The possible use of circRNAs as useful diagnostic, prognostic and therapeutic biomarkers in osteoporosis

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ABSTRACT

Bone health is ensured by coordinated work between osteoclasts and osteoblasts. When this balance is lacking, skeletal diseases, including osteoporosis (OP), arise. The latter is caused by an increase in erosion and a decrease in bone matrix deposition, resulting in low-quality bone that tends to have a higher risk of fracture. To date, despite the use of sophisticated software to diagnose OP, and the use of several drugs to mitigate the effects of this pathology, there are still limitations that drive the scientific community to search for new biomarkers. In recent years, epigenetics has attracted the attention of many researchers as a possible mechanism involved in the onset and progression of OP. In particular they have focused on circular RNAs (circRNAs), a particular class of non-coding RNAs. Precisely because of their properties, these molecules could open up new possibilities in the field of precision medicine. In conclusion, therefore, the aim of this review is to offer an overview on circRNAs, which could potentially become, in the future, useful diagnostic, prognostic and therapeutic biomarkers in OP routine clinical practice.

KEYWORDS

Osteoporosis, circRNAs, osteoblastogenesis, osteoclastogenesis.

Introduction

Osteoporosis: definition, causes, diagnosis and treatment

A healthy skeletal system is the result of continuous optimal bone remodelling, achieved through a harmonious balance between the processes of osteoblastogenesis and osteoclastogenesis. It is the lack of this functional balance that underlies osteoporosis (OP). OP is a chronic skeletal disease caused by a prevalence of osteoclastic activity over osteoblastic activity, leading to a progressive reduction in bone mass density and quality, with a consequent increase in the risk of fracture [1,2]. It is possible to distinguish between two types of OP: primary and secondary OP. The former includes postmenopausal (PMOP), male, juvenile and senile OP; secondary OP may be due to the pre-existence of other diseases and the intake of some drugs [3].

Today, the gold standard for diagnosis of OP is dual-energy X-ray (DEXA), which allows bone mineral density to be measured in the form of the T-score. In recent years, DEXA has been joined by new software-based tools, such as hip structural analysis and the trabecular bone score [3]. Laboratory tests are based principally on the dosage, in biological fluids, of bone turnover markers (BTMs) such as procollagen type I N-terminal propeptide, bone alkaline phosphatase (ALP), serum total osteocalcin, cathepsin K, deoxypyridinoline, pyridinoline and serum 5b isoenzyme of tartrate-resistant acid phosphatase [4,5]. Despite the use of sophisticated imaging instruments and the dosage of BTMs, there are still several limitations in OP diagnosis and fragility fracture assessment in patients with type 2 diabetes. Further issues are the considerable exposure of the patient to ionising radiation, and the low specificity and high cost of tests.

The basal treatment to prevent the risk of OP is increased vitamin D and calcium intake in the diet [3]. When OP has been diagnosed, it is the necessary to treat it through the intake of anti-osteoporotic drugs, which can be divided into anti-catabolic and anabolic drugs. The former include bisphosphonates [6] and denosumab [7], which have the ability to decrease bone matrix resorption, and selective oestrogen receptor modulators, which act as oestrogen agonists and promote antiresorptive activity [8]. Anabolic drugs include teriparatide, which stimulates bone matrix formation [9], and is administered to patients with severe OP. Although these drugs have some benefits for bone health, their use cannot be sustained over a long period of time because their adverse effects are not insignificant [6].

In view of all these gaps in the diagnosis and treatment of OP, there is a need to find useful new biomarkers that might

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enter routine clinical practice in this field.

In this regard, in recent years, researchers have found the study of epigenetics and its deregulation to be a possible and highly promising means to identify new biomarkers, since epigenetics and its deregulation is now described as the basis of the development of several diseases, including OP [10,11]. Among these molecules, circular RNAs (circRNAs) are an emerging class. circRNAs are non-coding RNAs (ncRNAs) with a length of between 100 and 1000 nucleotides and a circular structure that gives them greater stability and resistance to attack by exonucleases [12]. Biogenesis of circRNAs occurs mainly through a particular type of splicing called backsplicing, favoured by the presence of inverted repeat elements, long flanking introns and trans-acting RNA binding proteins [13,14]. As regards the mechanism of action of circRNAs, they function mainly as sponge factors for mRNAs and target proteins, associating with them and preventing them from carrying out their task, thus regulating gene expression at the post-transcriptional level [15]. Recent works reported that several circRNAs are involved in both osteoblastogenesis and osteoclastogenesis. Therefore, this review aims to provide a systematic overview of the state of art regarding research on the role of circRNAs in OP.

