential transcription factor for osteogenesis [49]. In cells from a patient displaying Runx2 haploinsufficiency, levels of miR-146a were depressed, further suggesting a connection between Runx2 and miR-146a [50]. Although other SNPs in the promoter region of miR-146a have been recognized (rs17057381, rs73318382, and rs6864584), their function has not yet been ascertained.

Interestingly, miR-146a has been also implicated in the pathogenesis of rheumatoid arthritis, another autoimmune disease affecting skeletal tissues [47, 51-54]. In rheumatoid arthritis, joint destruction and localized bone loss are caused by chronic inflammation; and miR-146a has been shown to suppress osteoclastogenesis [47, 52]. Although genetic determinants play a role in susceptibility to rheumatoid arthritis, an association between the SLE-linked miR-P-SNP rs57095329 and rheumatoid arthritis has not yet been examined.

A second functional SNP in miR-146a occurs in the pre-miRNA, SNP rs2910164. This common SNP (G>C, MAF 0.458 in CSAgilent Project) was mapped to the passenger strand in the stem of pre-miR-146a. This SNP was associated with risk of many cancer types, including papillary thyroid carcinoma, prostate cancer, breast cancer, gastric cancer, ovarian cancer, esophageal squamous cell carcinoma, and hepatocellular carcinoma [30, 33, 55-61]. The mechanism for SNP rs2910164 mediated alterations in mature miR-146a levels has been examined in studies of papillary thyroid carcinoma and nasopharyngeal carcinoma. GC heterozygosity was linked to high papillary thyroid carcinoma risk, while CC homozygosity was associated with increased nasopharyngeal carcinoma risk [30, 57, 61]. The risk-associated C allele resulted in lower miR-146a levels, due to impaired Drosha mediated processing of the pre-miRNA [57]. This led to decreased regulation of miR-146a target genes, TRAF6 and IRAK1. Apart from impairing Drosha processing, this SNP was also suggested to stabilize the passenger strand. In addition to the leading strand, papillary thyroid carcinoma patients with GC heterozygosity were shown to generate two passenger strands, -146a*G and -146a*C [55]. Bioinformatics suggest that these alternate passenger strands target different mRNAs, which might contribute to the risk of papillary thyroid carcinoma. Although rs2910164 was previously linked to rheumatoid arthritis, a later meta-analysis disproved this association [53, 54].

These seminal studies of miR-146a polymorphisms elegantly demonstrate the impact of miRNA-SNPs on miRNA transcription, processing and targeting. Given the role of miR-146a in regulating inflammation, autoimmunity, and skeletal homeostasis, it will be important to examine the function of these and other miR-146a SNPs, and determine whether they might be associated with skeletal diseases.

4.2. miR-125a

4.2.1 miR-125a function—The human miR-125 family is comprised of three homologs: miR-125b1, -125b2 and -125a. Both miR-125b1 (on chromosome 11q23) and -125b2 (on chromosome 21q21) genes produce identical mature miR-125b. The miR-125a gene is located on chromosome 19q13.41, within a cluster also containing miR-99b and let-7e [7, 62].

During RANKL-stimulated osteoclastogenesis in vitro, the expression of miR-125a is increased in both human and mouse cells [46, 63]. Upon RANKL stimulation, the p65 subunit of NF- κ B can bind near the transcription start site of the miR-125a gene cluster, inducing its expression. In turn, miR-125a positively regulates NF- κ B signaling by directly targeting TNFAIP3, a ubiquitin editing enzyme [63]. Remarkably, in B-cell lymphoma, ectopic expression of miR-125a was shown to suppress TNFAIP3 mediated ubiquitination of TRAF2 and I κ B α , and lead to elevated NF- κ B signaling [64]. Thus, impairment in miR-125a levels due to pri-miR-SNPs could modulate osteoclastogenesis and disrupt the balance in bone remodeling (Figure 4).

The other member of miR-125 family, miR-125b, acts as a negative regulator of osteoblastic differentiation, by targeting the Cbf (core-binding factor) family members Cbfb and Runx2 (Cbfa1) [65]. miR-125b levels plummet in response to BMP 2/4 induced osteoblast differentiation [65, 66]. Congruent with its negative role, increased miR-125b levels were detected in serum and bone samples of osteoporotic patients [67]. In cartilage, miR-125b was shown to confer protection against OA by directly targeting the cartilage degrading enzyme aggrecanase-1 (ADAMTS-4). Reportedly, IL-1 β suppresses miR-125b levels and upregulates aggrecanase-1 expression in OA chondrocytes [68].

4.2.2 miR-125a SNPs—Mechanistic studies examining the effect of a pri-miRNA SNP on processing have been described for miR-125a. SNP rs12975333 (G>T), located in the stem region of pri-mi125a, was shown to modulate miRNA expression and activity. The minor variant, rs12975333-T, decreases binding of pri-miR-125a to DGCR8, a component of the miRNA biogenesis machinery. This causes defective pri- to pre-miR processing and low mature miR-125a levels; and contributes to the resulting increase in levels of miR-125a targets observed in rs12975333-T expressing cells [7].

In addition to affecting processing, rs12975333 is retained by the mature miRNA and was shown to impair miR-125a mediated translational repression. Specifically, the T variant of rs12975333 impairs the seed-binding region of miR-125a, and reduces repression of the miR-125a target gene, Lin-28 [7]. Lin-28 is a RNA binding protein that acts as a translational enhancer of Igf2 [69]. SNPs that reside in the seed-binding of mature miRNAs are quite rare, which might reflect their profound impact on cell physiology [8, 9].

Although 6 polymorphisms in pri-miR-125a (rs41275794, rs12975333, rs12976445, rs10404453, rs78758318, rs143525573) have been reported, so far only rs12975333, described above, has been shown to be functional. Given the function of the miR-125 family in both the osteoblast and osteoclast lineage, it is of interest to determine whether these other SNPs display any association with BMD.

4.3. miR-196a2

4.3.1 miR-196a2 function—The miR-196 family consists of 3 members, miR-196a1, -196a2, and -196b that are embedded in the highly conserved clusters of Hox (homeobox) genes. Located on chromosome 17, the miR-196a1 gene lies within the Hoxb cluster, miR-196a2 lies on chromosome 12 within the Hoxc cluster, and miR-196b gene lies within the Hoxa cluster on chromosome 7. miR-196 isoforms show a high level of identity. Both miR-196a-1 and miR-196a-2 genes transcribe the same mature miR-196a sequence, while the mature miR-196b differs by a single base from miR-196a. This sequence similarity suggests that they may target the same panel of mRNAs.

Hox genes regulate skeletal patterning and limb development by coordinating morphogen signaling pathways including those for retinoic acid, BMP and TGF- β [70, 71]. By regulating the Hox genes (Hoxb8, Hoxc8, Hoxd8, Hoxa7), miR-196 family members play a crucial role in development of the axial skeleton, fine-tuning levels of HOX proteins in a temporal and spacial manner [70-76]. miR-196a promotes commitment of mesenchymal stem cells (MSCs) towards the osteoblast lineage by targeting Hoxc8 [77, 78]. In osteoblasts, Hoxc8 acts as a negative regulator of differentiation and transcriptionally suppresses osteopontin [72, 73, 76]. In turn, BMP signaling represses Hoxc8 activity through SMAD1 interaction with Hoxc8, relieving repression of osteopontin transcription. In addition to Hoxc8, miR-196a targets Hoxb7 in adult MSCs [79]. Over-expression of Hoxb7 enhances the proliferative and osteogenic capacity of MSCs. In aging MSCs, decreased osteogenesis due to low Hoxb7 was associated with increased levels of miR-196a.

4.3.2 miR-196a2 SNPs—Two miR-196a2 promoter region SNPs (rs35010275 and rs12304647) and one SNP in the pre-miRNA (rs11614913) have been studied in gastric cancer cohorts [80, 81]. Of these, miR-P-SNP rs35010275 (G>C; MAF 0.28 in 1000 Genomes Project) mapped within 1 kb of the miR-196a2 transcription start site and was associated with gastric cancer risk in a Chinese population [80]. High levels of miR-196a detected in these patients were linked to the risk-associated G variant. Functional analyses indicated that rs35010275 modulated the interaction of nuclear proteins with DNA, and promoter activity. Although the transcription factor complexes interacting with the SNP rs35010275 region were not identified, this study demonstrates that the allele-specific differences in promoter region of a miRNA gene can alter promoter activity and modulate miRNA expression [80].

Another variant in the miR-196a2 precursor rs11614913 (C>T MAF 0.334 in 1000 Genomes Project) was associated with breast cancer risk [82]. Specifically, the T-allele was associated with decreased cancer risk and low miR-196a levels. Functional analysis revealed that the T variant leads to decreased mature miR-196a, without affecting the pre-miR-196a2 levels, thereby indicating a miRNA processing defect [82]. Since Hox genes play a critical role in bone and cartilage, these common miR-196a variants could have an impact on skeletal phenotype.

4.4 miR-149

4.4.1 miR-149 function—The human miR-149 gene has been mapped to intron 1 of glypican1 (GPC1), an integral membrane proteoglycan. This highly conserved miRNA is expressed independently of its host gene in a wide variety of non-skeletal tissues [83]. In the skeleton, expression of miR-149 has only been reported in chondrocytes, where it is upregulated by the inflammatory cytokines IL-1 β , IL-6 and TNF- α . Furthermore, miR-149 can directly target these inflammatory cytokines in a feedback loop. In OA, dramatically decreased miR-149 expression has been suggested to exacerbate the inflammatory response [84, 85].

4.4.2 miR-149 SNPs—So far, two SNPs have been mapped to pre-miR-149 at rs2292832 (C>T MAF 0.387 in 1000 Genomes Project) and rs71428439 (A>G MAF 0.144 in 1000 Genomes Project). Although rs2292832 was extensively studied in the context of cancer risk, newer meta-analyses do not suggest an association between this SNP and cancer, and functional data on rs2292832 are lacking [86, 87].

