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Non-coding RNAs in Bone Remodelling and Bone Metastasis: Mechanisms of Action and Translational Relevance

Short title: Non-coding RNAs in Bone Metastasis

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Abbreviations

AGO	argonaute protein
APC	adenomatous polyposis coli
BMP	bone morphogenetic protein
ceRNA	competing endogenous RNA
circRNA	circular RNA
COX-2	cyclooxygenase-2
DKK-1	dickkopf-related protein-1
ECM	extracellular matrix
EV	extracellular vesicle
HSC	hematopoietic stem cell
IFN	interferon
IL	interleukin
lncRNA	long non-coding RNA
miRNA	microRNA
ncRNA	non-coding RNA
NSCLC	non-small cell lung cancer
NFATc1	nuclear factor of activated T-cells cytoplasmic 1
OB	osteoblast
OC	osteoclast
PDGF	platelet-derived growth factor
PPARγ	peroxisome proliferator-activated receptor gamma
PRC2	polycomb complex 2
pre-miRNA	precursor miRNA
pre-OB	precursor of osteoblast
pre-OC	precursor of osteoclast
pri-miRNA	primary miRNA
PTHrP	parathyroid hormone-related protein
RANKL	receptor activator of nuclear factor kappa-B ligand
RBP	RNA-binding protein
RISC	RNA-induced silencing complex
RNA-seq	RNA-sequencing
RUNX2	runt-related transcription factor 2
SOST-1	sclerostin-1
TKI	tyrosine kinase inhibitor
TNF-α	tumour necrosis factor-alpha
Wnt	wingless-INT

Abstract

Bone metastases are frequent complications in patients with advanced cancer, which can be fatal or may rapidly impede the quality of life of patients. Current treatments for patients with bone metastases are palliative. Therefore, a better understanding of the molecular mechanisms that precede the overt development of skeletal lesions could lead to better therapeutic interventions. In this review, we present evidence that non-coding RNAs (ncRNAs) such as long non-coding RNAs (lncRNAs), microRNAs (miRNAs), and circular RNAs (circRNAs) are emerging as master regulators of bone metastasis formation. We highlight potential opportunities for the therapeutic targeting of ncRNAs. Furthermore, we discuss the possibility that ncRNAs may be used as biomarkers in the context of bone metastases, which might provide insight for improving the response to current bone-targeting therapies.

Keywords

Non-coding RNA, lncRNA, miRNA, circRNA, bone physiology, bone metastasis, biomarkers.

Bullet point summary

What is already known:

- Most studies investigating bone metastasis have focused on the identification and characterization of proteins;
- The role of non-coding RNAs in bone metastasis is only to be unrevealed.

What this study adds:

- This study is a comprehensive review of non-coding RNA functions in bone metastasis.

Clinical significance:

- Non-coding RNAs emerge as potential therapeutic targets or biomarkers to predict risk of bone metastasis.

Introduction

Metastasis is a multi-step process by which cancer cells disseminate from primary tumour sites to distant organs (Chaffer *et al.*, 2016). Each type of cancer can metastasize to bone, although some cancer types, such as breast and prostate cancers, are more prone to disseminate to the skeleton (Suva *et al.*, 2011). In bones, metastatic cancer cells tend to colonize vascularized areas, within the red marrow of long bones, sternum, pelvis, ribs and vertebrae (Suva *et al.*, 2011). During bone metastasis formation, cancer cells (*i*) interfere with normal bone remodelling, (*ii*) compromise haematopoiesis, and (*iii*) alter the activity of the immune system, which leads to tumour cell outgrowth and bone degradation (Schmid-Alliana *et al.*, 2018). For these reasons, patients with bone metastases often show severe complications, such as bone pain, pathological fractures, and spinal cord compression, which substantially increase the morbidity and the mortality (Suva *et al.*, 2011).

Approved treatments for bone metastases include bisphosphonates (BPs), an anti-RANKL antibody, and radio-nucleotides, in addition to systemic therapies (Van Poznak *et al.*, 2017). Although bone-targeting agents and systemic therapies increase the overall quality of life of patients with bone metastases, they are only palliative (Van Poznak *et al.*, 2017). Thus, novel approaches to treat patients with bone metastases are needed in the clinics. In order to develop improved therapeutic interventions, it is important to better understand the molecular mechanisms underlying the cellular events preceding the development of overt skeletal lesions.

The majority of studies investigating the mechanisms of bone metastases have focused on the identification and characterization of coding proteins. However, non-coding RNAs (ncRNAs) are emerging as playing a key role in mediating bone metastasis formation (Croset *et al.*, 2015; Hesse & Taipaleenmaki, 2019).

Here we present important aspects of the biogenesis of ncRNAs, and summarise the current knowledge on the role of ncRNAs in bone physiology and bone metastasis formation, highlighting the potential translational impact of ncRNAs as therapeutic targets. Finally, we discuss the possibility of using ncRNAs as potential biomarkers to predict the risk of bone metastases in cancer patients.

Properties of ncRNAs

As suggested by the name, non-coding RNAs (ncRNAs) are a large class of transcripts with no apparent protein-coding potential, but characterized by a functional capacity (**Figure 1**). In general, ncRNAs are classified according to their length as *(i)* long non-coding RNAs (lncRNAs) (>200 nucleotides), which include long intervening non-coding RNAs (lincRNAs) and long ribosomal RNAs (rRNAs), 18S and 28S, and *(ii)* small non-coding RNAs (sncRNAs) (<200 nucleotides), which comprises microRNAs (miRNAs), P-element induced wimpy testis (PIWI)-interacting RNAs (piRNAs), short-interfering RNAs (siRNAs), transfer RNAs (tRNAs), 5.8S rRNAs, small nucleolar RNAs (snoRNAs), and small nuclear RNAs (snRNAs) (Bhartiya & Scaria, 2016). Recently, circular RNAs (circRNAs) have been identified as an another class of functional and abundant ncRNAs (Bhartiya & Scaria, 2016).

NcRNAs are evolutionary highly conserved and involved in major biological events during embryogenesis, organogenesis, and cell differentiation. In addition, ncRNAs are often implicated in deregulated molecular processes in the context of diseases, including cancer and metastasis (Flynn & Chang, 2014; Hesse & Taipaleenmaki, 2019). Furthermore, ncRNAs (*e.g.*, lncRNAs, miRNAs, and circRNAs) can be secreted by cells, being encapsulated within extracellular vesicles (*e.g.*, microvesicles and exosomes) or in association with high-density lipoproteins or with argonaute (AGO) proteins (Salido-Guadarrama *et al.,* 2014). These ncRNA-containing vesicles or ncRNA-(lipo-)protein complexes can be taken up by recipient cells in which these molecules may exert regulatory functions (Anfossi *et al.*, 2018; Li *et al.*,

2018b). Secreted ncRNAs can therefore contribute to cell-cell communication and regulate gene expression in recipient cells (Anfossi *et al.*, 2018).

Apart of a small number of lncRNAs such as XIST, HOTAIR, and TERC, the exact function of the vast majority of lncRNAs is unknown (Quinn & Chang, 2016). However, the aberrant expression of lncRNAs appears to play a critical role in tumorigenesis, and 1614 cancer-associated lncRNAs have been reported so far (Lnc2cancer database, http://www.bio-bigdata.com/lnc2cancer/statistics.html). The deregulated expression of lncRNAs may potentially contribute to each of the hallmarks of cancer cells (sustained proliferative signalling, evasion from growth suppressors, replicative immortality, invasion and metastasis, induction of angiogenesis, and resistance to cell death) (Quinn & Chang, 2016). This indicates the putative biological importance of these molecules in various cellular processes (Quinn & Chang, 2016). LncRNAs can functionally organize different nuclear sub-compartments, and regulate the chromatin structure accordingly (Akhade et al., 2017). LncRNAs also function as miRNA sponges, thereby acting as competing endogenous RNAs (ceRNAs) in order to regulate gene expression at the post-transcriptional level (Akhade et al., 2017). Furthermore, lncRNAs can trans-activate Staufen1 (STAU1)-mediated mRNA decay (SMD), and serve as a natural antisense transcript for mRNAs and other ncRNAs (Akhade et al., 2017). In addition, lncRNAs can bind to proteins to modulate their functions (Akhade et al., 2017; Pospiech et al., 2018). A further layer of complexity has been provided by recent evidence that some lncRNAs include short open reading frames (ORFs). Thus, these lncRNAs could potentially be translated into peptides and proteins, indicating the current classification of some lncRNAs has some limitations (Niazi & Valadkhan, 2012). Consistently, some annotated lncRNAs have been found to be associated with ribosomes, and to encode for short functional peptides (Anderson *et al.*, 2015; Choi *et al.*, 2018; Huang *et al.*, 2017; Matsumoto *et al.*, 2017; Rossi *et al.*, 2019). However, this ability for some lncRNAs to

be translated into proteins does not preclude their non-coding functions. Moreover, it has been reported that some lncRNAs and their related peptides can cooperate in the same cellular processes (Yu *et al.*, 2017; Rossi *et al.*, 2019).