Expression of circRNAs in OP: the first studies

Different studies found six differentially expressed circRNAs (DECs) in OP patients with respect to control group subjects [16-22]. In addition to this, some authors, using Gene Ontology and Kyoto Encyclopaedia of Genes and Genomes bioinformatic programs to construct circRNA-associated competing endogenous RNA (ceRNA) networks, discovered that some circRNAs could be involved in different physiological pathways and play an important role in the pathogenesis of various OP subtypes [23].

Finally, Lin *et al*. [24] evaluating the expression profile of circRNAs during osteoclast differentiation in the presence or absence of alendronate, discovered 110 DECs between osteoclast precursors (OPCSs) with respect to osteoclasts (OCs) and alendronate-treated OCs, and found that circ_0001776, circ_0000284, circ_0002922, circ_0000638, circ_0000994, circ_0007710 and circ_0113954 were upregulated in OCs compared to OPCSs.

However, these preliminary data need further investigations to validate the differential expression of these identified circRNAs, understand their role, and clarify which of them could be used as new diagnostic/prognostic biomarkers, but also as potential new therapeutic targets for OP.

circRNAs promoting OP

circ_0002060 was also studied by Liu *et al*. [25] .They tried to understand the function of this circRNA in the pathogenesis of OP, by studying its behaviour in osteoblast cells (hFOB 1.19) treated with dexamethasone (DEX). Functional analysis on viability and apoptosis of DEX-treated cells indicated that these parameters improved when circ_0002060 expression was totally suppressed. Moreover, the authors showed that this circRNA was able to bind and inhibit miR-198-5p, consequently increasing the expression of its target, Bax, a pro-apoptotic member of Bcl-2 family, which plays a key role in bone growth impairment. In addition, the studies in OVX-induced OP mouse models revealed that circ_0002060 knockdown ameliorated the progressive loss of osteoblasts in the animals, probably through the JNK signalling pathway. In conclusion, these data agree with Huang's previous work [21], underlining that circ_0002060 could be considered as a possible target in the treatment of OP.

Xu *et al*. [26], analysing the role of circ_0001275 in OP, observed that hFOB1.19 cells treated with DEX recovered their ability to proliferate once circ_0001275 silencing had been performed. Subsequently, it was seen that this circRNA was capable of inhibiting ALP activity and calcium nodule formation, probably acting through the mir-377/CDKN1B axis. This work thus showed that the role of circ_0001275 could be crucial for the onset and progression of OP.

Based on previous knowledge of melatonin's ability to induce osteogenic differentiation, Wang *et al*. [27] attempted to elucidate the role of circRNAs in this latter mechanism in bone marrow mesenchymal stem cells (BMSCs) stimulated by melatonin (MEL). circRNA profile analysis revealed that among the 209 DECs in MEL-treated BMSCs, circ_0003865 was particularly downregulated. Moreover, silencing of this molecule had a positive effect on osteogenic differentiation of the cells, whereas overexpression of circ_0003865 inhibited the differentiation process. Further studies demonstrated that this circRNA acted by sponging miR-3653-3p to increase GAS1 expression. Finally, it was also seen that silencing of circ_0003865 in an OP mouse model was able to reduce the disease features, confirming its negative effect in this pathology.

In 2019, Xu and his group [28] observed that circ_0011269 was particularly downregulated in OP patients. Further analysis showed that it was capable of sponging mir-122 and increasing the expression of its target gene runt-related transcription factor 2 (RUNX2), a key gene of pre-osteoblast differentiation. Furthermore, they observed that the expression of both circ_0011269 and RUNX2 was enhanced during the osteoblastogenesis process, while the expression of mir-122 gradually decreased. All these data provide evidence of the presence of a new signalling pathway, consisting of circ_0011269/mir-122/RUNX2, which could be important for the development of new therapies against OP progression.