In contrast, the mechanism for a SNP mediated effect on gene regulation has been elegantly described for rs71428439, in the context of myocardial infarction [88]. Specifically, the risk associated variant rs71428439-G was linked to decreased miR-149 expression. The SNP was predicted to alter the secondary structure of the pre-miR, yielding a less stable pre-miR-149-G. Functional analysis revealed that rs71428439-G modulates the pre- to mature miR processing, resulting in low levels of miR-149. In addition to defining mechanism, this study was unique in that it demonstrated the impact of rs71428439 on myocardial infarction susceptibility in vivo. In a mouse model of myocardial infarction, infarct size and myocyte apoptosis was low in mice injected with precursor for the miR-149- rs71428439-A variant, assigning a protective function against myocardial infarction. This SNP is a common variant, and since miR-149 targets inflammatory cytokines, an association between this SNP and OA phenotype could be possible [85].

4.5. miR-27a

4.5.1 miR-27a function—The human miR-27 family is comprised of two family members: miR-27a and b. miR-27a is transcribed within a cluster on chromosome 19, which also contains miR-23a and miR-24-2, whereas miR-27b is transcribed in cluster on chromosome 9, along with miR-23b, miR-3074 and miR-24-1. The mature miR-27a and b isoforms differ by a single nucleotide.

During osteoblastic differentiation in vitro, the expression of miR-27a is extensively regulated. miR-27a levels first decrease, as cells are proliferating and laying down extracellular matrix; miR-27a then increases as osteoblasts mature. However, in mineralizing cultures, the expression of miR-27a is nearly undetectable [89]. Runx2 was shown to negatively regulate the transcription of the miR-27a~miR-23a~miR-24-2 cluster. Moreover, miR-27a targets SATB2 (special AT-rich sequence binding protein 2), which interacts with Runx2 to promote osteoblast differentiation. Inhibition of the miR-27a~miR-23a~miR-24-2 cluster by Runx2 can promote a feed forward regulatory loop [89]. Further, miR-27a can target Hoxa10, which promotes osteoblast differentiation through

Runx2-dependent and -independent mechanisms [74]. Whereas these studies suggest that miR-27a has negative effects on osteoblastogenesis, others showed that the miR-27 family can promote this process by directly targeting inhibitors of Wnt/ β -catenin signaling, sFRP1 (soluble frizzled related protein 1) and APC (adenomatous polyposis coli) [90, 91]. It is likely that the timing of miR-27 expression is tightly regulated for optimal osteoblastic differentiation (Figure 5).

4.5.2 miR-27a SNPs—To date, two SNPs have been reported in pre-miR-27a: rs895819 (T>C MAF 0.364 in 1000 Genomes Project) and rs11671784 (G>A MAF 0.007 in 1000 Genomes Project). rs895819 is located in the terminal loop of pre-miR-27a, and increased expression of mature miR-27a has been observed in individuals homozygous for the minor allele [92, 93]. This SNP has been studied in the context of several cancers, most notably those of breast and colon. However, there are conflicting reports of whether the rs895819C variant can be associated with breast or colorectal cancer risk [87, 94].

In contrast, rs11671784 was strongly associated with risk of gastric cancer, and the A variant appears to have a protective effect [93, 95]. This SNP is located in the stem region of pre-miR-27a, where rs1167178-A introduces a mismatch. The presence of the A allele decreased processing of pre-miR-27a to the mature form by ~50%. Expression of miR-27a is frequently increased in gastric cancer, and patients homozygous or heterozygous for the A allele demonstrated decreased tumor/normal ratio for miR-27a, compared with patients homozygous for the G allele. Decreased miR-27a corresponded to increased levels of the miR-27a target Hoxa10. Moreover, in a mouse xenograft model, gastric cancer cells expressing the miR-27a rs1167178-A allele grew more slowly than those expressing rs1167178-G [95]. This study elegantly demonstrates the function of a miR-27a variant that could have an impact on osteoblastic differentiation by altering levels of mature miR-27a.

4.6 miR-124

4.6.1 miR-124 function—In humans, mature miR-124 arises from three different pri-miR structures encoded by independent genes. The genes encoding miR-124-1 and miR-124-2 have been mapped to chromosome 8, while miR-124-3 gene is located on chromosome 20. Mature miR-124 is evolutionarily conserved and highly expressed by neurons in brain [96]. In the skeleton, miR-124 is expressed by both osteoblasts and osteoclasts [97, 98]. In osteoblasts, miR-124 expression decreases during differentiation and it negatively regulates osteoblast formation by targeting *Dlx2*, *Dlx3* and *Dlx5*, which are critical for osteogenic commitment [98]. miR-124 has also been shown to suppress expression of chondrocyte marker genes such as *Sox9* and *Aggrecan* [99]. In contrast, miR-124 has been shown to increase dramatically during commitment of human mesenchymal stem cells to adipocyte lineage [98]. Thus, miR-124 may promote adipogenic differentiation at the expense of commitment to the osteo-chondral lineage. In osteoclasts, miR-124 can target NFATc1, key transcription factor; it also reduces osteoclast precursor proliferation and motility by targeting integrin B1 (ITGB1), RhoA and Rac1 [97]. In a rat inflammatory arthritis model, miR-124 mimic injection was shown to ameliorate progression of the arthritic lesion by decreasing osteoclast formation [100].

4.6.2 miR-124 SNPs—Polymorphism rs531564 (G>C MAF 0.130 in 1000 Genomes Project) was detected in pri-miR-124-1, and examined in a case-control study of Alzheimer's disease. Although the SNP was not linked to Alzheimer's disease, the study suggested that the rs531564-C variant might lead to decreased mature miRNA levels, possibly due an alteration in the pri-miR secondary structure [101]. Since rs531564C is a common variant, and because miR-124 impacts both osteoblast and osteoclast differentiation and function, it will be of interest to determine whether this SNP might be associated with bone phenotype.

5. miRNA variants directly implicated in skeletal diseases

Presently, miR-SNPs with a validated impact on bone mass have not been reported. However, a mutation in a miRNA precursor was recently associated with bone mass. The mechanism by which a mutation in miR-2861 affects miRNA processing and bone mass are discussed below.

5.1.1 miR-2861 function

miR-2861 is transcribed in a cluster with miR-3960, from a locus at 9q34.11. BMP2 was shown to induce expression of the miR-3960~2861 cluster and both miRNAs are positive regulators of osteoblast differentiation [102]. Indeed, systemic administration of a miR-2861 antagomiR decreased bone mass, trabecular bone volume and bone formation rate in mice [103]. miR-2861 was shown to target histone deacetylase 5 (HDAC5) which is known to deacetylate RUNX2, promoting its ubiquitin mediated degradation. Thus, miR-2861 targeting of Hdac5 increases RUNX2 acetylation and protein stability, providing a positive feedback loop contributing to BMP2-induced osteoblast differentiation and function [104] (Figure 6).

5.1.2 miR-2861 variant

Although SNPs in this miRNA have not been reported, a mutation in pre-miR-2861 was identified in one kindred with low bone mass. Functional analyses revealed that the mutation in this osteopenic kindred mapped to the leading strand in the stem of pre-miR-2861, which caused decreased mature miRNA levels due to reduced miRNA processing. High levels of the miR-2861 target Hdac5 were attributed to decreased RUNX2 protein in the bones of these patients. Although expansion of the linkage study to include unrelated osteoporotic patients and healthy controls did not reveal the presence of pre-miR-2861 mutations in either group, this study highlights a functional miR-SNP variant with a profound impact on bone mass [103].

6. miR-TS-SNPs: SNPs in miRNA target sites

Polymorphisms in miRNA target sites within a mRNA (miR-TS-SNPs) can either abrogate an existing miRNA binding site or create a novel site (Figure 7). Compared with SNPs in miRNA genes, miR-TS-SNPs in 3' UTRs are more abundant [8, 9]. Studies in the field of skeletal diseases such as osteoporosis and chondrodysplasia, as well as cancer genetics have identified the function of miR-TS-SNPs in several target genes with known functions in bone.

6.1 BMPRI

6.1.1 BMPRI function—BMPs are bone anabolic factors critical for development [105]. They signal through their membrane bound receptors, BMPRI (I-A and I-B) and BMPRII, which form a homo- or heterotrimeric complex upon ligand binding, and activate a canonical SMAD mediated transcriptional program [106]. Both type I BMP receptors have distinct spatial and temporal expression patterns during embryogenesis [107-109]. In osteoblasts, BMPRI-B is crucial for commitment of mesenchymal precursors towards the osteogenic lineage, while BMPRI-A appears to direct them towards an adipocytic fate [110, 111]. Conditional deletion of BMPRI-B in osteoblasts leads to decreased bone formation and growth [112]. In osteoclasts, inhibition of BMP signaling impairs osteoclastogenesis and bone resorption [113]. Overall, BMPs directly regulate both arms of the bone remodeling cycle.

6.1.2 BMPRI SNP—One case-control study linked SNP rs1434536 (C>T, MAF 0.404 in 1000 Genomes Project) in the BMPRI-B 3' UTR with increased risk of estrogen receptor-positive (ER+) breast cancer [114]. Previously, increased BMPRI-B mediated signaling in ER+ breast carcinoma was associated with high tumor grade, greater tumor cell proliferation, cytogenetic instability and poor prognosis [115]. Interestingly, SNP rs1434536 showed strong linkage with cancer associated SNPs from the CGEMS (Cancer Genetic Markers of Susceptibility) GWAS data set [114, 116]. Patients carrying the high risk rs1434536-T allele had increased BMPRI-B expression, and this allele demonstrated impaired binding of miR-125b to the BMPRI-B 3' UTR [114]. A second study demonstrated a similar mechanism for the rs1434536-T variant and higher prostate cancer risk [117]. Remarkably, the rs1434536-T allele was shown to impart BMPRI-B mediated protection against endometriosis, due to diminished miR-125b directed repression [118]. SNP rs1434536-T is a common variant, and increased BMPRI-B levels in individuals carrying this allele might contribute to higher bone mass.

