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What are microRNAs? Potential biomarkers and therapeutic targets in osteoporosis

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Summary

Micro-RNAs (miRs) are small non-coding RNA molecules that regulate gene expression at post-transcriptional level. Generally, they act on gene expression by silencing or degrading mRNAs, and are involved in regulating various biological processes, such as cell differentiation, proliferation, apoptosis and in embryonic and tissue development. They are currently a major focus of interest in the study of various diseases such as cancer or type 2 diabetes mellitus. At level of bone metabolism, various miRs are emerging that are involved in their regulation, opening an important research field to identify new biomarkers for diagnosis of osteoporosis and its development, and to design new drug therapies.

Key words: *epigenetics, micro-RNA, biomarkers and osteoporosis.*

Introduction

MicroRNAs were discovered in 1993 to study the regulation of the development of the nematode *Caenorhabditis elegans*¹. They are small noncoding RNAs (21-23 nucleotides) for proteins that constitute a large family of post-transcriptional regulatory genes. They are involved in the regulation of various biological processes such as cell differentiation, proliferation, and apoptosis in embryonic and tissue development². They function as an epigenetic control of an endogenous gene and, although they generally silence genes which differ from those which have been transcribed, there are also microRNAs that promote and / or co-activate other genes³.

Since their discovery, they have become one of the most studied in the field of epigenetic regulation of cells. Much of the current information has enhanced our understanding of the biological processes in which they are involved. Everything that has been carried out recently is a focus of interest for medicine as therapeutic targets in many diseases. To date, over 2,000 different human sequences of microRNAs have been described in the miRBase database (<http://www.mirbase.org>).

MicroRNAs represent only 2-3% of the human genome, and it is estimated they can regulate the expression of about 60% of genes⁴. One single miR can regulate about 200 different transcripts may act each in a different cellular pathway, and the same mRNA may be regulated by multiple microRNAs^{5,6}.

The genesis of microRNAs has been well studied and characterized by various authors⁷⁻⁹. First, inside the nucleus, the genes that encode for microRNAs are transcribed into long precursors, resulting in so-called primary microRNAs, varying in length from hundreds of base pairs. This precursor is cut by the Drosha and Pasha/DGCR8 ribonucleases in one or more RNA forked molecules, making it into pre-miRNAs of 60-70 nucleotides. The pre-miRNAs leave the nucleus and move toward the cytoplasm aided by exportin-5 where the miR maturation process takes place. In the cytoplasm, the pre-miRNA is carried by the RLC complex (RISC loading complex) formed by the RNase Dicer, TRBP (RNA binding protein in response to transactivation) pkrA (activating RNA dependent protein kinase) and Ago2. This complex produces the cleavage of pre-miR generating a duplex with a mature miR chain and its complementary. The mature chain with Ago2 form the RISC complex (RNA-induced silencing complex) and the complementary strand will be removed. RISC binds to an mRNA molecule (usually in the 3' untranslated) having a sequence complementary to the miR component and cuts the mRNA, leading to degradation of the mRNA or to modify its translation. Some microRNAs also serve as guides for the methylation of complementary sequences; both processes affect transcription. Biogenesis and mechanism by which they regulate microRNAs expression as shown in Figure 1.

The complementary sequence between mRNA and miR is only 7 nucleotides, it is believed that each miR could potentially mate with hundreds of different mRNAs. Similarly, a single RNA molecule could have multiple microRNAs binding sites. The translational inhibition binding must require several RISC complex to the same ARNm molecule¹⁰.

MicroRNAs as biomarkers

Osteoporosis is a disease characterized by low bone mass and a deterioration in its quality, with weakening of the microarchitecture, leading to increased risk of fractures with minimal trauma. Although we now have several tools for assessing the risk of osteoporotic fractures, many low-risk patients suffer fractures and vice versa. The microRNAs could provide information to improve risk prediction. One of the challenges in the field of osteoporosis is early disease detection, allowing timely action and obtaining better results in treatment. This requires developing more effective noninvasive methods that are predictors of bone loss, fracture risk and/or therapeutic response, and allow us to monitor and assess the effectiveness of drug therapy.