In the same year, Chen *et al*. [29] observed that RANKL and CSF1-treated bone marrow monocyte/macrophage cells induced in the osteoclastic direction presented a different expression profile of circRNAs and micro-RNAs (miRNAs) compared with untreated cells. In particular, it was reported that circRNA_28313 was upregulated in the first group of cells with respect to the controls. Furthermore, silencing of this circRNA decreased differentiation into osteoclasts *in vitro*, while it attenuated bone resorption in OVX mice, suggesting its negative function in OP.

The results obtained by Lin *et al*. [30] in 2020 showed that

expression of circ_SLC8A1 was upregulated in the OVX mice group compared with the control group. Moreover, this observation also occurred in OVX-derived BMSCs during osteogenic differentiation. In fact, overexpression of this circRNA promoted the expression of several genes involved in OP progression (i.e., BMP4, BGLAP, SPP1 and ALP). The opposite occurred when circ_SLC8A1 was silenced. At this point, it was seen that circ_SLC8A1 could bind mir-516b-5p and inhibit its action, increasing expression of its target gene AKAP2. In conclusion, the authors shed light on the possible involvement of the circ_SLC8A1/miR-516b-5p/AKAP2 axis in OP progression.

Although all the above-reported studies showed for the first time the possible involvement of various circRNAs in the promotion of OP, further investigations will need to be performed to better elucidate and detail their role in disease onset and progression, with a view to using them as potential therapeutic targets in the future clinical context.

circRNAs inhibiting OP

In parallel with the studies seeking to identify which circRNAs can promote the onset of OP, several studies were being conducted to establish which ones may instead be involved in mitigation of this disease.

In the last two years, four papers described how some circRNAs (i.e., circ_0007059, circ_0048211, circ_0000020 and circ_0016624) were able to mitigate the effects of OP, through their sponge action against different miRNAs (i.e., miR-93-5p, miR-378, miR-142-5p and miR-98, respectively), by promoting expression of BMP2 and other genes involved in the osteogenesis process [31-34].

Based on previous studies conducted in 2019 [34], Wen *et al.*[35] explored the possible function of circ_0076906 in OP. They observed that its expression levels were lower in both blood and tissue samples from OP patients than in samples from healthy patients. In addition, silencing of this circRNA in human-derived mesenchymal stem cells reduced the expression of osteogenesis-related genes (i.e., RUNX2 and OGN) and consequently inhibited osteogenesis. Subsequently, it was seen that circ_0076906 effectively regulated the expression of OGN through its action as a ceRNA against miR-1305. Thus, this study demonstrated that circ_0076906 positively influences osteogenic differentiation, probably by acting on the miR-1305/OGN pathway.

Based on Huang's study [21], Li *et al.*[36] saw that overexpression of circ_0062582 in BMSCs promoted their differentiation in the osteogenic direction and increased expression levels of osteoblastogenesis-associated marker proteins (i.e., osteocalcin, osterix, and collagen type 1). Later, *in silico* analyses predicted that miR-145 might be a target of circ_0062582, and this was subsequently confirmed by dual-luciferase experiments. Further investigation revealed that miR-145 inhibited osteogenesis by directly targeting core-binding factor subunit β (CBFB), an essential factor for transcription of genes associated with osteoblastogenesis. Therefore, the data reported in this work demonstrated that the circ_0062582/miR-145/CBFB axis could have an important function in the onset and progression of OP.

Guo *et al.*,[37] on the basis of the results obtained in their previous work, also decided to investigate the role of circ_0006766 in the osteogenic differentiation process of BMSCs. First, the upregulation of this circRNA during osteogenesis was reconfirmed. Then, bioinformatic analyses indicated several miRNA targets for circ_0006766, including miR-4739. Indeed, the authors found that circ_0006766 sponged miR-4739 to increase its downstream target Notch2. In summary, they found a new axis formed by circ_0006766/miR-4739/Notch2 that could be involved in the pathogenesis of OP.

Wang *et al.*[38] observed downregulated circ_0006393 expression in patients with glucocorticoid-induced OP (GIOP) compared with controls. Furthermore, overexpression of this circRNA in BMSCs increased the expression levels of four osteogenesis markers (i.e., OPG, BMP2, Sp7 and RUNX2). Further investigations revealed that the circ_0006393 mechanism of action was based on downregulation of miR-145-5p expression and upregulation of its target FOX1. Finally, the authors observed that high expression of circ_0006393 in a GIOP animal model was able to increase bone mass.