7. miR-TS-SNPs implicated in skeletal disease

Presently, there are only handful studies implicating miR-TS-SNPs in skeletal disease. These miR-TS-SNPs reside in the 3' UTR of genes that play an important role in osteoblast or chondrocyte function, and are discussed below.

7.1 FGF2

7.2.1 FGF2 function—Fibroblast growth factor 2 (FGF) is a potent mitogenic growth factor that is widely expressed in limb bud mesenchyme, chondrocytes, osteoblasts, osteoclasts and adipocytes. In osteoblasts, FGF2 promotes osteoblast differentiation by mechanisms that include regulation of BMP2 and ATF4 [119-121]. However, sustained FGF2 signaling can inhibit bone formation by maintaining osteoblastic precursors in a proliferative state, thereby preventing their differentiation [119, 120, 122]. Thus, in the osteoblast lineage, appropriate timing and levels of FGF2 signaling are critical for optimal differentiation. In the osteoclast lineage, FGF2 promotes recruitment of osteoclast precursors and their differentiation [120, 121, 123]. FGF2-null mice display osteopenia and decreased bone remodeling [124, 125].

7.2.2 FGF2 SNP—Recently, a miR-TS-SNP, rs6854081 (T>G, MAF 0.0855 in 1000 Genomes Project) located in the 3' UTR of Fgf2 was associated with BMD in a cohort of middle-aged Caucasian women. The minor allele, rs6854081-G, was associated with low BMD, and a subtle increase in FGF2 mRNA was found in monocytes or B cells isolated from the low BMD patients [126]. Using the poly-miRTS database, SNP rs6854081-G was shown to potentially interfere with miR-146a or miR-146b binding [126]. It was speculated that rs6854081-G might relieve translational suppression of Fgf2, leading to increased FGF2 levels and enhanced osteoclastogenesis. In this study, Fgf2 was not experimentally validated as a target of miR-146a and b, and it is possible that other miRNAs could be involved. However, the study did report a Fgf2 3' UTR SNP and provided an implication for the role of miR-TS-SNPs in mediating variations in BMD [126].

5.3 SPARC/Osteonectin

5.3.1 SPARC/Osteonectin function—Osteonectin is one of the most abundant non-collagenous extracellular matrix components in bone. It facilitates collagen fibril assembly and organization, and promotes osteoblast commitment, differentiation, and survival [127-129]. Global deficiency of osteonectin leads to development of low turnover osteopenia, with decreased bone formation [130]. Moreover, osteonectin-null and haploinsufficient mice display an attenuated bone-anabolic response to intermittent PTH treatment [131].

5.3.2 SPARC/Osteonectin SNP—A candidate gene study associated a miR-TS-SNP rs1054204 (C>G, MAF 0.387 in 1000 Genomes Project) in the 3' UTR of osteonectin with low bone mass in a cohort of Caucasian men with idiopathic osteoporosis [132, 133]. A subsequent study demonstrated a potential mechanism underlying this association, using in vitro and in vivo models. Specifically, a novel set of mouse knock-in models were created, in which the mouse osteonectin 3' UTR was replaced with the human 3' UTR, containing either rs1054204-C or -G. Mice carrying the minor allelic variant rs1054204-G had decreased levels of osteonectin in bone tissue, decreased trabecular bone volume, and a reduced bone-anabolic response to intermittent PTH (1-34) treatment [132, 133]. Moreover, in vitro studies demonstrated a cell-autonomous effect of the variant on osteoblastic differentiation and mineralized matrix production. Additional in vitro studies showed that the minor allele variant rs1054204-G enhances osteonectin post-transcriptional regulation by creating a novel miR-433 target site in its 3' UTR [133]. Other studies demonstrated that miR-433 could inhibit osteoblast differentiation and target Runx2 [133, 134]. Overall, these data provided a physiological function to a common miR-TS-SNP, and identified a potential mechanism [133].

5.4 HDAC6

5.4.1 HDAC6 function—Histone deacetylase 6 (HDAC6) functions as a negative regulator of osteoblast differentiation and a positive regulator of adipogenesis [135]. In osteoblasts, HDAC6 interacts with RUNX2 and promotes its ability to repress transcription of target genes [136]. In chondrocytes, HDAC6 may impact differentiation due to its ability to regulate RUNX2 activity. Moreover, since HDAC6 can deacetylate tubulin, it can destabilize ciliary microtubules. In articular chondrocytes, primary cilia responsive to

mechanical strain regulate hedgehog signaling, providing an additional potential mechanism for HDAC6 regulation of chondrogenesis [137].

5.4.2 HDAC6 variant—Although SNPs in Hdac6 have not yet been linked to skeletal disease, a mutation in the 3' UTR of this gene was suggested to be a likely molecular cause for disease in a kindred presenting with a new form of dominant X-linked chondrodysplasia characterized by platyspondyly, rhizomelic shortening of the members, specific brachydactyly, hydrocephaly, facial dysmorphism and microphthalmia. A linkage study mapped the disease locus to a pericentromeric region within Xp11.3–q13.1 (Lod score = 3.30). The HDAC6 gene was hypothesized to be a candidate gene in this locus, and exon sequencing revealed a variant (c.*281A>T) in the Hdac6 3' UTR [138].

In this chondrodysplasia study, miR-433 was demonstrated to target Hdac6. The mutation (c.*281A>T) was localized to a miR-433 seed binding region in the 3' UTR, and was shown to interfere with miR-433 targeting. Increased HDAC6 levels and activity were demonstrated in tissue from affected family members and in affected limbs of a female child displaying mosaicism, due to selective inactivation of the mutated allele. In the affected patients, upregulation of HDAC6 may indirectly regulate hedgehog signaling, among other pathways, and lead to phenotypic defects. Altogether, this study demonstrates that a Hdac6 3' UTR variant suppressed miR-433 mediated post-transcriptional regulation, causing overexpression of HDAC6, which could contribute to X-linked chondrodysplasia [138].

Conclusions

A growing number of GWAS have associated genomic loci with BMD and fracture risk. Within these intervals, SNPs in protein coding regions as well as UTRs and miRNA genes should be considered for functional validation. To date, only a limited number of studies have identified SNPs or mutations in miRNA genes and target binding sites relevant to BMD. However, miR-associated SNPs (miR-P-SNPs, miR-SNPs and miR-TS-SNPs) validated in other disease models have potential to inform studies of SNPs and bone mass. In particular, data on functional miR-associated SNPs (collated from other diseases), along with information on miRNA-target co-expression and quantitative trait locus (QTL) mapping could provide direction for investigation of miR-SNPs with the potential for relevance in skeletal diseases. Moreover, newer sophisticated bioinformatic tools (i.e. Patrocles Database, PolymiRTS Database 3.0) can be used in conjunction with existing GWAS data to determine whether a polymorphism of interest might also be miR-TS-SNP. Given the polygenic nature of skeletal diseases like osteoporosis, the addition of genetic profiling data that includes miRNA-associated SNPs could enhance the accuracy of fracture prediction tools that currently rely only on BMD and clinical risk factors [139, 140].

In addition to genetics, factors including age, lifestyle, hormonal status and co-morbidities can greatly influence BMD. Since a majority of SNP variants are likely to have a small impact on BMD, the evaluation of SNP function can be facilitated by the exclusion of possible confounding variables, such as differences in environment or genetic modifiers. This simplification can be achieved by creating novel in vitro and in vivo models to study SNP function. The increased usage of genome editing technology, such as the CrispR-Cas9

system, for the generation of new mouse models and cell lines will likely be instrumental in driving forward our understanding of SNP function.

In this review, we summarized the mechanisms for miR-associated SNP regulation of miRNA function, and provided evidence suggesting of potential impact that variants of several miRNAs might have in regulating skeletal phenotype (summarized Table 1, Figure 8). Lastly, we briefly described the current literature on known miR-associated SNPs or mutations in skeletal diseases. miRNA-based therapeutics are in clinical trials for selected malignancies, and the development of novel miRNA-based therapeutics for bone repair and regeneration is underway. Information on miRNA function in bone and cartilage, as well as information on genetic variants affecting miRNA function could help the clinicians develop a better plan for individualized treatment as well as predict the impact of drug efficacy, adverse reactions or toxicity along with disease risk.