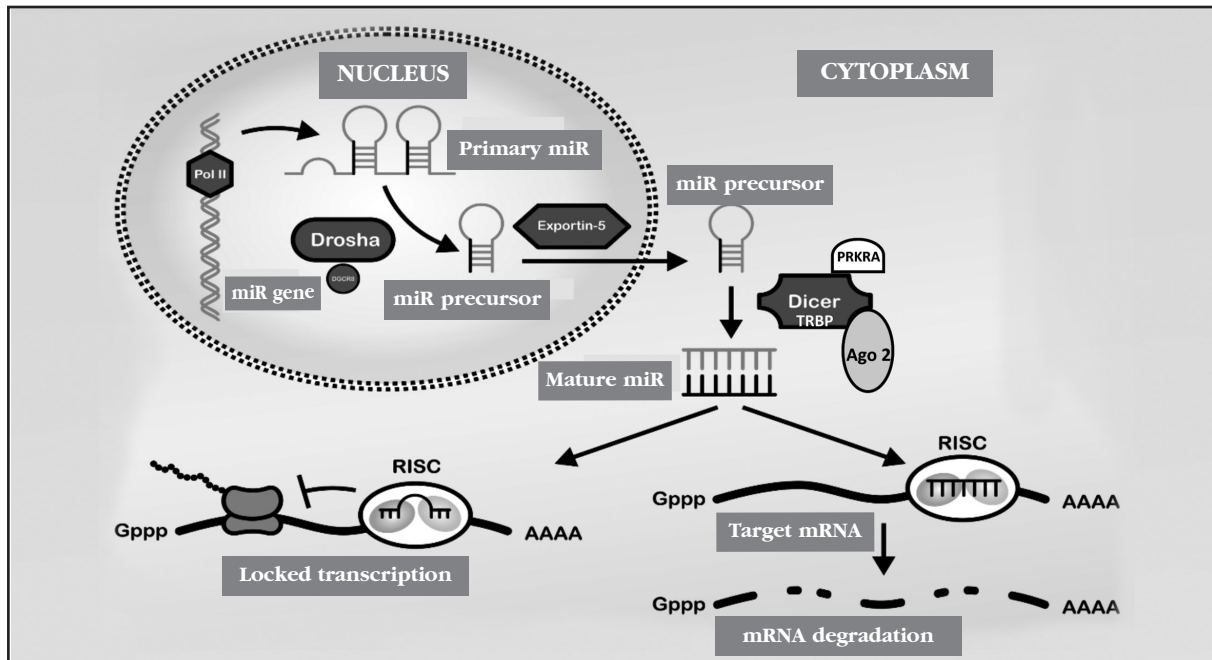
In this regard, the microRNAs may constitute new biomarkers of great interest, since they have been shown to resist RNase activity in peripheral blood, which affords them high stability in serum and plasma. On the other hand, they are reproducible and have high tissue specificity between individuals^{11,12}. We know that the levels of expression of several microRNAs vary with aging, and a specific miR can have a positive and negative effect on the same cell depending on its state of differentiation¹³. Moreover, various alterations have already been described in the expression of microRNAs strongly related to the occurrence and development of diseases such as cancer, type 2 diabetes mellitus, Alzheimer's, osteoarthritis, among others^{14,15}. Their quantification is already being used as biomarkers in the diagnosis and progression of these diseases¹⁶⁻¹⁸.

In recent years, different microRNAs have begun to be described related to osteoporotic disease and/or risk of fracture^{14,19-20}, although data are still scarce.

The miR-2861 was the first miR that attributed a clinical implication in human osteoporosis²¹. The miR-2861 as a target molecule has a histone deacetylase (HDAC5) which negatively regulates RunX2. Mutations at the encoding locus induced osteoporosis. Animals treated with miR-286 inhibitors have a low bone mass phenotype. Subsequently, other microRNAs may play an important role in regulating bone metabolism as the target having several genes encoding crucial transcription factors in bone remodeling. However, it is still not known which of them is related to a higher or lower rate of bone turnover and low bone mass. In figures 2a and 2b we see the different microRNAs acting at both cells as osteoclastic and osteoblastic line²²⁻²⁸.

Among the various microRNAs described to date, the role of some of them stand out as poten-

Figure 1. MiRNA biogenesis



tial biomarkers of osteoporotic fracture risk in our population. Specifically, we found that miR-21, miR-23a, miR-24, miR-25, miR-100, miR-125b, miR-miR-328-3p 518f are over-expressed in serum of women with fracture osteoporotic, whereas the expression of miR-187 is reduced^{1,13,29}. Other studies point to miR-133a and miR-194-5p as possible biomarkers associated with osteoporotic disease, showing that in the serum of postmenopausal women are higher levels of such microRNAs, and are also negatively correlated with spine BMD and femoral neck^{20,30}.