In the last year, Shen *et al.*[39] studied circRNA expression profiles in bone tissue of OP and healthy patients. circRNA microarray analysis showed that 2327 circRNAs and 2645 circRNAs were downregulated and upregulated, respectively, in tissues from the OP group compared with the healthy group. Furthermore, qPRC analysis confirmed that among the downregulated circRNAs, the expression of circFOXP1 was significantly lower. Further functional studies conducted in human adipose-derived mesenchymal stem cells revealed that this circRNA acted as a ceRNA for miR-33a-5p, increasing the expression and action of its target gene FOXP1 and the osteogenic differentiation process. In conclusion, the circFOXP1/miR-33a-5p/FOXP1 axis, too, could be involved in OP progression.

Two independent works demonstrated how two different circRNAs were able to promote osteogenesis by regulating expression of the same factor.

Indeed, Ji *et al.*[40] observed that circ_0006215 was downregulated in BMSCs isolated from patients with OP, and that its overexpression increased the osteogenic differentiation of these cells. Subsequently, they found that circ_0006215 behaved as a ceRNA for miR-942-5p, inhibiting its function and instead upregulating the expression of its targets, VEGF and RUNX2. In fact, further *in vivo* experiments investigating the osteogenesis coupled with the angiogenesis process showed that circ_0006215 overexpression in bone defect models allowed increased bone tissue repair.

Moreover, Ouyang *et al.*[41] saw that miR-942-5p was also targeted by circ_0074834 in BMSCs obtained from patients with bone nonunion. Indeed, this mechanism of action seemed to promote osteogenesis by regulating the expression of both the VEGF and the ZEB1 gene. In addition, experiments conducted on human umbilical vein endothelial cells revealed that circ_0074834 increased their migration and invasion capacities, demonstrating a proangiogenic effect and promoting angiogenesis-osteogenesis coupling. Finally, the

authors reported that upregulation of this circRNA *in vivo* exhibited a positive role in bone regeneration.

Hence, the results of these two works demonstrated that two different circRNAs, circ_0006215 and circ_0074834, could target the same pathway and play an important role in angiogenesis-osteogenesis coupling.

The purpose of the study carried out by Zhang *et al.*[42] was to understand the mechanism of action of circRNAs in the pathogenesis of OP. In their study, 5443 circRNAs were found to be deregulated during osteoblastic differentiation of BMSCs; subsequent miRNA microarray analysis revealed that 18 miRNAs were downregulated and 42 upregulated. These data allowed the authors to build a circRNA-miRNA interaction network and to further enrich it with mRNA targets of miRNAs, which were involved in several signalling pathways, including osteogenic differentiation. In addition, they validated the connection between circIGSF11 and miR-199-5p expression, observing that silencing of this circRNA increased miRNA expression and osteogenic differentiation of BMSCs, suggesting its involvement in OP pathogenesis.

In the study just mentioned, the circRNAs found to be upregulated during osteoblastic differentiation included also circ_0026827. Based on this, Ji *et al.*[43] investigated its possible role during osteogenic differentiation of dental pulp stem cells. First, the authors reconfirmed the upregulation of circ_0026827. Moreover, suppression of the expression of this circRNA led to a decrease in the osteoblastogenesis process. Furthermore, subsequent studies showed that circ_0026827 acted as a ceRNA for miR-188-3p, promoting osteogenic differentiation through the upregulation of runt-related transcription factor 1 (RUNX1) and beclin 1. Finally, the use of a heterotopic bone model helped to show that circ-0026827 also promotes bone formation *in vivo*.

Han *et al.*[44] demonstrated that expression of circ_0076690 was significantly decreased in samples collected from OP patients compared with the control group. Moreover, further investigations of the mechanism of action of this molecule led to the understanding that circ_0076690 inhibits miR-152 and increases the expression of its target gene RUNX2, promoting osteogenesis.