Acknowledgements

This work was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health under Award Number AR044877 (AMD) and by the Center for Molecular Medicine at UCONN Health. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Abbreviations

BMD	Bone mineral density
CDS	Coding sequences
DGCR8	DiGeorge critical region 8
GWAS	Genome wide association study
lncRNA	Long noncoding RNA
MSCs	Mesenchymal stem cells
miRNA, miR	microRNA
miR-SNPs	microRNA gene SNPs
miR-TS-SNPs	microRNA target site SNPs
MAF	Minor allele frequency
OA	Osteoarthritis
pre-miRNA	Precursor microRNA
pri-miRNA	Primary microRNA
RISC	RNA-induced silencing complex
SNP	Single nucleotide polymorphism
SLE	Systemic lupus erythematosus
TFBS	Transcription factor binding site

UTR

Untranslated region

References

- [1]. Genomes Project C. Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, Kang HM, Marth GT, McVean GA. An integrated map of genetic variation from 1,092 human genomes. *Nature*. 2012; 491:56–65. [PubMed: 23128226]
- [2]. Estrada K, Styrkarsdottir U, Evangelou E, Hsu YH, Duncan EL, Ntzani EE, Oei L, Albagha OM, Amin N, Kemp JP, Koller DL, Li G, Liu CT, Minster RL, Moayyeri A, Vandenput L, Willner D, Xiao SM, Yerges-Armstrong LM, Zheng HF, Alonso N, Eriksson J, Kammerer CM, Kaptoge SK, Leo PJ, Thorleifsson G, Wilson SG, Wilson JF, Aalto V, Alen M, Aragaki AK, Aspelund T, Center JR, Dailiana Z, Duggan DJ, Garcia M, Garcia-Giralt N, Giroux S, Hallmans G, Hocking LJ, Husted LB, Jameson KA, Khusainova R, Kim GS, Kooperberg C, Koromila T, Kruk M, Laaksonen M, Lacroix AZ, Lee SH, Leung PC, Lewis JR, Masi L, Mencej-Bedrac S, Nguyen TV, Nogues X, Patel MS, Prezelj J, Rose LM, Scollen S, Siggeirsdottir K, Smith AV, Svensson O, Trompet S, Trummer O, van Schoor NM, Woo J, Zhu K, Balcells S, Brandi ML, Buckley BM, Cheng S, Christiansen C, Cooper C, Dedoussis G, Ford I, Frost M, Goltzman D, Gonzalez-Macias J, Kahonen M, Karlsson M, Khusnutdinova E, Koh JM, Kollia P, Langdahl BL, Leslie WD, Lips P, Ljunggren O, Lorenc RS, Marc J, Mellstrom D, Obermayer-Pietsch B, Olmos JM, Pettersson-Kymmer U, Reid DM, Riancho JA, Ridker PM, Rousseau F, Slagboom PE, Tang NL, Urreizti R, Van Hul W, Viikari J, Zarrabeitia MT, Aulchenko YS, Castano-Betancourt M, Grundberg E, Herrera L, Ingvarsson T, Johannsdottir H, Kwan T, Li R, Luben R, Medina-Gomez C, Palsson ST, Reppe S, Rotter JJ, Sigurdsson G, van Meurs JB, Verlaan D, Williams FM, Wood AR, Zhou Y, Gautvik KM, Pastinen T, Raychaudhuri S, Cauley JA, Chasman DI, Clark GR, Cummings SR, Danoy P, Dennison EM, Eastell R, Eisman JA, Gudnason V, Hofman A, Jackson RD, Jones G, Jukema JW, Khaw KT, Lehtimäki T, Liu Y, Lorentzon M, McCloskey E, Mitchell BD, Nandakumar K, Nicholson GC, Oostra BA, Peacock M, Pols HA, Prince RL, Raitakari O, Reid IR, Robbins J, Sambrook PN, Sham PC, Shuldiner AR, Tybavsky FA, van Duijn CM, Wareham NJ, Cupples LA, Econs MJ, Evans DM, Harris TB, Kung AW, Psaty BM, Reeve J, Spector TD, Streeten EA, Zillikens MC, Thorsteinsdottir U, Ohlsson C, Karasik D, Richards JB, Brown MA, Stefansson K, Uitterlinden AG, Ralston SH, Ioannidis JP, Kiel DP, Rivadeneira F. Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. *Nat Genet*. 2012; 44:491–501. [PubMed: 22504420]
- [3]. Rivadeneira F, Styrkarsdottir U, Estrada K, Halldorsson BV, Hsu YH, Richards JB, Zillikens MC, Kavvoura FK, Amin N, Aulchenko YS, Cupples LA, Deloukas P, Demissie S, Grundberg E, Hofman A, Kong A, Karasik D, van Meurs JB, Oostra B, Pastinen T, Pols HA, Sigurdsson G, Soranzo N, Thorleifsson G, Thorsteinsdottir U, Williams FM, Wilson SG, Zhou Y, Ralston SH, van Duijn CM, Spector T, Kiel DP, Stefansson K, Ioannidis JP, Uitterlinden AG, Genetic Factors for Osteoporosis C. Twenty bone-mineral-density loci identified by large-scale meta-analysis of genome-wide association studies. *Nat Genet*. 2009; 41:1199–206. [PubMed: 19801982]
- [4]. Richards JB, Zheng HF, Spector TD. Genetics of osteoporosis from genome-wide association studies: advances and challenges. *Nat Rev Genet*. 2012; 13:576–88. [PubMed: 22805710]
- [5]. Arnold M, Ellwanger DC, Hartsperger ML, Pfeufer A, Stumpflen V. Cis-acting polymorphisms affect complex traits through modifications of microRNA regulation pathways. *PLoS One*. 2012; 7:e36694. [PubMed: 22606281]
- [6]. Keene JD. RNA regulons: coordination of post-transcriptional events. *Nat Rev Genet*. 2007; 8:533–43. [PubMed: 17572691]
- [7]. Duan R, Pak C, Jin P. Single nucleotide polymorphism associated with mature miR-125a alters the processing of pri-miRNA. *Hum Mol Genet*. 2007; 16:1124–31. [PubMed: 17400653]
- [8]. Saunders MA, Liang H, Li WH. Human polymorphism at microRNAs and microRNA target sites. *Proc Natl Acad Sci U S A*. 2007; 104:3300–5. [PubMed: 17360642]
- [9]. Sun G, Yan J, Noltner K, Feng J, Li H, Sarkis DA, Sommer SS, Rossi JJ. SNPs in human miRNA genes affect biogenesis and function. *RNA*. 2009; 15:1640–51. [PubMed: 19617315]

- [10]. Mishra PJ, Mishra PJ, Banerjee D, Bertino JR. MiRSNPs or MiR-polymorphisms, new players in microRNA mediated regulation of the cell: Introducing microRNA pharmacogenomics. *Cell Cycle*. 2008; 7:853–8. [PubMed: 18414050]
- [11]. Burke JM, Kelenis DP, Kincaid RP, Sullivan CS. A central role for the primary microRNA stem in guiding the position and efficiency of Drosha processing of a viral pri-miRNA. *RNA*. 2014; 20:1068–77. [PubMed: 24854622]
- [12]. Faller M, Toso D, Matsunaga M, Atanasov I, Senturia R, Chen Y, Zhou ZH, Guo F. DGCR8 recognizes primary transcripts of microRNAs through highly cooperative binding and formation of higher-order structures. *RNA*. 2010; 16:1570–83. [PubMed: 20558544]
- [13]. Ma H, Wu Y, Choi JG, Wu H. Lower and upper stem-single-stranded RNA junctions together determine the Drosha cleavage site. *Proc Natl Acad Sci U S A*. 2013; 110:20687–92. [PubMed: 24297910]
- [14]. Yi R, Qin Y, Macara IG, Cullen BR. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev*. 2003; 17:3011–6. [PubMed: 14681208]
- [15]. Bernstein E, Kim SY, Carmell MA, Murchison EP, Alcorn H, Li MZ, Mills AA, Elledge SJ, Anderson KV, Hannon GJ. Dicer is essential for mouse development. *Nat Genet*. 2003; 35:215–7. [PubMed: 14528307]
- [16]. Wilson RC, Tambe A, Kidwell MA, Noland CL, Schneider CP, Doudna JA. Dicer-TRBP Complex Formation Ensures Accurate Mammalian MicroRNA Biogenesis. *Mol Cell*. 2014
- [17]. Okamura K, Liu N, Lai EC. Distinct mechanisms for microRNA strand selection by *Drosophila* Argonautes. *Mol Cell*. 2009; 36:431–44. [PubMed: 19917251]
- [18]. Bartel B. MicroRNAs directing siRNA biogenesis. *Nat Struct Mol Biol*. 2005; 12:569–71. [PubMed: 15999111]
- [19]. Iwai N, Naraba H. Polymorphisms in human pre-miRNAs. *Biochem Biophys Res Commun*. 2005; 331:1439–44. [PubMed: 15883035]
- [20]. Cipollini M, Landi S, Gemignani F. MicroRNA binding site polymorphisms as biomarkers in cancer management and research. *Pharmacogenomics Pers Med*. 2014; 7:173–91. [PubMed: 25114582]
- [21]. Tye CE, Gordon JA, Martin-Buley LA, Stein JL, Lian JB, Stein GS. Could lncRNAs be the missing links in control of mesenchymal stem cell differentiation? *J Cell Physiol*. 2015; 230:526–34. [PubMed: 25258250]
- [22]. Zhu L, Xu PC. Downregulated lncRNA-ANCR promotes osteoblast differentiation by targeting EZH2 and regulating Runx2 expression. *Biochem Biophys Res Commun*. 2013; 432:612–7. [PubMed: 23438432]
- [23]. Gong J, Liu W, Zhang J, Miao X, Guo AY. lncRNASNP: a database of SNPs in lncRNAs and their potential functions in human and mouse. *Nucleic Acids Res*. 2014
- [24]. Kapinas K, Delany AM. MicroRNA biogenesis and regulation of bone remodeling. *Arthritis Res Ther*. 2011; 13:220. [PubMed: 21635717]
- [25]. Lian JB, Stein GS, van Wijnen AJ, Stein JL, Hassan MQ, Gaur T, Zhang Y. MicroRNA control of bone formation and homeostasis. *Nat Rev Endocrinol*. 2012; 8:212–27. [PubMed: 22290358]
- [26]. van Wijnen AJ, van de Peppel J, van Leeuwen JP, Lian JB, Stein GS, Westendorf JJ, Oursler M-J, Sampen H-JI, Taipaleenmaki H, Hesse E, Riester S, Kakar S. MicroRNA Functions in Osteogenesis and Dysfunctions in Osteoporosis. *Current osteoporosis reports*. 2013; 11:72–82. [PubMed: 23605904]
- [27]. Luo X, Yang W, Ye DQ, Cui H, Zhang Y, Hirankarn N, Qian X, Tang Y, Lau YL, de Vries N, Tak PP, Tsao BP, Shen N. A functional variant in microRNA-146a promoter modulates its expression and confers disease risk for systemic lupus erythematosus. *PLoS Genet*. 2011; 7:e1002128. [PubMed: 21738483]
- [28]. Alexiou P, Vergoulis T, Gleditsch M, Prekas G, Dalamagas T, Megraw M, Grosse I, Sellis T, Hatzigeorgiou AG. miRGen 2.0: a database of microRNA genomic information and regulation. *Nucleic Acids Res*. 2010; 38:D137–41. [PubMed: 19850714]
- [29]. Schmeier S, Schaefer U, MacPherson CR, Bajic VB. dPORE-miRNA: polymorphic regulation of microRNA genes. *PLoS One*. 2011; 6:e16657. [PubMed: 21326606]