MiRNAs regulate many cellular events through different molecular mechanisms (**Figure 1**). For instance, miRNAs exert key roles in the post-transcriptional regulation of gene expression by interacting with specific mRNA targets, thereby inhibiting translation and favouring mRNA degradation (Krol *et al.*, 2010). Importantly, one miRNA can modulate the abundance of hundreds of mRNA targets, whereas a single mRNA can be targeted by several miRNAs (Krol *et al.*, 2010). Biologically active miRNAs are the final product of a tightly regulated maturation process. Once a primary miRNA (pri-miRNA) is transcribed by RNA polymerase II, the Drosha-DGCR8 complex processes it as a precursor miRNA (pre-miRNA) (Han *et al.*, 2004). The pre-miRNA is then exported from the nucleus to the cytoplasm where it is cleaved by Dicer, generating a small RNA duplex composed by two RNA strands that are historically referred to 'guide' and 'passenger' strands (Ha & Kim, 2014). The RNA duplex is loaded onto an AGO protein (Salido-Guadarrama *et al.,* 2014) to form an effector complex called RNA-induced silencing Complex (RISC). RISC only retains the guide strand (or mature miRNA) and it is processed into a mature RISC (Nakanishi, 2016). RISC recognises its target(s), usually at the 3' untranslated region (UTR) of mRNAs, based on the base-pairing complementary to miRNA sequence as well as the accessibility of the target site (Jo *et al.*, 2015). Once bound to its target, RISC inhibits protein synthesis either by repressing the translation or by promoting mRNA deadenylation and degradation (Jo *et al.*, 2015). In addition to exerting a repressive function, miRNAs can also increase gene expression in certain cell types and under specific conditions using different mechanisms (Valinezhad Orang *et al.*, 2014). For example, miRNAs can interact with micro-ribonucleoproteins

(miRNPs). These are effector complexes, that can act in *trans*, thereby promoting the expression of mRNAs (Valinezhad Orang *et al.*, 2014). Furthermore, miRNAs can interact with AU-rich elements (ARE) of mRNAs and thus prevent the ARE-mediated mRNA decay (AMD) (Jing *et al.*, 2005). Changes in miRNA expression or defects in the biogenesis machinery are associated with numerous pathologies, including cancers (Croset *et al.*, 2015; Hesse & Taipaleenmaki, 2019). In particular, deregulated miRNAs can act either as oncogenes or as tumour suppressor genes depending on their target mRNAs (Croset *et al.*, 2015; Hesse & Taipaleenmaki, 2019).

In addition to lncRNAs and miRNAs, circRNAs also act as regulators of gene expression (**Figure 1**). The first circRNA was identified 40 years ago (Hsu & Coca-Prados, 1979). Since then, RNA-sequencing has shown that thousands of circRNAs are generated from protein-coding genes across various eukaryotes (Wilusz, 2018). CircRNAs are generated through a non-canonical splicing mechanism, called back splicing, by which the pre-mRNA splicing machinery covalently links a downstream 3' splice donor to an upstream 5' splice acceptor (which usually belongs to an exon), leading to the formation of a circRNA and a linear RNA (Quan & Li, 2018). CircRNA biogenesis is influenced by transcriptional elongation speed, and it is promoted by some RNA-binding proteins as well as by the presence of inverted repeats in exon extremities or inverted ALU repeat elements in exons (Conn *et al.*, 2015; Quan & Li, 2018). For example, the RNA-binding protein Quaking (QKI) positively regulates circRNA biogenesis during epithelial-to-mesenchymal transition (EMT) (Conn *et al.*, 2015), which is an important process during tumour progression. In general, high circRNA expression levels correlate with low cell proliferation rate and high cell differentiation state (Patop & Kadener, 2018). In tumour cells, the expression of circRNAs is usually reduced, compared to normal cells (Bachmayr-Heyda *et al.*, 2015). In addition to

these general findings, it has been discovered that the expression of specific circRNAs is associated with the expression of oncogenes and/or tumour suppressor genes, suggesting a role in tumour progression (Zhou *et al.*, 2018a). Furthermore, additional mechanisms of action have been proposed, including (*i*) the regulation of parental gene expression at a transcriptional or post-transcriptional level, (*ii*) the function as miRNA sponges (or ceRNAs), (*iii*) the complex formation with other proteins, and (*iv*) the translation into proteins/peptides (Zhou *et al.*, 2018a).

Functions of ncRNAs in bone physiology

Bone remodelling is a dynamic process that is based on the balanced activities of the bone-forming osteoblasts (OBs) and the bone-resorbing osteoclasts (OCs). The coordinated cellular activities of OBs and OCs are important to ensure bone integrity, repair, and calcium homeostasis (Suva *et al.*, 2011). Any imbalance between OB and OC activities can lead to bone diseases, such as osteoporosis and cancer-associated bone destruction (Suva *et al.*, 2011).

OBs differentiate from bone marrow-derived mesenchymal stem cells (MSCs) in a tightly regulated manner (Almeida *et al.*, 2017). Transforming growth factor-β (TGF-β), bone morphogenetic proteins (BMPs), and Wingless-INT (Wnt) proteins are local factors involved in OB differentiation (Long, 2011). Mature OBs secrete proteins, such as alkaline phosphatase and type I collagen, which are necessary for the formation of the bone extracellular matrix (also known as osteoid), which then becomes mineralized. Afterwards, while most of the OBs die by apoptosis, some reach quiescence as bone lining cells on bone surfaces or become embedded in the bone matrix as osteocytes (Dallas *et al.*, 2013). Osteocytes control calcium and phosphate homeostasis, and serve as mechano-sensors to respond to mechanical loading of the skeleton (Dallas *et al.*, 2013).

OCs are bone-specific multinucleated cells, which derive from the monocyte/macrophage lineage (Long, 2011). Like OBs, OC differentiation and function are regulated by various signals. For instance, the receptor activator of nuclear factor kappa-B ligand (RANKL) and the macrophage-colony stimulating factor (M-CSF) are two of the most important molecules that stimulate OC differentiation and activity (Long, 2011). OCs can also be activated by cytokines, such as interleukin-17 (IL-17), interferon-γ (IFN-γ), and tumour necrosis factor-α (TNF-α) (Long, 2011). OCs remove bone mineral and degrade the collagenous matrix by secreting protons and the proteolytic enzyme cathepsin K, respectively (Long, 2011). As bone is resorbed, growth factors embedded within the bone matrix, such as TGF-β and insulin-like growth factor-1 (IGF-1), are released and stimulate OB function and differentiation (Long, 2011). At the end of their life cycle, OCs undergo apoptosis (Long, 2011). In this respect, ncRNAs regulate OB and OC function, thereby participating in the maintenance of bone integrity, as described below and summarized in **Table 1**.

Role of lncRNAs in bone physiology

Several nuclear and cytoplasmic lncRNAs have been reported to be differentially expressed during osteogenesis (Tye *et al.*, 2018). The first well-characterized lncRNA acting in osteogenesis was Msh Homeobox 1 (Msx1) anti-sense RNA (Msx1-AS RNA) (Blin-Wakkach *et al.*, 2001). Msx1-AS RNA antagonizes the expression of the Msx1 homeodomain protein, which down-regulates the master gene of skeletal differentiation, *Runt-related transcription factor 2* (*Runx2*) (Blin-Wakkach *et al.*, 2001). Thus, the ratio between Msx1 sense and anti-sense RNAs is a very important determinant for the control of skeletal differentiation (Blin-Wakkach *et al.*, 2001).

HOTAIR is another lncRNA involved in skeletal development (Rinn *et al.*, 2007). It acts as a *trans*-regulator of *HoxD* gene expression by recruiting the polycomb repressive complex

2 (PRC2) to the HOXD locus (Rinn *et al.*, 2007). In contrast, a recent work suggests that HOTAIR activity in *trans* is not essential for mouse skeletal development, although significant changes in the expression of the neighbouring genes *Hoxc11* and *Hoxc12* are detected as a result of HOTAIR deletion in mutant mice, suggesting that HOTAIR can act in *cis* on the genomic locus (Amandio *et al.*, 2016). Furthermore, HOTAIR regulates the expression of other lncRNAs by targeting their loci, such as the *Dlk1-Meg3* locus, the *Igf2-H19 locus* and the *Hottip* gene in the *Hoxa* locus, all of them being involved in bone development (Huynh *et al.*, 2017).

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is an lncRNA that has been originally described in non-small cell lung cancer (NSCLC) (Ji *et al.*, 2003). However, MALAT1 expression is also increased during osteogenic differentiation of adipose-derived mesenchymal stem cells (ADSCs), while miR-30 expression is decreased (Yi *et al.*, 2019). Silencing of MALAT1 or overexpression of miR-30 in ADSCs suppressed the RUNX2-mediated OB differentiation. Overall, the authors showed that MALAT1 sponges miR-30 promote the RUNX2-mediated OB differentiation of ADSCs (Yi *et al.*, 2019).

Maternally expressed gene 3 (MEG3) directly interacts with the transcription factor SOX2, and causes its dissociation from the BMP4 promoter, thereby triggering BMP4 transcription and subsequent differentiation of MSCs into OBs (Zhuang *et al.*, 2015). Interestingly, MSCs from patients with multiple myeloma have frequently low expression of MEG3 in comparison to those obtained from normal donors, suggesting that its down-regulation might explain, at least in part, the lytic nature of skeletal lesions in multiple myeloma (Zhuang *et al.*, 2015).