Knowledge about the ability of circRNA Rtn4-modified BMSC-derived exosomes (Rtn4-Exos) to regulate osteoblast proliferation and apoptosis processes led Cao *et al.*[45] to elucidate the effects of these small vesicles on TNFα-induced apoptosis and cytotoxicity in murine MC3T3-E1 cells and to establish their potential role. The various experiments showed that TNFα-treated MC3T3-E1 cells increased the expression levels of both miR-146a and apoptosis-related factors such as caspase-3 and Bax, resulting in reduced cell viability. In contrast, these signs were reversed by downregulation of miR-146a. Moreover, subsequent evaluations of the effects of Rtn4-Exos on the consequences of TNFα treatment indicated that the vesicles were incorporated by MC3T3-E1 cells and were able to mitigate TNFα-induced cytotoxicity and apoptosis in a dose-dependent manner, suggesting that Rtn4-Exos could be a promising biomarker for the treatment of TNFα-induced OP. Obviously, further studies will have to be performed to verify possible effects of BMSCs-Exos on osteoblast mineralization and differentiation and bone mass recovery in an *in vivo* OP model.

Given the association of Yes-associated protein 1 (YAP1) with bone quality control, Huang *et al.*[46] analyzed its role in the differentiation of MC3T3-E1 cells and BMSCs. Initially, they observed that YAP1 expression increased during the differentiation process of both these cell types. Furthermore, the increase in its expression was correlated with the increase in ALP activity and the expression of osteogenic markers (i.e., OCN, OPN and RUNX2). Next, it was seen that YAP1 was a direct target of miR-376b-3p, which bound the 3'-UTR of YAP1 mRNA by inhibiting its transcription. In addition, circ_0024097 expression was also upregulated during differentiation of BMSCs and MC3T3-E1 cells, suggesting that it might act as a ceRNA for miR-376b-3p. Indeed, this mechanism was later confirmed by luciferase reporter assay, leading the authors to hypothesize, and then demonstrate, that circ_0024097 might facilitate osteoblast differentiation by sponging miR-376b-3p to elevate YAP1 expression and activate the Wnt/β-catenin pathway.

Finally, Liu *et al.*[47], conducted a study to elucidate the possible role of circRNAs in TNFα-related osteoclastogenesis and osteoblastogenesis. The results obtained from their *in vitro* and *in vivo* experiments showed that circHmbox1 expression is negatively affected by TNFα treatment. Furthermore, they found that circHmbox1 silencing significantly increased osteoclast differentiation in the presence of both RANK ligand and TNFα. Subsequently, they saw that pre-osteoclast-derived exosomes with low circHmbox1 expression were able to decrease osteoblast differentiation. The latter concept led the authors to understand that TNFα might inhibit osteoblastogenesis precisely by promoting the secretion, by osteoclasts, of exosomes with low circHmbox1 expression. Moreover, they found that circHmbox1 was able to directly interact with miR-1247-5p to inhibit its function in osteoclasts. This mechanism could also be reflected in osteoblast differentiation, because osteoclast-derived exosomes exhibiting low levels of circHmbox1 expression could affect the expression of miR-1247-5p in osteoblasts and consequently their differentiation. In addition, B cell lymphoma 6 (Bcl6) was identified as the target of miR-1247-5p, and was capable of regulating osteoclast differentiation negatively and the osteoblastogenesis process positively. Finally, OVX mice treated with circHmbox1 showed a recovery of microarchitecture and trabecular bone mass, with a marked improvement of the osteoporotic condition compared with the control group. All these results demonstrate that TNFα could regulate bone metabolism in PMOP through the circHmbox1/miR-1247-5p/Bcl6 axis.

In conclusion, most of the circRNAs mentioned in this section act mainly as a sponge for several miRNAs in order to increase the expression of crucial genes in osteogenic differentiation, and several studies identified different molecular axes, inclusive of circRNAs, which are positively involved in osteogenic differentiation. Thus, these studies could pave the way for conducting further investigations into the possible role of these molecules in the pathogenesis of OP, for the purpose of developing novel therapeutic strategies against the disease.

Analysis of circulating circRNAs in OP: the state of the art

It was 2015 when Memczak *et al.*[48] showed that circRNAs were present in human blood in greater amounts than other linear ncRNAs, thereby rendering accessible information about the activity of many genes that would not normally be accessible by measuring other molecules, and demonstrating that circulating circRNAs could enter clinical practice as useful diagnostic and prognostic biomarkers for several diseases.