- [30]. Jazdzewski K, Murray EL, Franssila K, Jarzab B, Schoenberg DR, de la Chapelle A. Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. *Proc Natl Acad Sci U S A*. 2008; 105:7269–74. [PubMed: 18474871]
- [31]. Sun Q, Zhao X, Liu X, Wang Y, Huang J, Jiang B, Chen Q, Yu J. miR-146a functions as a tumor suppressor in prostate cancer by targeting Rac1. *Prostate*. 2014; 74:1613–21. [PubMed: 25214035]
- [32]. He Y, Huang C, Sun X, Long XR, Lv XW, Li J. MicroRNA-146a modulates TGF-beta1-induced hepatic stellate cell proliferation by targeting SMAD4. *Cell Signal*. 2012; 24:1923–30. [PubMed: 22735812]
- [33]. Garcia AI, Buisson M, Bertrand P, Rimokh R, Rouleau E, Lopez BS, Lidereau R, Mikaelian I, Mazoyer S. Down-regulation of BRCA1 expression by miR-146a and miR-146b-5p in triple negative sporadic breast cancers. *EMBO Mol Med*. 2011; 3:279–90. [PubMed: 21472990]
- [34]. Taganov KD, Boldin MP, Chang KJ, Baltimore D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc Natl Acad Sci U S A*. 2006; 103:12481–6. [PubMed: 16885212]
- [35]. Park H, Huang X, Lu C, Cairo MS, Zhou X. MicroRNA-146a and MicroRNA-146b Regulate Human Dendritic Cell Apoptosis and Cytokine Production by Targeting TRAF6 and IRAK1 Proteins. *J Biol Chem*. 2015; 290:2831–41. [PubMed: 25505246]
- [36]. Rusca N, Monticelli S. MiR-146a in Immunity and Disease. *Mol Biol Int*. 2011; 2011:437301. [PubMed: 22091404]
- [37]. Li X, Gibson G, Kim JS, Kroin J, Xu S, van Wijnen AJ, Im HJ. MicroRNA-146a is linked to pain-related pathophysiology of osteoarthritis. *Gene*. 2011; 480:34–41. [PubMed: 21397669]
- [38]. Gravallese EM, Galson DL, Goldring SR, Auron PE. The role of TNF-receptor family members and other TRAF-dependent receptors in bone resorption. *Arthritis Res*. 2001; 3:6–12. [PubMed: 11178122]
- [39]. Kim JH, Jin HM, Kim K, Song I, Youn BU, Matsuo K, Kim N. The mechanism of osteoclast differentiation induced by IL-1. *J Immunol*. 2009; 183:1862–70. [PubMed: 19587010]
- [40]. Jin LEI, Zhao J, Jing W, Yan S, Wang XIN, Xiao C, Ma B. Role of miR-146a in human chondrocyte apoptosis in response to mechanical pressure injury in vitro. *International Journal of Molecular Medicine*. 2014; 34:451–463. [PubMed: 24939082]
- [41]. Li J, Huang J, Dai L, Yu D, Chen Q, Zhang X, Dai K. miR-146a, an IL-1beta responsive miRNA, induces vascular endothelial growth factor and chondrocyte apoptosis by targeting Smad4. *Arthritis Res Ther*. 2012; 14:R75. [PubMed: 22507670]
- [42]. Yamasaki K, Nakasa T, Miyaki S, Ishikawa M, Deie M, Adachi N, Yasunaga Y, Asahara H, Ochi M. Expression of MicroRNA-146a in osteoarthritis cartilage. *Arthritis Rheum*. 2009; 60:1035–41. [PubMed: 19333945]
- [43]. Huszar JM, Payne CJ. MIR146A inhibits JMJD3 expression and osteogenic differentiation in human mesenchymal stem cells. *FEBS Letters*. 2014; 588:1850–1856. [PubMed: 24726732]
- [44]. Cheung KSC, Sposito N, Stumpf PS, Wilson DI, Sanchez-Elsner T, Oreffo ROC. MicroRNA-146a Regulates Human Foetal Femur Derived Skeletal Stem Cell Differentiation by Down-Regulating SMAD2 and SMAD3. *PLoS ONE*. 2014; 9:e98063. [PubMed: 24892945]
- [45]. Schanen BC, Li X. Transcriptional regulation of mammalian miRNA genes. *Genomics*. 2011; 97:1–6. [PubMed: 20977933]
- [46]. Franceschetti T, Dole NS, Kessler CB, Lee SK, Delany AM. Pathway analysis of microRNA expression profile during murine osteoclastogenesis. *PLoS One*. 2014; 9:e107262. [PubMed: 25222202]
- [47]. Nakasa T, Shibuya H, Nagata Y, Niimoto T, Ochi M. The inhibitory effect of microRNA-146a expression on bone destruction in collagen-induced arthritis. *Arthritis Rheum*. 2011; 63:1582–90. [PubMed: 21425254]
- [48]. Ji J, Cha E, Lee W. Association of miR-146a polymorphisms with systemic lupus erythematosus: a meta-analysis. *Lupus*. 2014; 23:1023–1030. [PubMed: 24803388]
- [49]. Ducy P. Cbfa1: a molecular switch in osteoblast biology. *Dev Dyn*. 2000; 219:461–71. [PubMed: 11084646]

- [50]. Chen P, Wei D, Xie B, Ni J, Xuan D, Zhang J. Effect and possible mechanism of network between microRNAs and RUNX2 gene on human dental follicle cells. *J Cell Biochem.* 2014; 115:340–8. [PubMed: 24038414]
- [51]. Ceribelli A, Nahid MA, Satoh M, Chan EK. MicroRNAs in rheumatoid arthritis. *FEBS Lett.* 2011; 585:3667–74. [PubMed: 21600203]
- [52]. Nakasa T, Miyaki S, Okubo A, Hashimoto M, Nishida K, Ochi M, Asahara H. Expression of microRNA-146 in rheumatoid arthritis synovial tissue. *Arthritis Rheum.* 2008; 58:1284–92. [PubMed: 18438844]
- [53]. Chen HF, Hu TT, Zheng XY, Li MQ, Luo MH, Yao YX, Chen Q, Yu SY. Association between miR-146a rs2910164 polymorphism and autoimmune diseases susceptibility: a meta-analysis. *Gene.* 2013; 521:259–64. [PubMed: 23545308]
- [54]. Li K, Tie H, Hu N, Chen H, Yin X, Peng C, Wan J, Huang W. Association of two polymorphisms rs2910164 in miRNA-146a and rs3746444 in miRNA-499 with rheumatoid arthritis: a meta-analysis. *Hum Immunol.* 2014; 75:602–8. [PubMed: 24824381]
- [55]. Jazdzewski K, Liyanarachchi S, Swierniak M, Pachucki J, Ringel MD, Jarzab B, de la Chapelle A. Polymorphic mature microRNAs from passenger strand of pre-miR-146a contribute to thyroid cancer. *Proc Natl Acad Sci U S A.* 2009; 106:1502–5. [PubMed: 19164563]
- [56]. Gong J, Tong Y, Zhang HM, Wang K, Hu T, Shan G, Sun J, Guo AY. Genome-wide identification of SNPs in microRNA genes and the SNP effects on microRNA target binding and biogenesis. *Hum Mutat.* 2012; 33:254–63. [PubMed: 22045659]
- [57]. Lung RW, Wang X, Tong JH, Chau SL, Lau KM, Cheng SH, Woo JK, Woo J, Leung PC, Ng MH, Tang NL, To KF. A single nucleotide polymorphism in microRNA-146a is associated with the risk for nasopharyngeal carcinoma. *Mol Carcinog.* 2013; 52(Suppl 1):E28–38. [PubMed: 22711332]
- [58]. Wei WJ, Wang YL, Li DS, Wang Y, Wang XF, Zhu YX, Yang YJ, Wang ZY, Ma YY, Wu Y, Jin L, Ji QH, Wang JC. Association between the rs2910164 polymorphism in pre-Mir-146a sequence and thyroid carcinogenesis. *PLoS One.* 2013; 8:e56638. [PubMed: 23451063]
- [59]. Dai ZJ, Shao YP, Wang XJ, Xu D, Kang HF, Ren HT, Min WL, Lin S, Wang M, Song ZJ. Five Common Functional Polymorphisms in microRNAs (rs2910164, rs2292832, rs11614913, rs3746444, rs895819) and the Susceptibility to Breast Cancer: Evidence from 8361 Cancer Cases and 8504 Controls. *Curr Pharm Des.* 2015; 21:1455–63. [PubMed: 25483824]
- [60]. Xu X, Yang X, Ru G, Wu Y, Zhang S, Xing C, Wu Y, Cao J. miR-146a gene polymorphism rs2910164 and the risk of digestive tumors: A meta-analysis of 21 case-control studies. *Oncol Rep.* 2014; 31:472–9. [PubMed: 24247819]
- [61]. Huang GL, Chen ML, Li YZ, Lu Y, Pu XX, He YX, Tang SY, Che H, Zou Y, Ding C, He Z. Association of miR-146a gene polymorphism with risk of nasopharyngeal carcinoma in the central-southern Chinese population. *J Hum Genet.* 2014; 59:141–4. [PubMed: 24430575]
- [62]. Scott GK, Goga A, Bhaumik D, Berger CE, Sullivan CS, Benz CC. Coordinate suppression of ERBB2 and ERBB3 by enforced expression of micro-RNA miR-125a or miR-125b. *J Biol Chem.* 2007; 282:1479–86. [PubMed: 17110380]
- [63]. de la Rica L, Garcia-Gomez A, Comet NR, Rodriguez-Ubreva J, Ciudad L, Vento-Tormo R, Company C, Alvarez-Errico D, Garcia M, Gomez-Vaquero C, Ballestar E. NF-kappaB-direct activation of microRNAs with repressive effects on monocyte-specific genes is critical for osteoclast differentiation. *Genome Biol.* 2015; 16:2. [PubMed: 25601191]
- [64]. Kim S-W, Ramasamy K, Bouamar H, Lin A-P, Jiang D, Aguiar RCT. MicroRNAs miR-125a and miR-125b constitutively activate the NF-κB pathway by targeting the tumor necrosis factor alpha-induced protein 3 (TNFAIP3, A20). *Proceedings of the National Academy of Sciences.* 2012; 109:7865–7870.
- [65]. Huang K, Fu J, Zhou W, Li W, Dong S, Yu S, Hu Z, Wang H, Xie Z. MicroRNA-125b regulates osteogenic differentiation of mesenchymal stem cells by targeting Cbfbeta in vitro. *Biochimie.* 2014; 102:47–55. [PubMed: 24560795]
- [66]. Mizuno Y, Yagi K, Tokuzawa Y, Kanesaki-Yatsuka Y, Suda T, Katagiri T, Fukuda T, Maruyama M, Okuda A, Amemiya T, Kondoh Y, Tashiro H, Okazaki Y. miR-125b inhibits osteoblastic