H19 is involved in developmental biology, and operates through different mechanisms, which are not mutually exclusive (Gabory *et al.*, 2010; Raveh *et al.*, 2015). For example, H19, in concert with miR-675, promotes MSCs differentiation into OBs through inhibition of

the TGF-β1/Smad3/HDAC signalling pathway (Huang *et al.*, 2015). H19 acts also as a mediator of BMP9-induced osteogenic differentiation of MSCs by modulating Notch signalling-targeting miRNAs (including miR-449b, miR-107, miR-27b, miR-34a, miR-106b, miR-449a, miR-125a, and miR-17) (Liao *et al.*, 2017). Conversely, H19 can act as a ceRNA functioning as a miRNA sponge. For example, H19 activates the Wnt signalling pathway and promotes OB differentiation by binding and thus sequestering miR-141 and miR-22, both of which are negative regulators of osteogenesis (Liang *et al.*, 2016). Similarly, H19 is a positive regulator of mechanical tension-induced osteogenesis of human bone marrow MSCs by acting as a ceRNA for miR-138, which otherwise inhibits MSC differentiation (Wu *et al.*, 2018). Interestingly, H19 also acts as ceRNA for let-7 in primary breast carcinoma (Peng *et al.*, 2017), and let-7 is involved in regulating bone formation (Wei *et al.*, 2014). Although it is not proven yet, it is possible that H19 regulates let-7 function during osteogenesis.

Anti-differentiation ncRNA (ANCR), called also differentiation-antagonizing non-protein coding RNA (DANCR), is required to maintain cells in an undifferentiated state. Its expression is decreased during OB differentiation (Zhu & Xu, 2013). Conversely, its overexpression negatively regulates OB differentiation by inhibiting RUNX2 expression (Zhu & Xu, 2013). Along the same line, ANCR negatively regulates osteogenic differentiation of periodontal ligament stem cells by directly targeting miR-758 (Peng *et al.*, 2018).

RUNX2-AS1 is a lncRNA that has been described as being packed into exosomes secreted by myeloma cells (Li *et al.*, 2018a). These exosomes are transmitted into MSCs and repress OB differentiation of MSCs. More specifically, exosome-derived RUNX2-AS1 forms a duplex with RUNX2 pre-mRNA, thereby repressing RUNX2 expression and subsequent the osteogenic potential of MSCs (Li *et al.*, 2018a).

Linc-ROR is upregulated in MSCs during osteogenesis, and its presence is essential for the correct differentiation of OBs (Feng *et al.*, 2018). Mechanistically, it promotes the osteogenic differentiation of MSCs acting as a ceRNA of two miRNAs, miR-138 and miR-145, known to be negative regulators of osteogenesis by targeting ZEB2 (Feng *et al.*, 2018).

Owing to new bioinformatics techniques, such as next-generation sequencing (NGS) and high-throughput screenings, the list of lncRNAs involved in osteogenesis is expanding very rapidly. An example of a lncRNA identified using these methods is lncRNA-1, whose expression is down-regulated during myoblast differentiation, suggesting a potential role for lncRNA-1 in mesenchymal-to-osteogenic transition (Nardocci *et al.*, 2018). Several other studies used similar bioinformatics strategies, uncovering a potential role for additional lncRNAs in bone physiology and pathology (Gu *et al.*, 2017; Kim *et al.*, 2018; Xie *et al.*, 2016; Zuo *et al.*, 2013).

Function of miRNAs in bone physiology

The first evidence for an important role of miRNAs in skeletal development was obtained by an *in vivo* study that investigated the deletion of the miRNA processing endonuclease *Dicer* in chondrocytes (Kobayashi *et al.*, 2008). As a result of this deletion, animals had severe skeletal growth defects due to decreased chondrocyte proliferation and accelerated differentiation in post-mitotic hypertrophic chondrocytes (Kobayashi *et al.*, 2008). *Dicer* deletion in osteoprogenitor cells expressing *Osterix* also results in severe growth retardation in animals (Raaijmakers *et al.*, 2010). In contrast, conditional deletion of *Dicer* in mature, osteocalcin-expressing OBs only caused a delay of bone mineralization in mice, which resumed by one month (Gaur *et al.*, 2010). However, a second phenotype of significantly increased bone formation started to develop by two months of age and continued to increase for up to eight months of age, leading to a 2-fold increase in bone mass compared

to control mice (Gaur *et al.*, 2010; Kobayashi *et al.*, 2008; Raaijmakers *et al.*, 2010). Overall, these findings indicate that specific miRNAs must be up- or down-regulated in a timely manner during skeletal development and physiology.

Since the role of miRNAs in bone has already been described in several reviews (Bellavia *et al.*, 2019; Ell & Kang, 2014; Haider & Taipaleenmaki, 2018; Ji *et al.*, 2016), we focus on most recent findings regarding the characterization of miRNA functions. Several miRNAs regulate OB differentiation (Zhang *et al.*, 2011). For example, miR-29b promotes osteogenesis by directly down-regulating known inhibitors of OB differentiation (e.g., HDAC4, TGF-β3, ACVR2A, CTNNBIP1, DUSP2), through binding to the 3'-UTR seed region of target mRNAs (Li *et al.*, 2009). Along the same line, miR-218 promotes OB differentiation by down-regulating Wnt signalling inhibitors (such as SOST-1, DKK-2, and SFRP2) during the process of osteogenesis (Hassan *et al.*, 2012). Conversely, a panel of miRNAs (including miR-23a, miR-24-2, miR-27a, miR-30c, miR-34c, miR-133a, miR-135a, miR-137, miR-204, miR-205, miR-217, and miR-338) expressed in MSCs was found to negatively control the osteogenic activity of RUNX2 and to impede OB differentiation (Hassan *et al.*, 2010; Zhang *et al.*, 2011). For example, miR-23a, miR-24-2, and miR-27a target SATB2, blocking its synergistic action with the pro-osteogenic transcription factor RUNX2 (Hassan *et al.*, 2010). All of these miRNAs are inversely expressed relative to RUNX2 expression during osteogenesis (Hassan *et al.*, 2010; Zhang *et al.*, 2011). Overall, these findings underline how complex the interactions can be between mRNA targets and miRNAs, and how they can be part of tightly regulated feed-forward mechanisms during osteogenesis. Other miRNAs of particular interest, which have very recently been shown to regulate OB differentiation, are let-7c (Zhou *et al.*, 2018b), the miR-23a cluster (Godfrey *et al.*, 2018), miR-139-3p (Wang *et al.*, 2018b), miR-182-5p (Pan *et al.*, 2018), miR-219a-5p (Aquino-Martinez *et al.*, 2018), miR-221 (Zheng *et al.*, 2018), miR-342-3p (Han *et al.*, 2018),

miR-451a (Karvande *et al.*, 2018), and miR-874 (Lin *et al.*, 2018). In addition, it has recently been reported that miR-21 and miR-218 play a physiologic role in osteocytes, which are differentiated OBs embedded in the bone matrix (Davis *et al.*, 2017). The deletion of miR-21 expression in osteocytes is sufficient to increase apoptosis *ex vivo* (Davis *et al.*, 2017). Moreover, reduced levels of miR-21 in conjunction with increased miR-218 levels are associated with connexin 43(Cx43)-deficient osteocytes, thereby promoting apoptosis (Davis *et al.*, 2017).

There is ample evidence that miRNAs regulate OC differentiation and activity (Ell & Kang, 2014; Ji *et al.*, 2016). For example, miR-17 mediates the suppression of an osteoclastic protein-tyrosine phosphatase, which in turn promotes the inhibitory effect of EphA4 on OC activity (Lau & Sheng, 2018). MiR-27a is significantly increased during the process of estrogen-mediated inhibition of OC differentiation (Guo *et al.*, 2018b). Mechanistically, miR-27a inhibits peroxisome proliferator-activated receptor gamma (PPARγ) and adenomatous polyposis coli (APC) expression in OCs through a miR-27a binding site within the 3'-UTR region of PPARγ and APC. MiR-30a negatively regulates OC differentiation by inhibiting the DC-STAMP-c-Fos-nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1) signalling pathway (Yin *et al.*, 2017). MiR-145 inhibits OC differentiation by decreasing Smad3 expression (Yu *et al.*, 2018). MiR-182 is a negative regulator of protein kinase double-stranded RNA-dependent (PKR), a novel inhibitory factor of OC differentiation, which acts via regulation of the endogenous IFN-β-mediated autocrine feedback-loop (Inoue *et al.*, 2018). MiR-199a-5p expression levels are increased in OCs, compared to their precursors (Guo *et al.*, 2018a). It promotes OC differentiation *in vitro* by directly targeting transcription factor Mafb (Guo *et al.*, 2018a). Mafb was previously reported to negatively regulate OC differentiation, which suggests that there is a regulatory effect of miR-199a-5p on *Mafb* mRNA expression (Guo *et al.*, 2018a). MiR-218, beside its pro-osteogenic role (Hassan *et*

al., 2012), negatively regulates OC differentiation by attenuating the expression of p38 MAPK, c-Fos, and NFATc1 in OCs (Qu *et al.*, 2015). Furthermore, miR-218 directly targets TNF receptor-1, a cell membrane receptor for TNF, leading to the repression of the TNF/nuclear factor-κB signalling pathway (Wang *et al.*, 2018a). The expression levels of other miRNAs (including miR-33a-5p, miR-133a, miR-141-3p, miR-190, and miR-219-5p) were shown to be decreased after RANKL treatment of OC precursors (Ell *et al.*, 2013). Conversely, miR-16, miR-211, miR-378, and let-7a were significantly up-regulated during OC differentiation (Ell *et al.*, 2013). Additionally, miR-20a and miR-155 regulate autophagy in OCs (Sul *et al.*, 2018; Sun *et al.*, 2015).

As described above, both precursors and mature bone cells express a multitude of miRNAs, which fine-tune OB and OC differentiation. However, exosomal miRNAs derived from bone cells could also participate in bone homeostasis by mediating the crosstalk between OCs and OBs (Li *et al.*, 2016; Xie *et al.*, 2017). For example, OC-derived exosomal miR-214-3p is transferred to OBs to inhibit their activity *in vitro* and to reduce bone formation *in vivo* (Li *et al.*, 2016). Conversely, exosomal miR-503-3p from differentiated OBs inhibits RANKL-induced OC differentiation *in vitro* (Xie *et al.*, 2017).