Although there is increasing knowledge of more microRNAs involved in bone metabolism and biological function and its mechanism of action, the prior mechanism by which miRs reach the bloodstream is still not known, and their role in blood is not entirely understood. Future studies will clarify both aspects help us to find new and better biomarkers and more judiciously select those already proposed.

We should also bear in mind that, for the proper use of microRNAs as biomarkers in clinical practice, establishing a standard sample collection process and normalization techniques of real-time PCR is required.

The microRNAs as therapeutic targets

The microRNAs play a fundamental role in the regulation of bone metabolism. Variations in gene expression can lead to alterations in bone remodeling and have adverse effects on the skeleton. All this opens a new window of possibilities for the development of new therapeutic strategies for the treatment of various bone diseases such as osteoporosis.

The pharmaceutical industry is currently investigating drug targets aimed at normalizing the tis-

sue levels of specific microRNAs, silencing those which were over-expressed or increasing their levels in those with a deficit. The microRNAs can be silenced by molecules called anti-miRNAs (AMOs). These are synthetic antisense oligonucleotides which competitively inhibit interaction between the microRNAs and its target mRNA. The most widely used AMOs are 2'-O-methyl AMO, 2'-O-methoxyethyl AMO and the Locked Nucleic Acids (LNAs)³¹. On the other hand, microRNAs often work in groups to regulate the pathological processes, so that instead of designing different anti-microRNAs for equal treatment, microRNAs called "sponge" are being developed, which can set numerous microRNAs at once.

Conversely, if we want to restore decreased levels of miR, the strategy is to manage microRNAs mimetic (miR mimics), which are molecules of dsRNA chemically altered to mimic endogenous microRNAs. When introduced into cells, they are recognized by miR mimics machinery microRNAs biogenesis and processed as such.

MicroRNAs use as pharmacological agents is already accepted in some tumor and viral pathologies³¹. At present, there are different molecules with inhibitory pharmacological action of microRNAs being used in phase II and III for the treatment of hepatitis C, miR-1 (miravirsin)³² and RG-101³³.

As for the field of bone metabolism, advances in therapy are lower. We can find only a few isolated studies working in cell or animal models. Notably, a recent study published in Nature in which the miR-34a is designated as a new suppressor of osteoclast formation and bone resorption, which has important implications for the treatment of osteoporosis or bone metastases. This study shows how mice with increased levels

Figure 2a. Targets and function of the miRNA on the differentiation and proliferation of osteoblasts