The study performed by Xiang *et al.*[49] aimed to evaluate whether circ_0001445 could be a valid biomarker for patients with PMOP. The results obtained from an initial analysis revealed that the expression levels of circ_0001445 were significantly lower in the plasma of PMOP patients compared with patients with osteopenia (OPE) and healthy controls. Furthermore, although the Pearson correlation test showed that circ_0001445 expression levels showed no relationship with the different markers of OP, a positive relationship was found between circ_0001445 levels and T-score and a negative relationship with β-isomerized C-terminal telopeptides. Additionally, through ROC curve analysis the authors discovered that circ_0001445 levels could distinguish PMOP patients from OPE patients and healthy controls with a sensitivity of 81.82% and 97.62% and a specificity of 85.0% and 82.22%, respectively. Again, patients taking anti-osteoporotic drugs also showed increased expression of circ_0001445 in plasma at six months after the start of treatment. These data demonstrated that circ_0001445 could be a useful diagnostic and prognostic biomarker for OP patients.

Guan *et al.*[50] studied the expression of several circRNAs in peripheral blood mononuclear cells of PMOP women compared with healthy controls. Among the circRNAs found to be deregulated, the expression levels of circ_0021739 were significantly lower in the PMOP group than in the control group. Moreover, this circRNA expression correlated with the T-scores of the femur, lumbar vertebrae and forearm, but no correlation was found with clinical factors of OP, including age. In addition, the AUC obtained from ROC curve analysis was 0.845 with a specificity of 42.9% and sensitivity of 100%, showing that circ_0021739 might have good diagnostic value for OP. Finally, it was found that circ_0021739 could act as a ceRNA for miR-502-5p in order to regulate osteoclastogenesis.

Zhi *et al.*[51] analyzed circRNA expression profiles in OP patients through a circRNA microarray performed on exosomes obtained from serum of OP patients and control patients. The data obtained revealed the presence of 589 DECs between the two groups. Based on previous work, the authors chose to conduct further experiments with circ_006859. Indeed, the exosomal expression levels of circ_006859 were higher in OP patients compared with those of OPE patients or healthy controls. Moreover, this expression was negatively correlated with L1-L4 Z-score and T-score. Subsequently, the ROC curve analysis showed that circ_006859 was able to discriminate patients with OP from OPE and healthy controls, respectively, with a sensitivity of 75.0% and 93.1% and a specificity of 93.3 and 93.33%, indicating this molecule as a good potential diagnostic biomarker for OP. Finally, investigations on the role of circ_006859 in pathology suggested that this ncRNA could suppress osteoblast differentiation and promote adipocyte differentiation by sponging miR-431-5p. This mechanism would lead to increased expression of ROCK1 factor, which is capable of inhibiting osteoblastogenesis and increasing adipogenesis.

Taken together, these studies provided a new perspective on the use of circulating DECs in the biological fluids of OP and healthy subjects.