Relevance of circRNAs in bone physiology

CircRNAs are emerging as important factors that could regulate bone homeostasis. Using microarray analysis, several circRNAs were found to be differentially expressed in MSCs undergoing OB differentiation, compared to their undifferentiated counterparts (Zhang *et al.*, 2018b). Some of these circRNAs possess miRNA response elements enabling their interaction with specific miRNAs, which have osteogenic functions (Zhang *et al.*, 2018b). For example, miR-199b-5p is linked to circIGSF11, and silencing of circIGSF11 promotes OB differentiation and increases miR-199b-5p expression (Zhang *et al.*, 2018b). This study

suggests that circRNA-miRNA interaction actively contributes to the osteogenic differentiation of human MSCs.

Using high-throughput RNA-seq analysis, circRNA expression was measured during the osteogenic differentiation of MC3T3-E1 cells induced by BMP-2 (Qian *et al.*, 2017). Expression of circRNA.5846, circRNA.19142, and circRNA.10042 was significantly increased in BMP2-treated MC3T3E1 cells. However, the signalling pathways controlled by these circRNAs in osteoblastic cells are still unknown (Qian *et al.*, 2017). Similar analyses were conducted in OCs and their precursors following RANKL stimulation (Dou *et al.*, 2016). Over 20 circRNAs were found to be up- or down-regulated at different stages of OC differentiation (Dou *et al.*, 2016). However, no functional analysis was conducted. To further investigate the functions of circRNAs in bone remodelling as well as in cancer, additional investigations are required. The strategy to use high-throughput RNA-seq analyses followed by functional studies (*e.g.*, gene expression modulation of circRNAs *in vitro* and *in vivo*) could be useful to address this question.

Dysregulation of ncRNAs in bone metastases

Metastases are formed when cancer cells are released from the primary tumour site, travel through the blood or the lymphatic system, and finally form secondary tumours in distant organs. Bone is the most common metastatic site for primary breast, prostate, and lung tumours, where the bone marrow is a fertile soil for tumour cells to grow (Suva *et al.*, 2011). Tumour cells residing in the bone marrow alter the functions of OCs and OBs (Suva *et al.*, 2011). For example, breast and lung cancer cells cause bone destruction by enhancing OC activity, whereas prostate cancer cells form mixed lesions with osteosclerotic components by increasing OB activity (Suva *et al.*, 2011). Bones weakened due to skeletal metastases are prone to skeletal-related events, such as fractures, hypercalcaemia, cancer-induced bone pain

and spinal cord compression, which substantially increase the morbidity and mortality of patients with advanced cancer (Suva *et al.*, 2011).

At the bone metastatic site, tumour cells induce a distortion of bone remodelling, and bone-derived growth factors released from resorbed bone promote tumour growth (Guise *et al.*, 1996; Suva *et al.*, 2011). This concept of "vicious cycle" was first proposed by Dr Greg Mundy (University of Texas Health Science Centre, San Antonio, USA). Although it is difficult to identify a starting point for the "vicious cycle", it is well known that metastatic cancer cells are able to produce a number of factors, such as parathyroid hormone-related protein (PTHrP), interleukin (IL)-11, and prostaglandin E2 that mediate bone resorption by increasing the OB-dependent production of RANKL (Croucher *et al.*, 2016; Dougall, 2012). In turn, RANKL binds to its receptor RANK on OC precursors, leading to the formation of new OCs, as well as promotes the activity of mature OCs, thus favouring OC-mediated bone resorption (Croucher *et al.*, 2016). Under physiological conditions, osteoprotegerin (OPG) binds to RANKL and restricts its activity (Croucher *et al.*, 2016). PTHrP regulates RANKL expression in OBs and stromal cells (Dougall, 2012). Moreover, tumour-derived PTHrP reduces the OB-dependent OPG production, thereby further promoting bone resorption. Tumour cells also secrete IL-8 that has a direct effect on OC differentiation and activation (Suva *et al.*, 2011). Tumour cells not only stimulate OC activity, but also inhibit OB function, thereby worsening the imbalance between bone formation and bone resorption, which promotes bone destruction (Croucher *et al.*, 2016). Different factors produced by tumour cells have been shown to suppress OB differentiation. These include endothelin-1, dickkopf-related protein 1 (DKK-1), sclerostin-1 (SOST-1), activin A, and noggin (NOG) (Croucher *et al.*, 2016). Moreover, bone tissue is an abundant reservoir for growth factors, including TGF-β, IGF-1, BMPs, platelet-derived growth factor (PDGF), and epidermal growth factor (EGF), which are entrapped in the bone matrix and become released in the bone microenvironment

upon matrix degradation by active OCs (Croucher *et al.*, 2016). In turn, these bone-derived growth factors promote tumour growth.

A large body of evidence suggests that ncRNAs regulate the various molecular mechanisms of bone metastasis formation, although only a few studies investigated the role of lncRNAs and circRNAs in bone metastasis so far. By contrast, several articles already reviewed the role of miRNAs in bone metastasis (Baier & Wan, 2016; Browne *et al.*, 2014; Cheung *et al.*, 2017; Croset *et al.*, 2015; Ell & Kang, 2014; Hesse & Taipaleenmaki, 2019). In cancer and metastasis, a loss of tumour-suppressive miRNAs and an increased expression of oncogenic miRNAs are part of the disease mechanism. MiRNA functions as well as those of other ncRNAs in bone metastasis are summarized in **Table 2**. Herein, we will focus on most recent and/or pivotal findings, and show that the dysregulation of ncRNAs in tumour cells or bone cells as well as the shuttling of ncRNAs between tumour and bone cells may lead to the establishment of a cancer niche. This environment is conducive for tumour cells to grow and induce the formation of skeletal lesions.

OC- and OB-derived miRNAs and bone metastases

The role of OC-derived miRNAs in the development of osteolytic bone metastasis has been examined in detail by the group of Dr Yibin Kang (Princeton University, NJ, USA), hypothesizing that miRNAs might be possible therapeutic targets during the differentiation of OC precursors into mature OCs. In this respect, miRNA microarray profiling of mature OCs was performed after exposure of OC precursors to conditioned medium from breast cancer cells (Ell *et al.*, 2013). Four miRNAs (miR-133a, miR-141-3p, miR-190, and miR-219-5p) whose expression was consistently down-regulated were selected for further investigation (Ell *et al.*, 2013). Systemic treatment of healthy mice with each of these miRNAs (whose aim is to replenish the lost miRNA expression during OC differentiation) inhibited bone

resorption *in vivo*. Consistently, treatment of tumour-bearing mice with miR-141 or miR-219 significantly reduced the formation of osteolytic lesions caused by breast cancer cells (Ell *et al.*, 2013). In contrast, systemic treatment of tumour-bearing animals with miR-133a or miR-190 had no effect on the development of bone metastases, compared to control (Ell *et al.*, 2013). Nevertheless, these miRNAs were inhibiting OC differentiation *in vitro* through the functional targeting of essential OC genes, including *Mitf*, *Traf6*, *Calcr*, and *Mmp14*. The observation that miR-133a and miR-190 did not inhibit the formation of lytic bone metastases, whereas they did inhibit normal bone resorption, was of particular interest. Of note, the half-lives of miR-133a and miR-190 in the circulation were shorter than those of miR-141 and miR-219 (Ell *et al.*, 2013). These differences in pharmacokinetics of miRNAs could therefore explain these unexpected results, suggesting that inhibition of osteolytic lesions may require greater miRNA levels to reach the bone than those required for the inhibition of normal bone resorption (Ell *et al.*, 2013).

MiR-34a is another miRNA that is down-regulated during OC differentiation. It inhibits OC differentiation by directly targeting the pro-osteoclastic factor TGIF2 (TGF-β-induced factor 2) (Krzeszinski *et al.*, 2014). The pharmacological administration of a miR-34a mimic delivered in nanoparticles attenuates the formation of breast cancer bone metastases in mice (Krzeszinski *et al.*, 2014). A phase I, open-label, dose-escalation study investigated the safety, pharmacokinetics, and pharmacodynamics of a miR-34a mimic (MRX34), encapsulated in lipid nanoparticles, in patients with advanced or metastatic cancer (Beg *et al.*, 2017). However, the clinical trial was terminated prematurely due to cases of immune-related serious adverse events.

MiR-214-3p expression in OCs is significantly up-regulated in bone specimens from breast cancer patients with bone metastases (Liu *et al.*, 2017). Additionally, the conditioned medium from MDA-MB-231 breast cancer cells stimulates miR-214 expression in mature

OCs during OC differentiation. Mechanistically, miR-214 directly targets the TNF receptor associated factor 3 (TRAF3), a negative regulator of OC differentiation, thereby promoting the formation of mature OCs. The treatment of animals with antagomiR-214-3p encapsulated within an OC-targeting delivery system (D-Asp_8-liposome) attenuates the formation of osteolytic lesions caused by breast cancer cells (Liu *et al.*, 2017). Additionally, OC-derived exosomal miR-214-3p was transferred to OBs, and inhibited OB activity *in vitro* and reduced bone formation *in vivo* (Li *et al.*, 2016). Of note, miR-214 is expressed by OBs and inhibits OB differentiation *in vitro* and bone formation *in vivo,* through repression of *activating transcription factor 4* (*Atf4*), which is one of the main transcription factors required for OB function (Wang *et al.*, 2013). Taken together, these findings suggest that the therapeutic inhibition of OC- or OB-derived miR-214-3p could be a strategy to impede, respectively, the development of lytic or sclerotic bone metastases (Haider & Taipaleenmaki, 2018; Li *et al.*, 2016; Wang *et al.*, 2013).