- differentiation by down-regulation of cell proliferation. *Biochem Biophys Res Commun*. 2008; 368:267–72. [PubMed: 18230348]
- [67]. Seeliger C, Karpinski K, Haug AT, Vester H, Schmitt A, Bauer JS, van Griensven M. Five freely circulating miRNAs and bone tissue miRNAs are associated with osteoporotic fractures. *J Bone Miner Res*. 2014; 29:1718–28. [PubMed: 24431276]
- [68]. Matsukawa T, Sakai T, Yonezawa T, Hiraiwa H, Hamada T, Nakashima M, Ono Y, Ishizuka S, Nakahara H, Lotz MK, Asahara H, Ishiguro N. MicroRNA-125b regulates the expression of aggrecanase-1 (ADAMTS-4) in human osteoarthritic chondrocytes. *Arthritis Res Ther*. 2013; 15:R28. [PubMed: 23406982]
- [69]. Poleskaya A, Cuvellier S, Naguibneva I, Duquet A, Moss EG, Harel-Bellan A. Lin-28 binds IGF-2 mRNA and participates in skeletal myogenesis by increasing translation efficiency. *Genes Dev*. 2007; 21:1125–38. [PubMed: 17473174]
- [70]. Zakany J, Duboule D. The role of Hox genes during vertebrate limb development. *Curr Opin Genet Dev*. 2007; 17:359–66. [PubMed: 17644373]
- [71]. Alexander T, Nolte C, Krumlauf R. Hox Genes and Segmentation of the Hindbrain and Axial Skeleton. *Annual Review of Cell and Developmental Biology*. 2009; 25:431–456.
- [72]. Lei H, Wang H, Juan AH, Ruddle FH. The identification of Hoxc8 target genes. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102:2420–2424. [PubMed: 15699330]
- [73]. Juan AH, Lei H, Bhargava P, Lebrun M, Ruddle FH. Multiple roles of hoxc8 in skeletal development. *Ann N Y Acad Sci*. 2006; 1068:87–94. [PubMed: 16831908]
- [74]. Hassan MQ, Tare R, Lee SH, Mandeville M, Weiner B, Montecino M, van Wijnen AJ, Stein JL, Stein GS, Lian JB. HOXA10 controls osteoblastogenesis by directly activating bone regulatory and phenotypic genes. *Mol Cell Biol*. 2007; 27:3337–52. [PubMed: 17325044]
- [75]. McGlinn E, Yekta S, Mansfield JH, Soutschek J, Bartel DP, Tabin CJ. In ovo application of antagomiRs indicates a role for miR-196 in patterning the chick axial skeleton through Hox gene regulation. *Proc Natl Acad Sci U S A*. 2009; 106:18610–5. [PubMed: 19846767]
- [76]. Zheng YJ, Chung HJ, Min H, Kang M, Kim SH, Gadi J, Kim MH. In vitro osteoblast differentiation is negatively regulated by Hoxc8. *Appl Biochem Biotechnol*. 2010; 160:891–900. [PubMed: 19214787]
- [77]. Yekta S, Shih IH, Bartel DP. MicroRNA-directed cleavage of HOXB8 mRNA. *Science*. 2004; 304:594–6. [PubMed: 15105502]
- [78]. Kim YJ, Bae SW, Yu SS, Bae YC, Jung JS. miR-196a regulates proliferation and osteogenic differentiation in mesenchymal stem cells derived from human adipose tissue. *J Bone Miner Res*. 2009; 24:816–25. [PubMed: 19063684]
- [79]. Candini O, Spano C, Murgia A, Grisendi G, Veronesi E, Piccinno MS, Ferracin M, Negrini M, Giacobbi F, Bambi F, Horwitz EM, Conte P, Paolucci P, Dominici M. Mesenchymal Progenitors Aging Highlights a miR-196 Switch Targeting HOXB7 as Master Regulator of Proliferation and Osteogenesis. *Stem Cells*. 2015; 33:939–50. [PubMed: 25428821]
- [80]. Xu M, Qiang F, Gao Y, Kang M, Wang M, Tao G, Gong W, Zhu H, Wu D, Zhang Z, Zhao Q. Evaluation of a novel functional single-nucleotide polymorphism (rs35010275 G>C) in MIR196A2 promoter region as a risk factor of gastric cancer in a Chinese population. *Medicine (Baltimore)*. 2014; 93:e173. [PubMed: 25474430]
- [81]. Hua HB, Yan TT, Sun QM. miRNA polymorphisms and risk of gastric cancer in Asian population. *World J Gastroenterol*. 2014; 20:5700–7. [PubMed: 24914331]
- [82]. Hoffman AE, Zheng T, Yi C, Leaderer D, Weidhaas J, Slack F, Zhang Y, Paranjape T, Zhu Y. microRNA miR-196a-2 and Breast Cancer: A Genetic and Epigenetic Association Study and Functional Analysis. *Cancer Research*. 2009; 69:5970–5977. [PubMed: 19567675]
- [83]. Chamorro-Jorganes A, Araldi E, Rotllan N, Cirera-Salinas D, Suarez Y. Autoregulation of glypican-1 by intronic microRNA-149 fine tunes the angiogenic response to FGF2 in human endothelial cells. *J Cell Sci*. 2014; 127:1169–78. [PubMed: 24463821]
- [84]. Diaz-Prado S, Cicione C, Muinos-Lopez E, Hermida-Gomez T, Oreiro N, Fernandez-Lopez C, Blanco FJ. Characterization of microRNA expression profiles in normal and osteoarthritic human chondrocytes. *BMC Musculoskelet Disord*. 2012; 13:144. [PubMed: 22883423]