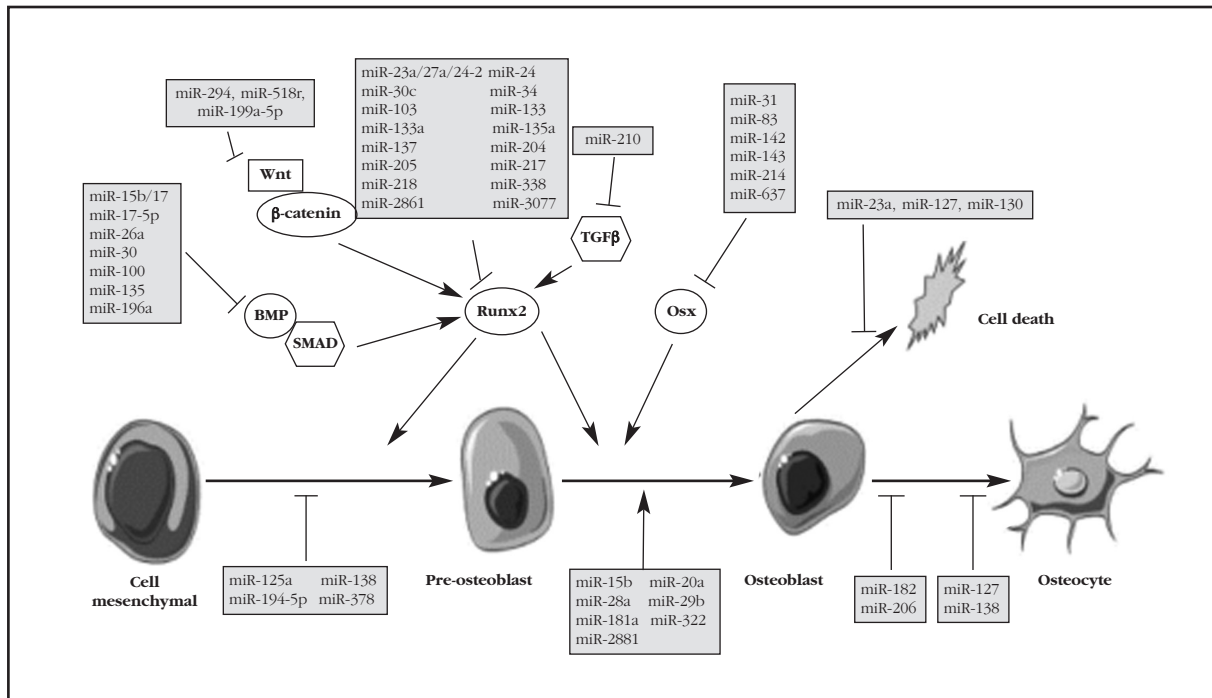
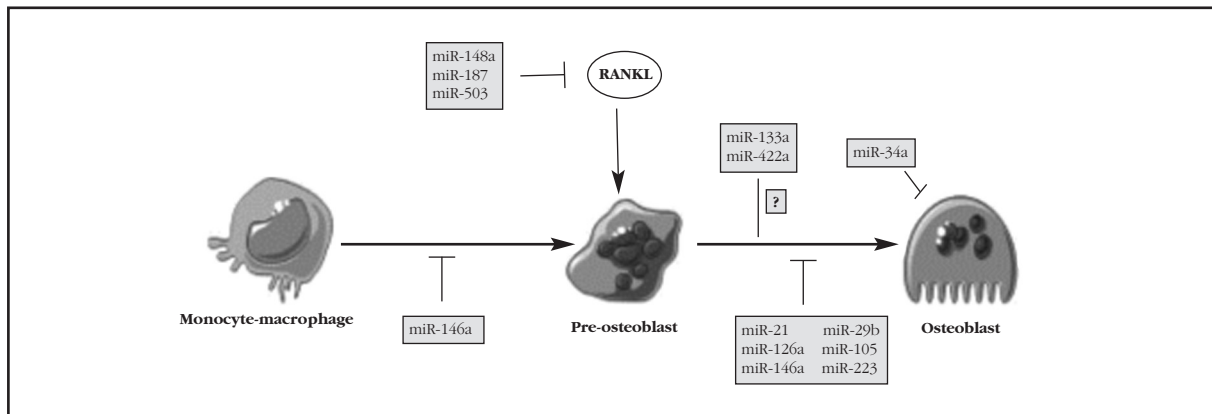


Figure 2b. Targets and function of the miRNA on the differentiation and proliferation of osteoblasts



of miR-34a have a higher bone density and lower rate of bone fractures. After injection of nanoparticles containing microRNA, both bone loss in mice with postmenopausal osteoporosis and bone metastasis in mouse models of reduced breast or skin cancer were reduced¹⁹. Wang et al. injected anti-miR-214 in mice and observed a lower loss of bone mass in treated animals¹³. Currently there are two major limitations to the use of microRNAs as pharmacological agents. The first is that one miR tends to have different target genes at a time and also can act as an inhibitor or promoter, depending on the target gene and the stage of cell differentiation time. This complexity explains the difficulties in predicting the spectrum of action and toxicity profiles associated with microRNAs therapy. To avoid this issue, recent research focuses on testing the stability of the microRNAs and direct its action to target tissues or cells. The second limitation is

that the unmodified microRNAs may trigger nonspecific reactions of interferon in tissues. The presence of anti-miR or miR mimics modulates the stimulators of interferon gene expression, causing changes in the immune response.

Conclusions

1. The role of microRNAs in gene regulation is essential. They are involved in the regulation of various biological processes such as cell differentiation, proliferation and apoptosis in embryonic development and tissue.

2. The differential expression of microRNAs induces changes in most stages of skeletal development, so that the process of bone remodeling is also regulated by different microRNAs.

3. The study of the diverse differential expression profiles of microRNAs in bone metabolism disorders lead us to identify new biomarkers of osteoporotic disease and its development.

4. Given that microRNAs have a crucial role in bone tissue, better knowledge could lead us to set new therapeutic targets.

5. Better understanding of biogenesis microRNAs and their role in the pathogenic processes provide new tools for the diagnosis and prognosis of osteoporotic disease and new therapeutic targets.

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