Tumour-derived miRNAs and homing of tumour cells to the bone marrow

Searching for regulators of tumour spreading, Tavazoie *et al.* (2008) identified a series of miRNAs (miR-126, miR-206, and miR-335) that were poorly expressed in lung- or bone-tropic breast cancer cells. Overexpression of these miRNAs in breast cancer cells reduced lung and bone metastasis formation in animals (Tavazoie *et al.*, 2008). MiR-126 suppressed metastases by inhibiting tumorigenesis *in vivo* and tumour cell proliferation *in vitro*. In contrast, miR-206 and miR-335 did not affect tumorigenesis, yet suppressed metastasis by inhibiting tumour cell migration and invasion *in vitro*. MiR-335 directly targets the 3'UTR of mRNAs for transcription factor SOX4 and tenascin C (TNC), both factors being involved in promoting tumour cell migration and invasion (Tavazoie *et al.*, 2008). Elevated expression levels of let-7 in breast cancer cells were also reported to inhibit bone metastasis formation

by directly targeting HMGA2, a chromatin remodelling protein that activates pro-invasive genes (Ell & Kang, 2014). Conversely, miR-10b activated by transcription factor Twist facilitates lung and bone metastasis formation in animals by promoting MDA-MB-231 breast cancer cell invasion through direct targeting of the mRNA encoding homeobox D10 (HOXD10), resulting in an increased expression of the pro-metastatic gene *Ras homolog gene family member C* (*Rhoc*) (Croset *et al.*, 2015; Ell & Kang, 2014; Ma *et al.*, 2007). Similarly, miR-17 promotes breast cancer cell migration/invasion *in vitro* and bone metastases *in vivo* through repression of the type II TGF-β receptor (TβR2) (Croset *et al.*, 2015). Along the same line, miR-21 promotes the colonization of breast cancer cells in bone by inhibiting the expression of the anti-metastatic genes PTEN, PDCD4, and SPRY2, which in turn leads to an enhanced tumour cell invasion and metastasis formation (Sahay *et al.*, 2015).

In prostate cancer, the ectopic expression of miR-143 and miR-145 in PC-3 tumour cells inhibits bone metastasis formation *in vivo* by preventing EMT, and by reducing tumour cell migration (Croset *et al.*, 2015; Ell & Kang, 2014). These inhibitory effects of miR-145 on EMT and tumour cell migration occur through repression of the oncogenic protein HEF1 (Croset *et al.*, 2015; Ell & Kang, 2014). MiR-203 is also a tumour suppressor whose expression is inversely correlated with prostate cancer bone metastases (Siu *et al.*, 2018). Reconstitution of metastatic prostate cancer cells with miR-203 suppresses bone metastasis formation in animals by inhibiting EMT, invasion, and motility (Saini *et al.*, 2011). The capacity of miR-203 to inhibit bone metastasis formation has been associated with its ability to directly repress *Survivin/BIRC5* expression, an inhibitor of apoptosis (Saini *et al.*, 2011). Additionally, miR-203 represses the activation of the EGFR signalling pathway in prostate cancer cells (Siu *et al.*, 2018). A role for miR-203 in the inhibition of bone metastases caused by non-small cell lung cancer has also been reported (Wei *et al.*, 2017). Several other

miRNAs have been shown to control early stages of bone metastasis formation, whose functions are summarized in **Table 2**.

MSC-derived miRNAs

Entering a foreign environment, such as bone, poses tumour cells with numerous challenges regarding survival and proliferation. It is therefore likely that tumour cells undergo dormancy as a mechanism to facilitate survival until the environmental conditions are sufficiently permissive for tumour outgrowth.

Exosomal miR-23b and miR-222/223 released from MSCs have been reported to stimulate breast cancer dormancy in the bone marrow (Bliss *et al.*, 2016; Ono *et al.*, 2014). For example, miR-23b induces dormancy through the suppression of the *myristoylated alanine-rich C-kinase substrate* (*MARCKS*) target gene, which encodes a protein that promotes cell proliferation and motility (Ono *et al.*, 2014).

MiRNAs regulate the adaptation of tumour cells to the bone microenvironment

While residing in the bone marrow, tumour cells exit and re-enter a dormant state. During this phase, cancer cells undergo a selection and acquire the capacity for metastasis formation. Specifically bone metastatic cancer cells express genes that are normally expressed by bone cells (a process called osteomimicry), which endow cancer cells with a full competence for outgrowth in the bone marrow (Croset *et al.*, 2015; Hesse & Taipaleenmaki, 2019). As described below, several miRNAs were reported to regulate cancer cell osteomimicry.

RUNX2 plays a pivotal role in OB differentiation and it is aberrantly expressed in bone-tropic breast- and prostate cancer cells (Haider & Taipaleenmaki, 2018). In bone-tropic tumour cells, RUNX2 promotes the progression of bone metastases and the development of osteolytic lesions (Haider & Taipaleenmaki, 2018). RUNX2 expression is repressed by

several miRNAs, including miR-135, miR-203, and miR-466. In this respect, miR-135 and miR-203 expression is absent in bone-tropic breast cancer cells and in tissue biopsies from breast cancer bone metastases that express RUNX2 (Haider & Taipaleenmaki, 2018). Conversely, transfection of miR-135 and miR-203 mimics in bone-tropic breast cancer cells results in a decreased skeletal tumour growth and decreased formation of osteolytic lesions in tumour-bearing mice (Taipaleenmaki *et al.*, 2015). By repressing RUNX2, miR-135 and miR-203 indirectly inhibit the expression of genes (*Il-11*, *Mmp-13*, *PthrP*) that are associated with bone metastases (Taipaleenmaki *et al.*, 2015). Similarly, miR-203 and miR-466 suppress prostate cancer bone metastases by directly targeting *RUNX2* (Colden *et al.*, 2017; Saini *et al.*, 2011). Furthermore, restoring miR-203 in bone-tropic prostate cancer cells represses the expression of other genes associated with osteomimicry, including *homeobox protein Dlx-5* (*DLX5*), *osteopontin* (*SPP1*), and *osteocalcin* (*BGLAP*) (Saini *et al.*, 2011). Similarly, reconstitution of prostate cancer cells with miR-466 represses *SPP1* and *BGLAP* expression (Colden *et al.*, 2017).

MiRNA members of the miR-30 family (miR-30a, miR-30b, miR-30c, miR-30d, and miR-30e) are suppressors of breast cancer bone metastasis (Croset *et al.*, 2018). Expression of these miRs in bone-tropic triple-negative breast cancer cells was profoundly decreased. Conversely, overexpression of miR-30 family members in triple-negative breast cancer cells results in the reduction of bone metastasis formation *in vivo*. *In vitro*, miR-30 family members inhibit tumour cell invasion, and restore bone homeostasis by reversing the effects of tumour cell-conditioned medium on OC and OB formation. Mechanistically, miR-30s directly target a number of genes associated with the stimulation of OC differentiation (IL-8, IL-11), the inhibition of OB differentiation (DKK-1), tumour cell osteomimicry (RUNX2, CDH11), and invasiveness (CTGF, ITGA5, ITGB3) (Croset *et al.*, 2018). Among these genes, silencing of CDH11 or ITGA5 in triple-negative breast cancer cells recapitulates the

inhibitory effects of miR-30 family members on bone metastasis formation *in vivo*. Overall, these findings provide evidence that miR-30 family members employ multiple mechanisms to impede breast cancer bone metastases, including osteomimicry (Croset *et al.*, 2018). Of note, lncRNA MALAT1 acts as a sponge for miR-30, thus promoting OB differentiation of ADSCs (see section on bone physiology) (Yi *et al.*, 2019). MALAT1 is overexpressed in several cancers, including triple-negative breast cancer (Jadaliha *et al.*, 2016). It is therefore tempting to speculate that in triple-negative breast cancer MALAT1 overexpressing cells may be responsible for miR-30 down-regulation, which then facilitates tumour cell osteomimicry.

As previously described, miR-218-5p promotes OB differentiation by down-regulating Wnt signalling inhibitors (*e.g.*, SOST-1, DKK-2, and sFRP2) during the process of osteogenesis (Hassan *et al.*, 2012). MiR-218-5p also enhances Wnt activity in osteotropic breast cancer cells. Moreover, the expression of genes associated with osteomimicry (*CXCR-4*, *BSP,* and *SSPP1*) and OC-mediated bone resorption (PTHrP) is increased in miR-218-expressing breast cancer cells (Haider & Taipaleenmaki, 2018; Hassan *et al.*, 2012; Taipaleenmaki *et al.*, 2016). Transfection of bone-tropic breast cancer cells with anti-miR-218-5p decreases Wnt activity, and reduces skeletal tumour burden and cancer-induced osteolysis *in vivo* (Taipaleenmaki *et al.*, 2016). Furthermore, miR-218 secreted from breast cancer cells can directly down-regulate type I collagen expression by OBs, thereby impairing bone integrity (Liu *et al.*, 2018). Taken together, these results (Haider & Taipaleenmaki, 2018; Liu *et al.*, 2018) indicate that inhibition of miR-218-5p can attenuate the activity of the "vicious cycle" during bone metastasis progression, suggesting that it could be an attractive therapeutic target.