- [85]. Santini P, Politi L, Vedova PD, Scandurra R, Scotto d'Abusco A. The inflammatory circuitry of miR-149 as a pathological mechanism in osteoarthritis. *Rheumatol Int.* 2014; 34:711–6. [PubMed: 23595570]
- [86]. He B, Pan Y, Cho WC, Xu Y, Gu L, Nie Z, Chen L, Song G, Gao T, Li R, Wang S. The association between four genetic variants in microRNAs (rs11614913, rs2910164, rs3746444, rs2292832) and cancer risk: evidence from published studies. *PLoS One.* 2012; 7:e49032. [PubMed: 23155448]
- [87]. Hu Y, Yu C-Y, Wang J-L, Guan J, Chen H-Y, Fang J-Y. MicroRNA sequence polymorphisms and the risk of different types of cancer. *Sci. Rep.* 2014:4.
- [88]. Ding SL, Wang JX, Jiao JQ, Tu X, Wang Q, Liu F, Li Q, Gao J, Zhou QY, Gu DF, Li PF. A pre-microRNA-149 (miR-149) genetic variation affects miR-149 maturation and its ability to regulate the Puma protein in apoptosis. *J Biol Chem.* 2013; 288:26865–77. [PubMed: 23873935]
- [89]. Hassan MQ, Gordon JA, Beloti MM, Croce CM, van Wijnen AJ, Stein JL, Stein GS, Lian JB. A network connecting Runx2, SATB2, and the miR-23a~27a~24-2 cluster regulates the osteoblast differentiation program. *Proc Natl Acad Sci U S A.* 2010; 107:19879–84. [PubMed: 20980664]
- [90]. Guo D, Li Q, Lv Q, Wei Q, Cao S, Gu J. MiR-27a targets sFRP1 in hFOB cells to regulate proliferation, apoptosis and differentiation. *PLoS One.* 2014; 9:e91354. [PubMed: 24625818]
- [91]. Wang T, Xu Z. miR-27 promotes osteoblast differentiation by modulating Wnt signaling. *Biochem Biophys Res Commun.* 2010; 402:186–9. [PubMed: 20708603]
- [92]. Sun Q, Gu H, Zeng Y, Xia Y, Wang Y, Jing Y, Yang L, Wang B. Hsa-mir-27a genetic variant contributes to gastric cancer susceptibility through affecting miR-27a and target gene expression. *Cancer Sci.* 2010; 101:2241–7. [PubMed: 20666778]
- [93]. Song B, Yan G, Hao H, Yang B. rs11671784 G/A and rs895819 A/G polymorphisms inversely affect gastric cancer susceptibility and miR-27a expression in a Chinese population. *Med Sci Monit.* 2014; 20:2318–26. [PubMed: 25399405]
- [94]. Kupcinskas J, Bruzaite I, Juzenas S, Gyvyte U, Jonaitis L, Kiudelis G, Skieceviciene J, Leja M, Pauzas H, Tamelis A, Pavalkis D, Kupcinskas L. Lack of association between miR-27a, miR-146a, miR-196a-2, miR-492 and miR-608 gene polymorphisms and colorectal cancer. *Sci. Rep.* 2014:4.
- [95]. Yang Q, Jie Z, Ye S, Li Z, Han Z, Wu J, Yang C, Jiang Y. Genetic variations in miR-27a gene decrease mature miR-27a level and reduce gastric cancer susceptibility. *Oncogene.* 2014; 33:193–202. [PubMed: 23246964]
- [96]. Zhou Q, Long L, Shi G, Zhang J, Wu T, Zhou B. Research of the Methylation Status of miR-124a Gene Promoter among Rheumatoid Arthritis Patients. *Clinical and Developmental Immunology.* 2013; 2013:4.
- [97]. Lee Y, Kim HJ, Park CK, Kim YG, Lee HJ, Kim JY, Kim HH. MicroRNA-124 regulates osteoclast differentiation. *Bone.* 2013; 56:383–9. [PubMed: 23867221]
- [98]. Qadir AS, Um S, Lee H, Baek K, Seo BM, Lee G, Kim GS, Woo KM, Ryoo HM, Baek JH. miR-124 Negatively Regulates Osteogenic Differentiation and In vivo Bone Formation of Mesenchymal Stem Cells. *J Cell Biochem.* 2015; 116:730–42. [PubMed: 25424317]
- [99]. Laine SK, Alm JJ, Virtanen SP, Aro HT, Laitala-Leinonen TK. MicroRNAs miR-96, miR-124, and miR-199a regulate gene expression in human bone marrow-derived mesenchymal stem cells. *J Cell Biochem.* 2012; 113:2687–95. [PubMed: 22441842]
- [100]. Nakamachi Y, Ohnuma K, Uto K, Noguchi Y, Saegusa J, Kawano S. MicroRNA-124 inhibits the progression of adjuvant-induced arthritis in rats. *Annals of the Rheumatic Diseases.* 2015
- [101]. Qi L, Hu Y, Zhan Y, Wang J, Wang BB, Xia HF, Ma X. A SNP site in pri-miR-124 changes mature miR-124 expression but no contribution to Alzheimer's disease in a Mongolian population. *Neurosci Lett.* 2012; 515:1–6. [PubMed: 22430032]
- [102]. Hu R, Liu W, Li H, Yang L, Chen C, Xia ZY, Guo LJ, Xie H, Zhou HD, Wu XP, Luo XH. A Runx2/miR-3960/miR-2861 regulatory feedback loop during mouse osteoblast differentiation. *J Biol Chem.* 2011; 286:12328–39. [PubMed: 21324897]
- [103]. Li H, Xie H, Liu W, Hu R, Huang B, Tan YF, Xu K, Sheng ZF, Zhou HD, Wu XP, Luo XH. A novel microRNA targeting HDAC5 regulates osteoblast differentiation in mice and contributes to primary osteoporosis in humans. *J Clin Invest.* 2009; 119:3666–77. [PubMed: 19920351]

- [104]. Jeon E-J, Lee K-Y, Choi N-S, Lee M-H, Kim H-N, Jin Y-H, Ryoo H-M, Choi J-Y, Yoshida M, Nishino N, Oh B-C, Lee K-S, Lee YH, Bae S-C. Bone Morphogenetic Protein-2 Stimulates Runx2 Acetylation. *Journal of Biological Chemistry*. 2006; 281:16502–16511. [PubMed: 16613856]
- [105]. Wang EA, Rosen V, D'Alessandro JS, Bauduy M, Cordes P, Harada T, Israel DI, Hewick RM, Kerns KM, LaPan P, et al. Recombinant human bone morphogenetic protein induces bone formation. *Proc Natl Acad Sci U S A*. 1990; 87:2220–4. [PubMed: 2315314]
- [106]. Ashique AM, Fu K, Richman JM. Signalling via type IA and type IB bone morphogenetic protein receptors (BMPR) regulates intramembranous bone formation, chondrogenesis and feather formation in the chicken embryo. *Int J Dev Biol*. 2002; 46:243–53. [PubMed: 11934153]
- [107]. Suzuki A, Thies RS, Yamaji N, Song JJ, Wozney JM, Murakami K, Ueno N. A truncated bone morphogenetic protein receptor affects dorsal-ventral patterning in the early *Xenopus* embryo. *Proc Natl Acad Sci U S A*. 1994; 91:10255–9. [PubMed: 7937936]
- [108]. Dewulf N, Verschueren K, Lonnoy O, Moren A, Grimsby S, Vande Spiegle K, Miyazono K, Huylebroeck D, Ten Dijke P. Distinct spatial and temporal expression patterns of two type I receptors for bone morphogenetic proteins during mouse embryogenesis. *Endocrinology*. 1995; 136:2652–63. [PubMed: 7750489]
- [109]. Zou H, Wieser R, Massague J, Niswander L. Distinct roles of type I bone morphogenetic protein receptors in the formation and differentiation of cartilage. *Genes Dev*. 1997; 11:2191–203. [PubMed: 9303535]
- [110]. Chen D, Ji X, Harris MA, Feng JQ, Karsenty G, Celeste AJ, Rosen V, Mundy GR, Harris SE. Differential roles for bone morphogenetic protein (BMP) receptor type IB and IA in differentiation and specification of mesenchymal precursor cells to osteoblast and adipocyte lineages. *J Cell Biol*. 1998; 142:295–305. [PubMed: 9660882]
- [111]. Wan M, Cao X. BMP signaling in skeletal development. *Biochem Biophys Res Commun*. 2005; 328:651–7. [PubMed: 15694398]
- [112]. Zhao M, Harris SE, Horn D, Geng Z, Nishimura R, Mundy GR, Chen D. Bone morphogenetic protein receptor signaling is necessary for normal murine postnatal bone formation. *J Cell Biol*. 2002; 157:1049–60. [PubMed: 12058020]
- [113]. Broege A, Pham L, Jensen ED, Emery A, Huang TH, Stemig M, Beppu H, Petryk A, O'Connor M, Mansky K, Gopalakrishnan R. Bone morphogenetic proteins signal via SMAD and mitogen-activated protein (MAP) kinase pathways at distinct times during osteoclastogenesis. *J Biol Chem*. 2013; 288:37230–40. [PubMed: 24235143]
- [114]. Saetrom P, Biesinger J, Li SM, Smith D, Thomas LF, Majzoub K, Rivas GE, Alluin J, Rossi JJ, Krontiris TG, Weitzel J, Daly MB, Benson AB, Kirkwood JM, O'Dwyer PJ, Sutphen R, Stewart JA, Johnson D, Larson GP. A risk variant in an miR-125b binding site in BMPRI1B is associated with breast cancer pathogenesis. *Cancer Res*. 2009; 69:7459–65. [PubMed: 19738052]
- [115]. Helms MW, Packeisen J, August C, Schitteck B, Boecker W, Brandt BH, Buerger H. First evidence supporting a potential role for the BMP/SMAD pathway in the progression of oestrogen receptor-positive breast cancer. *J Pathol*. 2005; 206:366–76. [PubMed: 15892165]
- [116]. Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. *Nat Rev Cancer*. 2006; 6:259–69. [PubMed: 16557279]
- [117]. Feng N, Xu B, Tao J, Li P, Cheng G, Min Z, Mi Y, Wang M, Tong N, Tang J, Zhang Z, Wu H, Zhang W, Wang Z, Hua L. A miR-125b binding site polymorphism in bone morphogenetic protein membrane receptor type IB gene and prostate cancer risk in China. *Mol Biol Rep*. 2012; 39:369–73. [PubMed: 21556765]
- [118]. Chang CY, Chen Y, Lai MT, Chang HW, Cheng J, Chan C, Chen CM, Lee SC, Lin YJ, Wan L, Tsai PW, Yang SH, Chung C, Sheu JJ, Tsai FJ. BMPRI1B up-regulation via a miRNA binding site variation defines endometriosis susceptibility and CA125 levels. *PLoS One*. 2013; 8:e80630. [PubMed: 24339876]
- [119]. Naganawa T, Xiao L, Coffin JD, Doetschman T, Sabbieti MG, Agas D, Hurley MM. Reduced expression and function of bone morphogenetic protein-2 in bones of Fgf2 null mice. *J Cell Biochem*. 2008; 103:1975–88. [PubMed: 17955502]