Discussion and conclusions

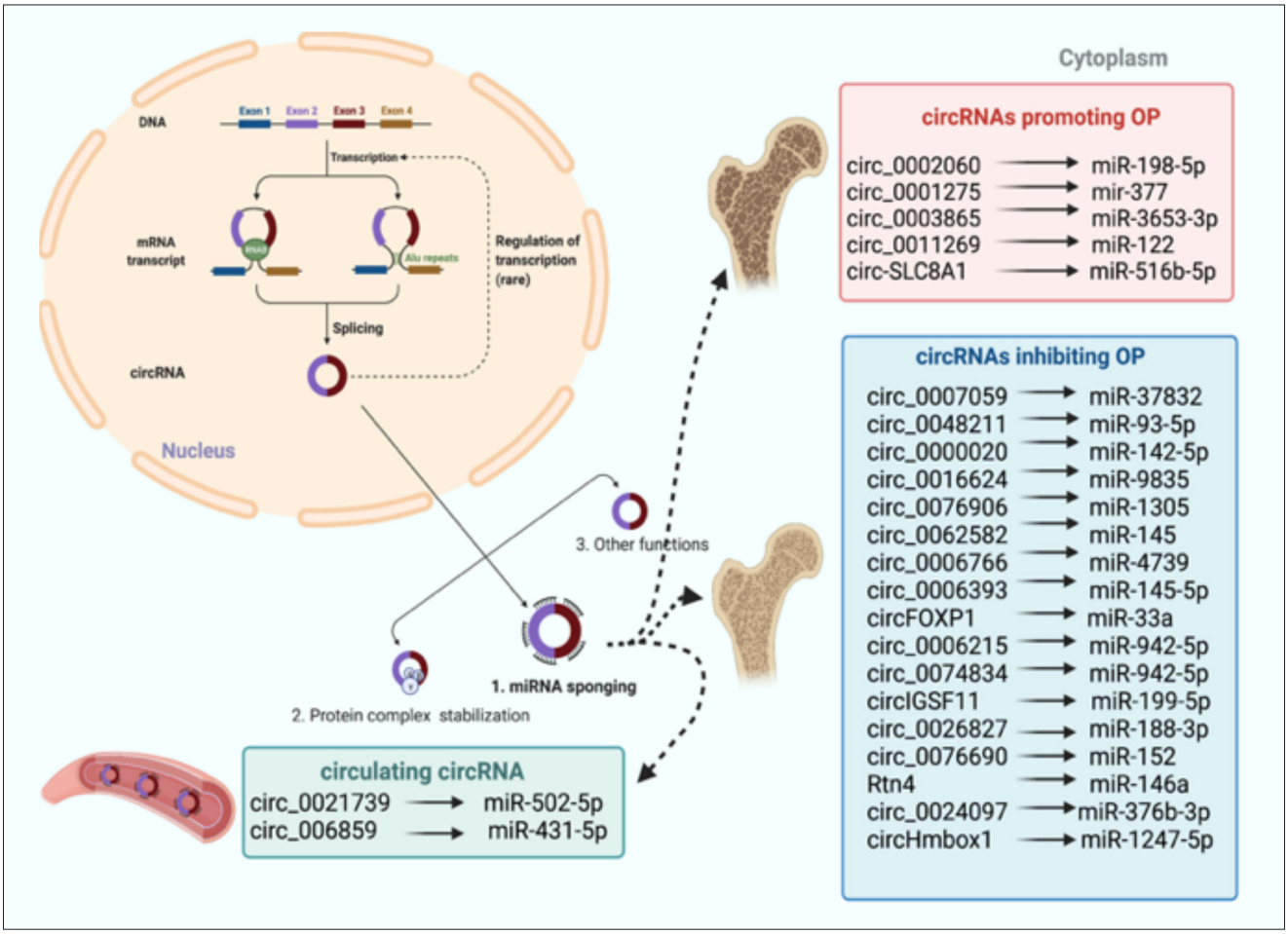
Osteoporosis is a skeletal system disease characterized by progressive loss of the microarchitecture of bone trabeculae and of bone mass. OP itself and the resulting fragility fractures have a strong impact on morbidity and mortality rates of the entire world population, as well as on health care costs [52].

Although clinical practice in this field is trying to evolve, both from a diagnostic and therapeutic point of view, there are still limitations that do not allow accurate diagnosis of OP in some types of patients, and make it difficult to correctly and accurately calculate the risk of fragility fractures. Until recently, it was thought that ncRNAs, including circRNAs, had no biological function, but recent advances in high-throughput RNA sequencing analysis have led the scientific community to reconsider their possible involvement in the pathogenesis of several diseases, including OP.

The World Health Organization has defined a biomarker as "any substance, structure, process or its products that can be measured in the body and that impacts foresees the incidence of outcome or disease" [53]. The ability of circRNAs to regulate gene expression is an emerging area of interest for scientists [13]. Indeed, as reported in this review, circRNAs act principally as ceRNAs for several miRNAs in order to upregulate or downregulate the expression of their target genes that are generally involved in the processes of osteoblastogenesis and osteoclastogenesis, and thus in the onset and progression of OP (Fig.1).

On the basis of the studies reported herein, there seems to be an aberrant expression of these ncRNAs in patients with OP compared with healthy ones. For the diagnosis of OP, circRNAs such as circ_006859, circ 0001445 and circ_0021739 could be considered good candidates to become biomarkers to be measured in plasma. Other molecules, however, such as circ_0001275, circ_0000020, etc., could become possible targets for the development of new drug therapies for this disease. In conclusion, despite the encouraging data, research on the use of circRNAs as possible future diagnostic, prognostic and therapeutic biomarkers for OP is still in its infancy, so further studies should be carried out in the coming years to find new diagnostic and prognostic biomarkers for OP.

Figure 1 circRNAs involved in osteoporosis (*This image was created with Biorender software*).



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