Tumour-derived ncRNAs impair OB and OC function

Several studies have shown that lncRNAs and miRNAs expressed and/or released by tumour cells can be directly involved in the formation of osteolytic or osteoblastic lesions (**Table 2**).

The lncRNA MAYA mediates a crosstalk between the ROR1-HER3 and Hippo/Yes-associated protein (YAP) pathways in breast cancer cells to promote lytic bone metastases (Li *et al.*, 2017). Mechanistically, neuregulin induces the heterodimerization of receptor tyrosine kinase-like orphan receptor (ROR)-1 and receptor tyrosine kinase erbB-3 (HER3), which leads to the phosphorylation of HER3 in breast cancer cells. In turn, HER3 phosphorylation recruits a MAYA-containing complex that methylates the serine/threonine kinase Hippo/MST1 and activates YAP. This results in the translocation of YAP to the nucleus and the increased expression of connective tissue growth factor (CTGF) (Li *et al.*, 2017). CTGF is a factor that promotes OC differentiation and activity. *In vivo*, the treatment of animals with an antibody against CTGF prevents the formation of osteolytic metastases caused by human MDA- MB-231 breast cancer cells (Shimo *et al.*, 2006).

Increased MALAT1 expression in NSCLC is associated with a poor clinical outcome, including bone metastases (Liu *et al.*, 2016). MALAT1 is overexpressed in several other cancers, where it promotes tumour cell invasion and proliferation (Liu *et al.*, 2016). It has been reported that MALAT1 expression is increased in PC3 prostate cancer cells if co-cultured with primary mouse OBs (Sebastian *et al.*, 2015). Moreover, MALAT1 expression in PC3 cells is further enhanced upon co-culture with mouse OBs lacking *Sost* (Sebastian *et al.*, 2015). However, the underlying mechanisms by which SOST regulates MALAT1 expression in tumour cells are unknown.

RUNX2-AS1 is released by myeloma cells and is transferred to MSCs. In MSCs, RUNX2-AS1 represses RUNX2 expression through the formation of a duplex with RUNX2 pre-

mRNA, which then blocks OB differentiation (Li *et al.*, 2018a). MiR-124 is a tumour suppressor whose expression is down-regulated in several cancer types (Cai *et al.*, 2018). Its expression is also substantially reduced in breast cancer bone metastases (Cai *et al.*, 2018). Experimentally, restoration of miR-124 expression in bone-tropic breast cancer cells reduces the progression of bone metastases in animals (Cai *et al.*, 2018). Mechanistically, miR-124 represses the expression of IL-11 by breast cancer cells, which is a tumour-derived factor promoting bone resorption (Cai *et al.*, 2018; Croucher *et al.*, 2016). Of note, IL-11 expression is inversely correlated with the expression of miR-124, and high expression of IL-11 in bone metastases is associated with an overall shorter survival of patients (Cai *et al.*, 2018).

Tumour-derived exosomal miRNAs can also affecting the function of bone cells. For example, exosomal miR-141 and miR-940 produced by prostate cancer cells are taken up by OB precursors and promote OB differentiation and proliferation, thereby facilitating the formation of sclerotic bone metastases (Hashimoto *et al.*, 2018; Ye *et al.*, 2017). Mechanistically, miR-141 activates p38-MAPKinases and increases OPG expression in OBs while miR-940 promotes osteogenic differentiation of MSCs *via* targeting ARHGAP1 (Rho GTPase Activating Protein 1) and FAM134A (Family with Sequence Similarity 134 Member A) (Hashimoto *et al.*, 2018; Ye *et al.*, 2017). Conversely, miR-218 secreted from breast cancer cells directly inhibits type I collagen deposition by OBs (Liu *et al.*, 2018). Similarly, lung adenocarcinoma cell-derived exosomal miR-21 facilitates OC differentiation *in vitro* (Hu *et al.*, 2017). MiR-21 exerts its promoting effect on OC differentiation *via* targeting programme cell death 4 (PDCD4), a repressor of c-Fos, which is an important transcription factor for OC differentiation (Hu *et al.*, 2017).

Circulating ncRNAs and their value as biomarkers

NcRNAs that are released into body fluids (*e.g.*, serum, plasma, urine, saliva) are either encapsulated within extracellular vesicles or are associated with high-density lipoproteins or AGO proteins, which provides protection from nuclease-mediated degradation (Anfossi *et al.*, 2018; Li *et al.*, 2018b). Thus, circulating ncRNAs are rather stable, and a growing body of evidence suggests that they might be useful as biomarkers in cancer (Anfossi *et al.*, 2018; Kang, 2016; Mishra, 2014; Wan-Ibrahim *et al.*, 2016). However, the prognostic value of ncRNAs in predicting the risk of bone metastases in patients with cancer clearly needs to be addressed.

Ell and colleagues identified a series of 4 miRNAs (miR-16, miR-211, miR-378, and let-7a) that were up-regulated during OC differentiation and induced by conditioned medium from breast cancer cells (Ell *et al.*, 2013). The authors thought to investigate this group of miRNAs as potential biomarkers associated with lytic bone metastases in patients. They found that the expression of miR-16 and miR-378 was consistently increased in the serum of breast cancer patients with bone metastases (n = 38), compared to healthy controls (n = 21) (Ell *et al.*, 2013). The amount of miR-10b in the serum was also significantly increased in breast cancer patients with bone metastases (n = 22), compared to breast cancer patients without bone metastases (n = 100) or healthy control subjects (n = 59) (Zhao *et al.*, 2012). In prostate cancer, the abundance of miR-218 was greatly reduced in the serum of patients with bone metastases (n = 38), compared to patients with no metastases (n = 115) (Peng *et al.*, 2019). Conversely, the abundance of miRNAs (miR-409-3p/5p, miR-154*, and miR-379) in the delta-like 1 homolog-deiodinase, iodothyronine 3 (DLK1-DIO3) cluster was increased in the serum of patients with metastatic prostate cancer, and an elevated expression of miR-154 and miR-379 was observed in bone metastatic prostate cancer cells *in situ*. This suggests that

miRNAs of the DLK1-DIO3 cluster could serve as biomarkers in patients with advanced prostate cancer (Gururajan *et al.*, 2014).

Of note, the identification of circulating bone-derived miRNAs to predict specific stages of the disease has been suggested for bone-related pathologies other than cancer. For example, Seeliger and colleagues identified five miRNAs (miR-21-5p, miR-23a-3p, miR-24-3p, miR-100-5p, and miR-125b-5p) whose expression levels are increased in both the serum and bone tissue of osteoporotic patients with osteoporotic fractures (Seeliger *et al.*, 2014). A correlation with bone mineral density in osteoporotic patients was also observed for miR-21-5p, miR-24-3p, miR-93-5p, miR-100-5p, and miR-125b-5p (Kelch *et al*., 2017). Additionally, intracellular studies revealed a simultaneous up-regulation of miR-21-5p, miR-93-5p, miR-100-5p and miR125b-5p in OBs and in OCs, whereas miR-148a-3p up-regulation was specific for osteoporotic OCs (Kelch *et al*., 2017). Overall, these findings (Seeliger *et al*., 2014; Kelch *et al*., 2017; Cheng *et al.*, 2019) suggest these miRNAs may be useful biomarkers for early diagnosis of osteoporosis. None of these miRNAs associated with osteoporosis did however overlap with those associated with breast cancer-induced bone destruction (Ell *et al.*, 2013).

Not only miRNAs, but also lncRNAs and circRNAs could potentially serve as biomarkers to predict the risk of bone metastases in patients with advanced cancer. For example, MALAT1 is measurable in the cellular fraction of peripheral human blood, and it has been proposed to be useful as a biomarker for the diagnosis of NSCLC (Weber *et al.*, 2013). Given that MALAT1 is highly expressed in tissues from NSCLC patients with bone metastases compared to tissues from patients without bone metastases (Liu *et al.*, 2016), it is likely that circulating MALAT1 could serve as a biomarker to predict the onset of skeletal-related events in NSCLC.

Interestingly, circRNAs are more stable than miRNAs and lncRNAs (Suzuki & Tsukahara, 2014), and they are enriched in exosomes compared to cells from where they are secreted (Li *et al.*, 2015), suggesting they might be used as biomarkers for human cancers (Kristensen *et al.*, 2018; Meng *et al.*, 2017; Wang *et al.*, 2016).

Concluding remarks

In this review, we describe the properties of ncRNAs, and we show that the dysregulation of ncRNAs in tumour cells and/or bone cells as well as the shuttling of ncRNAs between tumour cells and bone cells lead to the construction of a cancer niche in bone, which is essential for tumour progression (**Figure 2**). Interestingly, the identification of specific ncRNAs involved in bone metastasis formation and progression raises the possibility of developing novel biomarkers, which could provide important insight to predict the risk of relapse in patients with cancer. Likewise, the regulatory activity of ncRNAs in bone homeostasis and their dysregulation in bone metastasis encourage the development of ncRNA-based therapeutics.

Nomenclature of Targets and Ligands:

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding *et al.*, 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander *et al.*, 2017).

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Conflict of interest:

The authors declare no conflict of interest.

Declaration of transparency and scientific rigour

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the *BJP* guidelines for Design & Analysis, and as recommended by funding agencies, publishers and other organisations engaged with supporting research.

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Table 1- Noncoding RNAs (ncRNAs) involved in normal osteoblast (OB) and osteoclast (OC) functions.