- [120]. Fei Y, Xiao L, Hurley MM. The impaired bone anabolic effect of PTH in the absence of endogenous FGF2 is partially due to reduced ATF4 expression. *Biochem Biophys Res Commun.* 2011; 412:160–4. [PubMed: 21806973]
- [121]. Su N, Jin M, Chen L. Role of FGF/FGFR signaling in skeletal development and homeostasis: learning from mouse models. *Bone Research.* 2014; 2:14003. [PubMed: 26273516]
- [122]. Kalajzic I, Kalajzic Z, Hurley MM, Lichtler AC, Rowe DW. Stage specific inhibition of osteoblast lineage differentiation by FGF2 and noggin. *J Cell Biochem.* 2003; 88:1168–76. [PubMed: 12647299]
- [123]. Okada Y, Montero A, Zhang X, Sobue T, Lorenzo J, Doetschman T, Coffin JD, Hurley MM. Impaired osteoclast formation in bone marrow cultures of Fgf2 null mice in response to parathyroid hormone. *J Biol Chem.* 2003; 278:21258–66. [PubMed: 12665515]
- [124]. Montero A, Okada Y, Tomita M, Ito M, Tsurukami H, Nakamura T, Doetschman T, Coffin JD, Hurley MM. Disruption of the fibroblast growth factor-2 gene results in decreased bone mass and bone formation. *J Clin Invest.* 2000; 105:1085–93. [PubMed: 10772653]
- [125]. Homer-Bouthiette C, Doetschman T, Xiao L, Hurley MM. Knockout of Nuclear High Molecular Weight FGF2 Isoforms in Mice Modulates Bone and Phosphate Homeostasis. *Journal of Biological Chemistry.* 2014
- [126]. Lei SF, Papsian CJ, Deng HW. Polymorphisms in predicted miRNA binding sites and osteoporosis. *J Bone Miner Res.* 2011; 26:72–8. [PubMed: 20641033]
- [127]. Boskey AL, Moore DJ, Amling M, Canalis E, Delany AM. Infrared analysis of the mineral and matrix in bones of osteonectin-null mice and their wildtype controls. *J Bone Miner Res.* 2003; 18:1005–11. [PubMed: 12817752]
- [128]. Delany AM, Kalajzic I, Bradshaw AD, Sage EH, Canalis E. Osteonectin-null mutation compromises osteoblast formation, maturation, and survival. *Endocrinology.* 2003; 144:2588–96. [PubMed: 12746322]
- [129]. Kapinas K, Lowther KM, Kessler CB, Tilbury K, Lieberman JR, Tirnauer JS, Campagnola P, Delany AM. Bone matrix osteonectin limits prostate cancer cell growth and survival. *Matrix Biol.* 2012; 31:299–307. [PubMed: 22525512]
- [130]. Delany AM, Amling M, Priemel M, Howe C, Baron R, Canalis E. Osteopenia and decreased bone formation in osteonectin-deficient mice. *J Clin Invest.* 2000; 105:915–23. [PubMed: 10749571]
- [131]. Machado do Reis L, Kessler CB, Adams DJ, Lorenzo J, Jorgetti V, Delany AM. Accentuated osteoclastic response to parathyroid hormone undermines bone mass acquisition in osteonectin-null mice. *Bone.* 2008; 43:264–73. [PubMed: 18499553]
- [132]. Delany AM, McMahon DJ, Powell JS, Greenberg DA, Kurland ES. Osteonectin/SPARC polymorphisms in Caucasian men with idiopathic osteoporosis. *Osteoporos Int.* 2008; 19:969–78. [PubMed: 18084690]
- [133]. Dole NS, Kapinas K, Kessler CB, Yee SP, Adams DJ, Pereira RC, Delany AM. A single nucleotide polymorphism in osteonectin 3' untranslated region regulates bone volume and is targeted by miR-433. *J Bone Miner Res.* 2014
- [134]. Kim EJ, Kang IH, Lee JW, Jang WG, Koh JT. MiR-433 mediates ERRgamma-suppressed osteoblast differentiation via direct targeting to Runx2 mRNA in C3H10T1/2 cells. *Life Sci.* 2013; 92:562–8. [PubMed: 23353875]
- [135]. Huang S, Wang S, Bian C, Yang Z, Zhou H, Zeng Y, Li H, Han Q, Zhao RC. Upregulation of miR-22 promotes osteogenic differentiation and inhibits adipogenic differentiation of human adipose tissue-derived mesenchymal stem cells by repressing HDAC6 protein expression. *Stem Cells Dev.* 2012; 21:2531–40. [PubMed: 22375943]
- [136]. Westendorf JJ, Zaidi SK, Cascino JE, Kahler R, van Wijnen AJ, Lian JB, Yoshida M, Stein GS, Li X. Runx2 (Cbfa1, AML-3) interacts with histone deacetylase 6 and represses the p21(CIP1/WAF1) promoter. *Mol Cell Biol.* 2002; 22:7982–92. [PubMed: 12391164]
- [137]. Thompson CL, Chapple JP, Knight MM. Primary cilia disassembly down-regulates mechanosensitive hedgehog signalling: a feedback mechanism controlling ADAMTS-5 expression in chondrocytes. *Osteoarthritis Cartilage.* 2014; 22:490–8. [PubMed: 24457103]

- [138]. Simon D, Laloo B, Barillot M, Barnette T, Blanchard C, Rooryck C, Marche M, Burgelin I, Coupry I, Chassaing N, Gilbert-Dussardier B, Lacombe D, Grosset C, Arveiler B. A mutation in the 3'-UTR of the HDAC6 gene abolishing the post-transcriptional regulation mediated by hsa-miR-433 is linked to a new form of dominant X-linked chondrodysplasia. *Hum Mol Genet.* 2010; 19:2015–27. [PubMed: 20181727]
- [139]. Nguyen TV, Eisman JA. Genetics and the individualized prediction of fracture. *Curr Osteoporos Rep.* 2012; 10:236–44. [PubMed: 22851044]
- [140]. Mitchell BD, Streeten EA. Clinical impact of recent genetic discoveries in osteoporosis. *Appl Clin Genet.* 2013; 6:75–85. [PubMed: 24133373]
- [141]. Hu Y, Liu CM, Qi L, He TZ, Shi-Guo L, Hao CJ, Cui Y, Zhang N, Xia HF, Ma X. Two common SNPs in pri-miR-125a alter the mature miRNA expression and associate with recurrent pregnancy loss in a Han-Chinese population. *RNA Biol.* 2011; 8:861–72. [PubMed: 21788734]

Highlights

- miRNAs are key post-transcriptional regulators in skeletal cells.
- SNPs in miRNA genes or miRNA binding sites have the potential to alter gene regulation.
- Functionally validated SNPs in miRNA genes and miRNA targets are described.
- The potential impact of these variants on the function of osteoblasts, osteoclasts and chondrocytes is discussed.
- SNPs in miRNA genes and miRNA binding sites likely contribute to genetic variation in bone mass.

Figure 1. Schematic diagram of miR-P-SNP function

SNPs in a transcription factor-binding site (TFBS) near the promoter of miRNA gene can alter miRNA expression.

Figure 2. Schematic diagram of miR-SNP function

SNPs in pri-miRNAs (A) and pre-miRNAs (B) are likely to affect miRNA processing and subsequent mature miRNA levels. SNPs in mature miRNA can alter their activity (C).

Figure 3. Potential model for miR-146a role in osteoarthritis

In early OA lesions, levels of miR-146a are high (depicted by large font size), which can suppress SMAD4/TGF- β signaling and enhance chondrocyte apoptosis. With progression of OA into later stages, miR-146a levels decline (depicted by small font size). Decreased targeting of IRAK1 and TRAF6 could result in increased IL-1 β /NF- κ B signaling, and induction MMPs and ADAMTS5, with suppression of Col II and aggrecan. Together this exacerbates cartilage matrix degradation. Inflammatory cytokines, TNF- α , LPS and IL- β , promote NF- κ B mediated transcription of miR-146a. Note that the displayed regulatory interactions represent only some of the relevant concepts for the role of miR-146a in OA.

Figure 4. Potential model for miR-125a role in osteoclasts

miR-125a has a feed forward regulatory mechanism with the NF-κB pathway, miR-125a directly targets TNFAIP3, a negative regulator of NF-κB signaling. Note that the indicated interaction represents only some of the several regulatory mechanisms for miR-125a function in osteoclasts.

Figure 5. Potential model for miR-27a role in osteoblasts

miR-27 fine tunes osteoblast differentiation by positively regulating canonical Wnt signaling and negatively regulating Runx2 activity. miR-27a targets inhibitors of canonical Wnt pathway, APC and sFRP1, as well as promoters of Runx2 activity, SATB2 and Hoxa10. The scope of this figure is limited to include only specific targets discussed in the text.

Figure 6. Potential model for miR-2861 role in osteoblasts

miR-2861 is induced by BMP2. It increases Runx2 levels indirectly by targeting HDAC5, a deacetylase that promotes ubiquitin mediated Runx2 degradation. Note that for simplification several other miR-2861-target interactions have been excluded from the model.

miR-TS-SNPs: SNPs in miR- binding site

Wild type

Variant

Figure 7. Schematic diagram of miR-TS-SNP function

SNPs in miRNA target site (TS) can strengthen or reduce interaction between the miRNA and its mRNA target. Moreover, such SNPs can create a novel site or destroy an existing target binding sites, ultimately modulating target gene expression.

Figure 8. Schematics of regulatory networks of SNP associated miRNA

Regulatory networks of microRNAs and miRNA targets (*) containing SNPs and their potential impact on osteoblasts (top), osteoclasts (middle) and chondrocytes (bottom).

Table 1.1
Genetic variants associated with miRNAs

	miRNAs	SNP #	Alleles	Description	Reference
miR-P-SNPs	miR-146a	rs57095329	A>G	G-decreased miR levels; TFBS modification	[27]
	miR-196a-2	rs35010275	G>C	C- decreased miR levels; TFBS modification	[80]
miR-SNPs	Pri-miR				
	miR-125a	rs12975333	G>T	T-decreased miR levels; DGCR8 binding	[7]
	miR-124	rs531564	G>C	G-decreased miR levels	[101]
	Pre-miR				
	miR-146a	rs2910164	G>C	G-increased miR levels; stable alternative passenger strands	[30, 55]
	miR-196a-2	rs11614913	C>T	T-reduced miR levels	[82]
	miR-149	rs71428439	A>G	G-reduced miR levels	[88]
	miR-27a	rs11671784	G>A	G-reduced miR levels	[95]
	miR-2861 *	mutation	C>G	G-reduced miR levels	[103]
	mature-miR				
	miR-125a	rs12975333	G>T	T- impairs miR binding to lin-28	[7], [141]
miR-TS-SNPs	BMPR-IB	rs1434536	C>T	T-loss of miR-125b binding site	[114]
	FGF2	rs6854081	T>G	G-loss of miR-146a, -b binding site	[126]
	SPARC	rs1054204	C>G	G-creates miR-433 binding site	[133]
	HDAC6 *	mutation	A>T	T-loss of miR-433 binding site	[138]