Function	ncRNA name	Target(s)	References
Long non-coding RNAs (lncRNAs)			
Inhibition of OB differentiation	ANCR (DANCR)	*Runx2*, miR-758	(Zhu & Xu, 2013; Peng *et al.*, 2018)
	lnc-RUNX2-*AS1*	*Runx2*	(Li *et al.*, 2018a)
Promotion of OB differentiation	H19	TGF-β1/Smad3/HDAC signalling pathway, miR-449b, miR-107, miR-27b, miR-34a, miR-106b, miR-449a, miR-125a, miR-17, miR-141, miR-22, miR-138, let-7	(Gabory *et al.*, 2010; Raveh *et al.*, 2015; Huang *et al.*, 2015; Liao *et al.*, 2017; Liang *et al.*, 2016; Wu *et al.*, 2018; Peng *et al.*, 2017; Wei *et al.*, 2014)
	HOTAIR	*HoxD, Hoxc11, Hoxc12, Dlk1-Meg3, Igf2-H19, Hottip*	(Rinn *et al.*, 2007; Amandio *et al.*, 2016; Huynh *et al.*, 2017)
	Linc-ROR	miR-138, miR-145	(Feng *et al.*, 2018)
	lncRNA-1	*Runx2/p57, Sp7*	(Nardocci *et al.*, 2018)
	MALAT1	*miR-30*	(Yi *et al.*, 2019)
	MEG3	*Sox2*	(Zhuang *et al.*, 2015)
Promotion of dental and craniofacial development	Msx1-*AS*	*Msx1*	(Blin-Wakkach *et al.*, 2001)
MicroRNAs (miRNAs)			
(1) MiRNAs and osteoblasts (OBs):			
Inhibition of OB differentiation	let-7c	*Scd-1*	(Zhou *et al.*, 2018)
	miR-100	*Bmpr2*	(Ell *et al.*, 2014)
	miR-106	*Bmp2*	(Ell *et al.*, 2014)
	miR-133a	*Runx2*	(Zhang *et al.*, 2011)
	miR-135a	*Runx2*	(Zhang *et al.*, 2011)
	miR-137	*Runx2*	(Zhang *et al.*, 2011)
	miR-138	*Fak*	(Ell *et al.*, 2014)
	miR-139-3p	*Elk1*	(Wang *et al.*, 2018b)
	miR-141	*Dlx5*	(Ell *et al.*, 2014)
	miR-143	*Osterix*	(Ell *et al.*, 2014)

	miR-155	*Socs1, Mitf*	(Ell *et al.*, 2014)
	miR-17-5p	*Bmp2*	(Ell *et al.*, 2014)
	miR-182-5p	*Adcy6*	(Pan *et al.*, 2018)
	miR-204	*Runx2*	(Zhang *et al.*, 2011)
	miR-205	*Runx2*	(Zhang *et al.*, 2011)
	miR-200a	*Dlx5*	(Ell *et al.*, 2014)
	miR-206	*Cx43*	(Ell *et al.*, 2014)
	miR-208	*Ets1*	(Ell *et al.*, 2014)
	miR-214	*Atf4, Osterix*	(Ell *et al.*, 2014)
	miR-217	*Runx2*	(Zhang *et al.*, 2011)
	miR-23a	*Fak, Runx2, Satb2*	(Ell *et al.*, 2014)
	miR-23a cluster	*HoxA* cluster function	(Godfrey *et al.*, 2018)
	miR-24-2	*Fak, Runx2, Satb2*	(Ell *et al.*, 2014)
	miR-27a	*Fak, Runx2, Satb2*	(Ell *et al.*, 2014)
	miR-30	*Smad1, Runx2*	(Ell *et al.*, 2014)
	miR-30c	*Runx2*	(Zhang *et al.*, 2011)
	miR-338	*Runx2*	(Zhang *et al.*, 2011)
	miR-34	*Satb2, Runx2,* Notch pathway	(Ell *et al.*, 2014)
	miR-34c	*Runx2*	(Zhang *et al.*, 2011)
	miR-542-3p	*Bmp7*	(Ell *et al.*, 2014)
	miR-764-5p	*Chip/Stub1*	(Ell *et al.*, 2014)
	miR-93	*Sp7*	(Ell *et al.*, 2014)
Promotion of OB differentiation	miR-15b	*Smurf1*	(Ell *et al.*, 2014)
	miR-17~92		(Ell *et al.*, 2014)
	miR-181a	*Tgfbi*	(Ell *et al.*, 2014)
	miR-20a	*PPARg, Bambi, Crim1*	(Ell *et al.*, 2014)
	miR-218	*Sost-1, Dkk-2, Sfrp2*	(Hassan *et al.*, 2012)
	miR-219a-5p		(Aquino-Martinez *et al.*, 2018)
	miR-221	*Zzpm2*	(Zheng *et al.*, 2018)
	miR-29a	*ON, Dkk1, Krm2, sFrp2*	(Ell *et al.*, 2014)
	miR-332	*Tob2*	(Ell *et al.*, 2014)
	miR-342-3p	*Atf3*	(Han *et al.*, 2018)
	miR-335-5p	*Dkk1*	(Ell *et al.*, 2014)
	miR-451a	*Osr1*	(Karvande *et al.*, 2018)
	miR-874	*Sufu*	(Lin *et al.*, 2018)
(2) MiRNAs and osteocytes:			
Promotion of osteocytes apoptosis	miR-21	*Pten*	(Davis et al., 2017)
	miR-218	*IκB kinase B*	(Davis et al., 2017)

(3) MiRNAs and osteoclasts (OCs):			
Inhibition of OC differentiation	miR-124-3p	*NFATc1, RhoA, Rac1*	(Ji *et al.*, 2016)
	miR-125a-5p	*Traf6*	(Ji *et al.*, 2016)
	miR-145	*Sox9, Smad3*	(Yu *et al.*, 2018)
	miR-146a-5p	*Traf6, Stat1*	(Ji *et al.*, 2016)
	miR-155	*Socs1, Mitf*	(Ell *et al.*, 2014)
	miR-17	*EphA4*	(Lau & Sheng, 2018)
	miR-218	*p38 MAPK, NFATc1*	(Qu *et al.*, 2015)
	miR-218-5p	*p38MAPK-c-Fos-NFATc1*	(Ji *et al.*, 2016)
	miR-26a-5p	*Ctgf*	(Ji *et al.*, 2016)
	miR-27a	*Runx2*	(Guo *et al.*, 2018b)
	miR-30a		(Yin *et al.*, 2017)
	miR-34a-5p	*Tgif2*	(Ji *et al.*, 2016)
	miR-503-5p	*Rank*	(Ji *et al.*, 2016)
	miR-7b-5p	*DC-Stamp*	(Ji *et al.*, 2016)
Promotion of OC differentiation	miR-148a-3p	*MafB*	(Ji *et al.*, 2016)
	miR-183-5p	*HO-1*	(Ji *et al.*, 2016)
	miR-199a-5p	*Mafb*	(Guo *et al.*, 2018a)
	miR-29	*Cdc42, Srgap2, Nfia, Cd93, Calcr*	(Ji *et al.*, 2016)
	miR-31-5p	*RhoA*	(Ji *et al.*, 2016)
	miR-422a	*Cbl, Cd226, Igf1, Pag1, Tob2*	(Ji *et al.*, 2016)
	miR-9718	*Pias3*	(Ji *et al.*, 2016)
Promotion/inhibition of OC differentiation	miR-21-5p	*FasL, Pdcd4*	(Ji *et al.*, 2016)
	miR-223-3p	*Nfia, Ikk*	(Ji *et al.*, 2016)
	miR-29b	*Cdc42, Srgap2*	(Ell *et al.*, 2014)
Promotion of OC activity	miR-133a-3p	*Cxcl11, Cxcr3, Slc39A1*	(Ji *et al.*, 2016)
	miR-182	*Pkr*	(Inoue *et al.*, 2018)
Promotion of OC autophagy	miR-155	*Mitf*	(Sul *et al.*, 2018)
	miR-20a	*PPARg, Bambi, Crim1*	(Sun *et al.*, 2015)
(4) MiRNAs and chondrocytes:			
Inhibition of chondrocyte differentiation	miR-145	*Sox9*	(Ell *et al.*, 2014)
	miR-34a		(Ell *et al.*, 2014)
	miR-199a-3p	*Smad1*	(Ell *et al.*, 2014)
Promotion of chondrocyte activity	miR-140	*Hdac4, Dnpep*	(Ell *et al.*, 2014)

Promotion of chondrocyte development	miR-17~92	(Ell *et al.*, 2014)
CircularRNA (CircRNA)		
Inhibition of OB differentiation	circIGSF11	(Zhang *et al.*, 2018b)

Table 2- Dysregulated non-coding RNAs (ncRNAs) involved in bone metastasis.

NcRNA (expression level)	Function(s)	Target(s)	References
Osteoclasts:			
miR-133a (up)	Promotes bone resorption *in vivo*	*Mitf, Traf6, Calcr, Mmp14*	(Ell *et al.*, 2013)
miR-141-3p (up)	Promotes bone resorption *in vivo*	*Mitf, Traf6, Calcr, Mmp14*	(Ell *et al.*, 2013)
miR-190 (up)	Promotes bone resorption *in vivo*	*Mitf, Traf6, Calcr, Mmp14*	(Ell *et al.*, 2013)
miR-21 (up)	Promotes bone resorption *in vitro*	*Pdcd4*	(Hu *et al.*, 2017)
miR-214-3p (up)	Promotes bone resorption *in vivo*	*Traf3*	(Liu *et al.*, 2017)
miR-219-5p (up)	Promotes bone resorption *in vivo*	*Mitf, Traf6, Calcr, Mmp14*	(Ell *et al.*, 2013)
miR-34a (up)	Inhibits bone resorption *in vivo* (MRX34 in clinical trial)	*Tgif2*	(Krzeszinski *et al.*, 2014)
Osteoblasts:			
miR-141 (up)	Promotes bone formation *in vivo*	*p38-Mapk, Opg*	(Ye *et al.*, 2017)
miR-214-3p (up)	Inhibits bone formation *in vivo*	*Atf4*	(Haider & Taipaleenmaki, 2018)
miR-218 (up)	Promotes bone disruption *in vitro*	Collagen-type I	(Liu *et al.*, 2018)
Breast cancer cells:			
let-7g (up)	Inhibits bone metastasis *in vivo*	*Hmga2*	(Ell & Kang, 2014)
MALAT1 (up)	Promotes bone metastasis *in vitro*	miR-30 sponge (?)	(Jadaliha *et al.*, 2016)
MAYA (up)	Promotes bone metastasis *in vivo*	*CTGF*	(Li *et al.*, 2017a)
miR-10b (up)	Promotes bone metastasis *in vivo*	*HoxD10*	(Croset *et al.*, 2015; Ell & Kang, 2014; Ma *et al.*, 2007)
miR-124 (down)	Promotes bone metastasis *in vivo*	*Il-11*	(Cai *et al.*, 2018)
miR-126 (down)	Promotes bone metastasis *in vivo*		(Tavazoie *et al.*, 2008)
miR-135 (down)	Promotes bone metastasis *in vivo*	*Runx2*	(Taipaleenmaki *et al.*, 2015)
miR-17 (up)	Promotes bone metastasis *in vivo*	*Tbr2*	(Croset *et al.*, 2015; Ell & Kang, 2014)
miR-203 (down)	Promotes bone metastasis *in vivo*	*Runx2*	(Taipaleenmaki *et al.*, 2015)
miR-206 (down)	Promotes bone metastasis *in vivo*		(Tavazoie *et al.*, 2008)
miR-21 (up)	Promotes bone metastasis *in vitro*	*Pten, Pdcd4, Spry2*	(Sahay *et al.*, 2015)
miR-218 (up)	Promotes bone metastasis *in vivo*	WNT activity	(Liu *et al.*, 2018; Hassan *et al.*, 2012; (Taipaleenmaki *et al.*, 2015)
miR-30s (up)	Inhibits bone metastasis *in vivo*	*Il-8, Il-11, Dkk-1 Runx2, Cdh11, Ctgf, Itga5, Itgb3*	(Croset *et al.*, 2018)
miR-335 (down)	Promotes bone metastasis *in vivo*	*Sox4, Tnc*	(Tavazoie *et al.*, 2008)
Prostate cancer cells:			

MALAT1 (up)	Promotes bone metastasis *in vitro*		(Sebastian *et al.*, 2015)
miR-143 (up)	Inhibits bone metastasis *in vivo*	*Hef1*	(Croset *et al.*, 2015; Ell & Kang, 2014)
miR-145 (up)	Inhibits bone metastasis *in vitro*	*Hef1*	(Croset *et al.*, 2015; Ell & Kang, 2014)
miR-146a (up)	Inhibits proliferation and adhesion *in vitro*	*Rock*	(Ell & Kang, 2014)
miR-16 (up)	Inhibits bone metastasis *in vivo*		(Ell & Kang, 2014)
miR-203 (up)	Inhibits bone metastasis *in vivo*	*Survivin/Birc5* EGFR signaling pathway, *Runx2, Dlx5, Spp1, Bglap*	(Siu *et al.*, 2018; Saini *et al.*, 2011)
miR-466 (up)	Inhibits bone metastasis *in vivo*	*Runx2, Spp1, Bglap*	(Colden *et al.*, 2017; Saini *et al.*, 2011)
Non-small cell lung cancer cells:			
MALAT1 (up)	Promotes bone metastasis *in vivo*		(Liu *et al.*, 2016)
miR-203 (down)	Promotes bone metastasis *in vivo*	*Bax, Casp-3, p53, pSmad2, Tgf-β1*	(Wei *et al.*, 2017)
Mesenchymal stem cells:			
let-7 (up)	Promotion of cancer progression *in vivo*	*Il-6*	(Ell & Kang, 2014)
miR-23b (up)	Promotes breast cancer dormancy *in vitro*	*Marcks*	(Ono *et al.*, 2014)
miR-222/223 (up)	Promotes breast cancer dormancy *in vivo*		(Bliss *et al.*, 2016)
RUNX2-AS1 (up)	Inhibits osteoblast differentiation *in vivo*	*Runx2*	(Li *et al.*, 2018a)

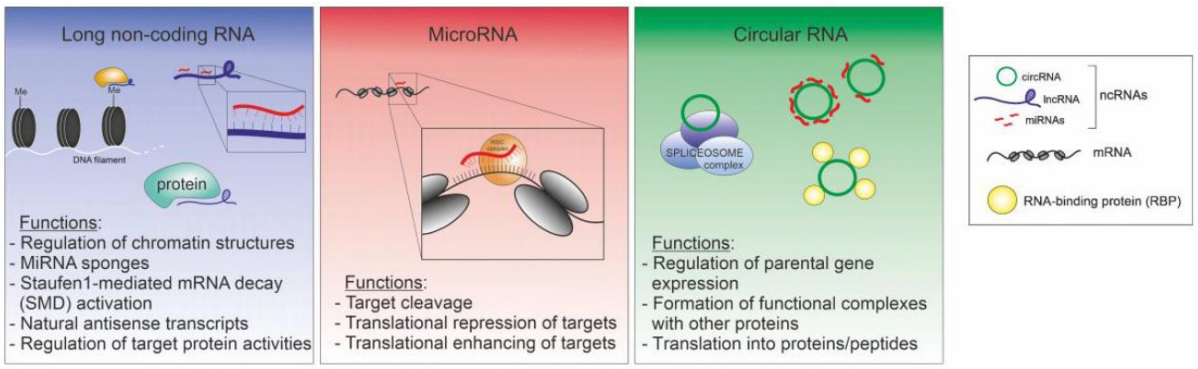

Figure 1- Schematic representation of ncRNA mechanisms of action.
NcRNAs regulate gene expression both at transcriptional and post-transcriptional levels. LncRNA (*left box*), miRNA (*middle box*), and circRNA (*right box*) act in a variety of ways promoting or inhibiting the expression of specific targets. Mechanisms of action of ncRNAs are summarized within each box.

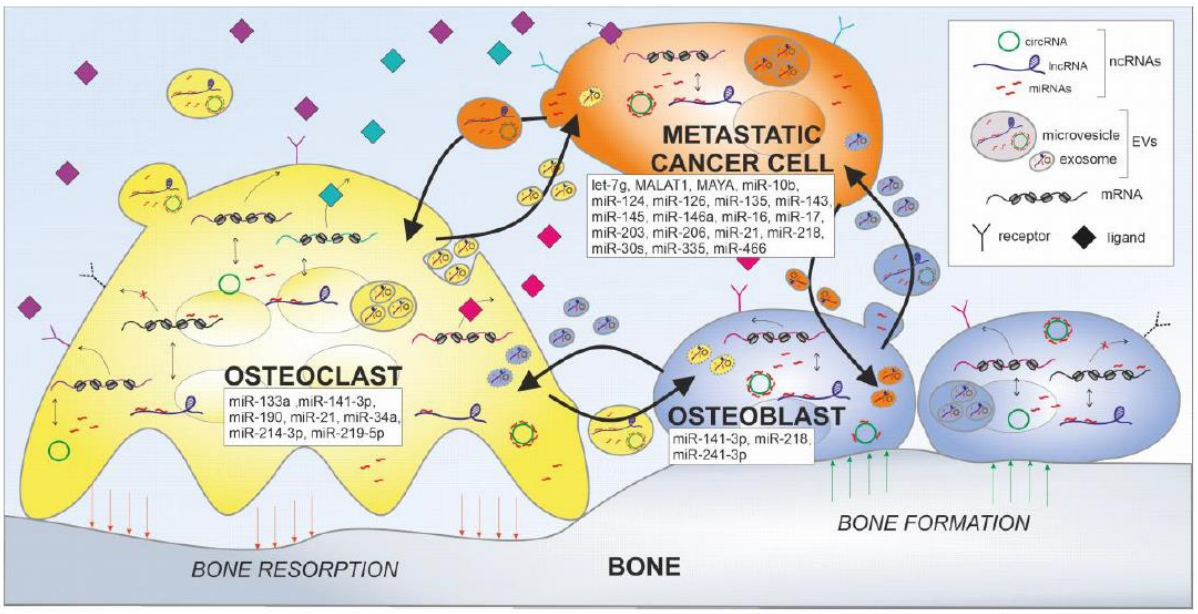

Figure 2- Involvement of ncRNAs in 'vicious cycle' of bone metastases.
A metastatic cancer cell displays a different gene expression pattern in comparison to a normal cell in part as a result of dysregulated expression of lncRNAs, miRNAs, and circRNAs, which in turn alters the expression of proteins involved in metastasis formation. Moreover, ncRNAs may serve as inter-cellular messengers for cell-cell communication. Specifically, ncRNAs can be secreted by cancer cells and bone cells (osteoclast, osteoblast) either encapsulated within extracellular vesicles (EVs), such as microvesicles and exosomes, or as ncRNA-(lipo-)protein complexes, which are taken up by recipient cells. These ncRNAs can then reprogram recipient cells to facilitate bone metastases formation. NcRNAs involved in bone metastasis formation and progression are listed for each cell type (metastatic cancer cell, osteoblast, osteoclast).